



# 30. Arbeitstagung der Anatomischen Gesellschaft in Würzburg

# 25.09.2013 bis 27.09.2013

Titel: TGFβ1 and matricellular protein expression in desmoglein 2-mutant mice, a model for arrhythmogenic right ventricular cardiomyopathy

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## Abstract:

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a rare predominantly genetic heart muscle disorder, characterized by cardiomyocyte death, dilation of ventricles and fibrofatty replacement of the myocardium. In juvenile ARVC patients myocardial infarction like scar formation is observed, a feature that is recapitulated in our ARVC mouse model. These knock-in mice contain mutant alleles of the gene coding for the desmosomal cadherin Desmoglein 2 (Dsg2mt/mt). The mutant polypeptide lacks major parts of the extracellular domains 1 and 2. We examined the expression and cardiac localization of the pro-fibrotic cytokine transforming growth factor beta 1 (TGFbeta1) and of the matricellular proteins tenascin C (TNC) and thrombospondin 1 (THBS1) because of their pivotal roles in tissue repair after myocardial infarction. We analyzed DSG2mt/mt and healthy control mice at 2, 6, 8 and 12 weeks after birth.

Cardiac mRNA expression of TGFbeta1 was significantly upregulated in 8 and 12- week-old DSG2mt/mt mice. TGFbeta1 mRNA and protein expression were both detected in leukocytes, endothelial cells and connective tissue of the myocardial lesions.

Cardiomyocytes next to these lesions express little TGFbeta1 mRNA but show strong TGFbeta1 immunostaining. THBS1 and TNC mRNA expression was significantly increased in 6, 8 and 12-week-old DSG2mt/mt mice. In addition, strong TNC immunostaining was observed in the extracellular matrix of the fibrotic myocardial lesions.

We conclude that the synthesis of TGFbeta1 and TGFbeta1-regulated extracellular matrix proteins is directly related to the extensive replacement fibrosis in our ARVC mouse model. Thus, targeting TGFbeta1 signaling is a promising therapeutic strategy in ARVC.

Titel:Functional imaging of desmoglein molecules on human keratinocytes using atomic force microscopy

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## Abstract:

Atomic force microscopy (AFM) is a technique to visualize the topography of hard and soft surfaces by nanometer scale. Furthermore, by functionalizing the scanning tip it enables to investigate the binding properties of a specific adhesion molecule. Here we used an AFM setup combined with light microscopy to simultaneously visualize the topography of human keratinocytes and delineate the distribution of the Ca2+-dependent adhesion molecule desmoglein 3 (Dsg3) on the cell surface. By linking recombinant Dsg3 to the AFM scanning tip, we detected adhesion events which were distributed in clusters on the cell surface and at areas of cell-cell contact. These events were blocked by removing Ca2+ and by specific Dsg3 autoantibodies. The binding forces were similar to those observed by studies using recombinant Dsg3 molecules in a cell-free environment. We further studied changes in the topography of HaCaT cells induced by autoantibodies derived from patients with the blistering skin disease pemphigus vulgaris. The autoantibody fractions, which target Dsg3, induced separation of plasma membranes between desmosomal adhesive complexes as soon as after 30 min. This was followed by retraction of intermediate filaments, the latter of which are detectable as bundles of fiber-like structures attached to desmosomes in the topography image. These data are in line with well-established morphological changes induced by pemphigus autoantibodies such as interdesmosomal widening and collapse of the keratin filament cytoskeleton. Taken together, the combination of topographical and functional AFM imaging on living cells represents a powerful tool for research of cell adhesion receptors.

Titel:Plakoglobin but not desmoplakin regulates keratinocyte cohesion via modulation of p38mapk signaling

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## Abstract:

Plakoglobin (Pg) and desmoplakin (Dp) are adapter proteins within the desmosome, providing a mechanical link between desmosomal cadherins as transmembrane adhesion molecules and the intermediate filament cytoskeleton. Because in the severe skin blistering disease pemphigus autoantibodies against desmosomal adhesion molecules induce loss of keratinocyte cohesion at least in part via p38MAPK activation and depletion of desmosomal components, we evaluated the roles of Pg and Dp in p38MAPK-dependent loss of cell adhesion. Silencing of either Pg or Dp reduced cohesion of cultured human keratinocytes in dissociation assays. However, Pg but not Dp silencing caused activation of p38MAPK-dependent keratin filament collapse and cell dissociation. Interestingly, extranuclear but not nuclear Pg rescued loss of cell adhesion molecule desmoglein 3 and tethered p38MAPK to desmosomal adhesion molecule desmoglein 3 and tethered p38MAPK to desmosomal complexes. Our data demonstrate a novel role of extranuclear Pg in controlling cell adhesion via p38MAPK-dependent regulation of keratin filament organization.

Titel:Fatty acid β-oxidation: novel insights from a phylogenetic approach

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#### Abstract:

Lipid metabolism is of major concern not only for obesity but also because a controlled assembly of lipid species is crucial for a healthy eukaryotic cell. In human, peroxisomes and mitochondria catabolize fatty acids of different chain length involving large sets of enzymes. Such a division of tasks requires a coordination of beta-oxidation in both organelles, particularly because peroxisomes generate cytotoxic H2O2 using acyl-CoA oxidases, while mitochondria surpass electrons to the respiratory chain involving acyl-CoA dehydrogenases. As a matter of genome complexity, the functional analysis of such coordinative processes is challenging in mammals whereas genetically easily accessible models like yeast do not possess a mitochondrial pathway. Introducing the fungus Ustilago maydis as a new model, we explored its genome for the presence of mitochondrial and peroxisomal beta-oxidation systems and verified that those enzymes localize to their respective organelle. Moreover, a peroxisome-less mutant still grows on short/medium-chain fatty acids but not on longer ones. Thus, Ustilago mimics the metabolic situation found in human implying that parallel organellar beta-oxidation pathways are an ancient eukaryotic trait. A phylogenetic analysis of > 30 fungal and animal genomes showed that this assumption is indeed valid. Concerning the H2O2 production in peroxisomal beta-oxidation, it is tempting to ask, why this pathway was not suppressed during evolution. Importantly, our phylogenetic reconstruction revealed that a subset of acyl-CoA dehydrogenases possess conserved peroxisomal targeting sequences. Verifying that these enzymes target to peroxisomes, we propose that peroxisomal acyl-CoA dehydrogenases balance H2O2 production during beta-oxidation. Consequently, H2O2 may not just be cellular threat but kept at leveled cellular concentrations in order to act as a regulator of subcellular redox states.

Titel: A PPAR $\gamma$  knockout in Clara cells of the mouse lung leads to peroxisomal proliferation and high induction of peroxisomal genes

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## Abstract:

PPARy is a member of the nuclear hormone receptor superfamily involved in the regulation of cellular differentiation, lung maturation and inflammation. PPARy is expressed strongly in Clara and AECII cells of the mouse lung, where peroxisomes are also mainly present. Clara cell specific PPARy knockout mice (ccsPparyKO) displayed enlarged airspaces, an altered lung physiology and reduced extracellular matrix gene expression. However, so far no knowledge is available on the alterations of peroxisomal compartment induced by a PPARy deficiency. Therefore, in this study, we used lungs of ccsPparyKO mice to investigate its effect on peroxisomal compartment and its role in distal airway morphogenesis. We characterized ccsPparyKO mouse lungs by means of 1) double-IF for peroxisomal proteins 2) cryosectioning, laser-assisted microdissection and RT-PCR analysis 3) lipid profiling (plasmalogens content). Our results revealed a strong proliferation of peroxisomes in the lungs of ccsPparyKO mice. Moreover, significant changes in peroxisomal proteins and their targeting receptors as well as strong induction of corresponding gene expression were observed. A strong upregulation of the mRNA for PEX7p, encoding the peroxisome targeting signal 2 receptor and PEX7p dependent matrix proteins import, such as enzymes for plasmalogen synthesis (GNPAT and AGPS) or peroxisomal  $\beta$ -oxidation (ACAA1) and  $\alpha$ oxidation (PHYH) was observed in Clara cells, suggesting a high turnover of peroxisomal lipid substrates. In addition, increase of peroxisomal antioxidative enzyme catalase was observed revealing oxidative stress in the Clara cells. In conclusion, our study provides strong evidence that the deficiency of PPARy in Clara cells induces peroxisomal proliferation and their metabolic enzymes, suggesting a protective role in Clara cells in the control of ROS and lipid homeostasis.

Titel:Water homeostasis of the inner ear can be influenced by the anti-diuretic hormone

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#### Abstract:

Hearing and balance functions of the inner ear rely on homeostasis of the inner ear fluid spaces. Disturbance of this equilibrium leads to pathologic conditions, for example the endolymphatic hydrops (EH) often observed in Menière's disease (MD). While several specific transmembran proteins have been identified that facilitate endolymphatic ion homoestasis, the knowledge about the molecular determinants of endolymphatic water homoestasis and its volume regulation is rather limited. A positive correlation between the presence of EH and blood concentrations of the anti-diuretic hormone (ADH) was reported from clinical observations and animal experiments. Therefore, the inner ear has been suggested as a direct target of ADH.

Using a confocal laser scanning technique the transepithelial and transcellular water flow in the endolymphatic sac (ES) epithelium was measured in response to osmotic challenge under different pharmacological treatment conditions.

Our data show that the inner ear is a direct target of ADH and that the endolymphatic water homeostasis may be influenced by pharmacological substances which are involved in an ADH-mediated signal transduction cascade. Besides Aquaporins, Na+-K+-CI--

cotransporters (NKCC) facilitiate water transport across cell membranes along osmotic gradients and are shown to be regulated by ADH.

As knowledge of the expression pattern of NKCC subtypes in the ES ist restricted to the cellular level, we analysed the subellular localization in the cells of the ES.

These results add the understanding of the mechanisms of water related volume regulation of the inner ear and have direct implications for potential treatment options in MD.

Titel:Two-photon microscopy reveals stationary podocytes in living zebrafish larvae

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## Abstract:

Podocytes are an essential component of the glomerular filtration barrier and cover the outer aspect of glomerular capillaries. They form a complex actin-based cytoskeleton in vivo and show a prominent motility in vitro. Since a long time it has been speculated whether podocytes are stationary or mobile in vivo. To address this question we performed twophoton microscopy (2PM) of the pronephros of translucent zebrafish larvae (casper) expressing eGFP specifically in podocytes (wt1a:eGFP larvae) over extended periods of time. By intravital 2PM, podocyte cell bodies as well as the interdigitating branching pattern of major processes could be resolved in zebrafish larvae at 5-7 dpf (days post fertilization) with a resolution of about 1 µm in the xy-plane. Time-lapse imaging demonstrated that podocytes neither migrated nor changed their branching pattern of major processes over a time period of up to 18 h. Podocyte motility was neither detected by recording at low rates (2-5 images per h) nor at high rates (up to 5 images per s). By contrast, weakly GFP-positive non-podocyte cells close to the pronephric glomerulus exhibited vigorous motility in 2PM time-lapse recording. In summary, we have generated a translucent zebrafish with fluorescently labeled podocytes for intravital 2PM revealing that podocytes are stationary cells in the intact glomerulus.

Titel:Crown-like structures are a new source of adipose tissue macrophages

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## Abstract:

Obesity is frequently associated with low-grade inflammation of adipose tissue (AT) and increase of AT macrophages (ATMs) is linked to an increased risk of type 2 diabetes. Macrophages have been regarded as terminally differentiated cells and hence post-mitotic, but recent observations challenged this view. Here, we tested the hypothesis that macrophages proliferate within AT in three different models of mouse obesity and in humans. We show that ATMs undergo local proliferation within the tissue, predominantly on sites of local inflammation around dead adipocytes, the so-called crown-like structures (CLS). Upon feeding mice with a high fat diet (HFD), we found a time-dependent increase of ATM proliferation using histochemistry and flow cytometry. Up-regulation of CD206 and CD301 of proliferating ATMs indicated preferential M2-polarization, suggesting a more antiinflammatory immune phenotype. Live-imaging within AT explants from obese mice revealed that macrophages emigrate out of the CLS to become resident in the interstitium. In humans, we confirmed the increased expression of proliferation markers of CD68-positive macrophages in CLS and demonstrate a higher Ki67 mRNA expression in AT from obese and diabetic patients (n=239). Thus, local proliferation contributes to the increase of ATMs in obesity. Further, our data identify CLS as the primary site of ATM proliferation and supports a model of different recruitment mechanisms for classically-activated (M1) and alternativelyactivated (M2) macrophages in obesity.

This work was supported by the DFG-SFB 1052 "obesity mechanisms" and the Helmholtz alliance "Imaging and Curing Environmental Metabolic Disease".

Titel:Bidirectional cellular communication: reverse signaling of the transmembrane chemokines CXCL16 and CX3CL1

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#### Abstract:

Reverse transduction of signals can occur when transmembrane ligands bind to their classical receptors. This "reverse signaling" has been initially observed with ligands of the tumor necrosis factor (TNF) family after binding to their cognate receptors (or to antibodies). Thus, bidirectional responses are produced in the targeted and in the ligand-exposing cells. Since transmembrane chemokines bear potential signal-transducing motifs in their intracellular domains, we explored whether or not reverse signaling occurs also after binding of CXCL16 or CX3CL1/fractalkine to their classical G-protein-coupled receptors CXCR6 and CX3CR1. As shown by quantitative RT-PCR and immunocytochemistry, the ligands are abundantly expressed in many types of tumor cells and endothelial cells, whereas the receptors are mostly restricted to activated T cells (CXCR6) respectively monocytes/macrophages (CX3CR1). To study classical and reverse signaling we exposed adherent cells expressing or transfected with the transmembrane ligand to non-adherent cells expressing or transfected with the receptor. Signal transduction was monitored either morphologically by co-staining phosphorylation of kinases with cell-type specific markers, or biochemically by separation of cells (washing away) followed by analysis of kinase phosphorylation by Western blots. In these experiments reverse and classical signaling were clearly observed. Mutation and cell biology experiments are ongoing elucidating the signal transduction mechanism of transmembrane chemokines and further biological functions. Reverse signaling may thus be an alternative to an autocrine secondary signaling loop with classical receptors.

Titel:Chronic Nrf2 activation in hepatocytes results in tumourigenesis

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## Abstract:

Hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCC) occurs in the background of the pro-oxidative environment found in chronic liver disease. Nrf2 and its cellular repressor Keap1 is well established as the major regulators of oxidative stress defence with anticarcinogenic effect and is known to be activated during chronic liver disease. In contrast, somatic mutations of the Nrf2 gene (NFE2L2) leading to constitutive activation of Nrf2 by disrupting the Nrf2 Keap1 interaction are found in various carcinomas. Recently, analysis of somatic mutations of HCC reveals an interaction of the Nrf2/Keap1 pathway with the Wnt/Bcatenin pathway, which is also involved in HCC development and progression. However, the molecular mechanisms of this interplay leading to HCC development are unknown. Here we show that chronic activation of Nrf2 in hepatocytes spontaneously induces HCC formation and CCC up to large hepatic cysts via epithelial-mesenchymal transition. Specifically, constitutive Nrf2 signalling in the liver leads to β-catenin up-regulation via an antioxidant response element within the  $\beta$ -catenin promoter and to nuclear translocation of  $\beta$ -catenin. Consequently,  $\beta$  catenin target gene SOX9 are constantly up-regulated in Nrf2 active livers inducing EMT of hepatocytes. Our data establish a previously unexpected oncogenic role for an Nrf2- $\beta$ -catenin interplay in liver cancer development. This study provides a new explanatory approach for the fact that chronic liver diseases and NFE2L2 mutations lead to cancer development.

Titel:Tumor and endothelial cell-derived microvesicles carry distinct CEACAMs and influence T-cell behaviour

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#### Abstract:

Normal and malignant cells release a variety of different vesicles into their extracellular environment. The most prominent vesicles are the extracellular microvesicles (MVs, 100-1 000 nm in diameter), which are shed of the plasma membrane, and the exosomes (70-120 nm in diameter), derivates of the endosomal system. MVs have been associated with intercellular communication processes and transport numerous proteins, lipids and RNAs. As essential component of immune-escape mechanisms tumor-derived MVs suppress immune responses. Additionally, tumor-derived MVs have been found to promote metastasis, tumorstroma interactions and angiogenesis. Since members of the carcinoembryonic antigen related cell adhesion molecule (CEACAM)-family have been associated with similar processes, we studied the distribution and function of CEACAMs in MV fractions of different human epithelial tumor cells and of human and murine endothelial cells. Here we demonstrate that in association to their cell surface phenotype, MVs released from different human epithelial tumor cells contain CEACAM1, CEACAM5 and CEACAM6, while human and murine endothelial cells were positive for CEACAM1 only. Furthermore, MVs derived from CEACAM1 transfected CHO cells carried CEACAM1. In terms of their secretion kinetics, we show that MVs are permanently released in low doses, which are extensively increased upon cellular starvation stress. Although CEACAM1 did not transmit signals into MVs it served as ligand for CEACAM expressing cell types. We found evidence that CEACAM1-positive MVs significantly increase the CD3 and CD3/CD28-induced T-cell proliferation. Thus our data demonstrate that MV-bound forms of CEACAMs play important roles in intercellular communication processes, which can modulate the immune response.

Titel:Endothelial damage contributes to bbb breakdown in different models of ischemic stroke

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## Abstract:

The term 'blood- brain barrier' (BBB) relates to the ability of the vasculature of the central nervous system (CNS) to prevent entrance of hydrophilic molecules into the CNS parenchyma proper. This specific feature of CNS vessels was initially reported to depend on tight junction contacts between overlapping endothelial cells. Therefore, as a converse argument, BBB breakdown associated with ischemic stroke was often exclusively attributed to an opening of endothelial tight junctions, which consequently leads to edema and hemorrhages thereby critically impacting on the clinical outcome of concerned patients. However, clinical studies often failed to adopt preclinical treatment concepts into daily routine as experimental models often fail to mirror the clinical situation. We therefore investigated the fate of endothelial tight junctions in areas of BBB breakdown in an embolic model of ischemic stroke in rats. Contrary to our expectations, we were not able to demonstrate changes of the staining patterns for critical tight junction proteins such as occludin and claudin-5 in affected areas. Furthermore, ultrastructural analysis revealed novel evidence questioning the impact of altered tight junction complexes on BBB breakdown as we could regularly show endothelial degeneration to cause BBB damage whereas an opening of tight junctions was never observed. To rule out that the observed mechanism only holds true for the applied model, we also used a permanent and a transient model of stroke in mice, which confirmed our previous findings. Hence, protection of endothelial cells may turn into focus of future therapeutic strategies.

Titel: TGFBR2 conditional knock-out in the developing telencephalon reveals neurovascular defects

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Abstract:

To understand the role of transforming growth factor beta (TGFbeta) in forebrain development, we characterize the function of TGFbeta signaling in vivo via a Foxg1-cre knock-in mouse to conditionally knock-out Tgfbr2. Although FOXG1 is expressed in progenitors and neurons of the telencephalon, the ventral telencephalon of Foxg1cre/+;Tgfbr2flox/flox (Tgfbr2-cKO) mutants displayed intracerebral hemorrhages. Impaired vasculature due to clustered appearance of vessels and reduced branching is observed in mutant embryos. Pericyte coverage and cell junctions existed in endothelial cells. In contrast, integrity of the extracellular matrix seemed disturbed in Tgfbr2-cKO. Expressions of several IGF-ligand and binding proteins, TGFbeta, THBS2, ADAMTS1 and ID-1 as well as distribution of VEGFA and some integrins in the tissue were altered. Endothelial cells and pericytes did not express Foxg1 in significant amounts. Thus, the observed vascular defects arose from disturbed signaling between neurons and vessels. involving an altered secretome. Hence, we stimulated HUVECs with conditioned medium from primary neuronal cultures of different regions derived from Tgfbr2-cKO and control forebrains, they displayed less branching points. VEGFA supplementation reversed this defect, whereas application of TGFbeta severed this condition. Migration of HUVECs towards conditioned medium of mutants compared to medium of controls was also impaired. This phenotype was partially rescued by supplementation of conditioned media with soluble factors VEGFA, FGF2, IGF1 and IGF2, whereas TGFbeta supplementation also increased the migration defect. In conclusion, we discovered disturbed neural secretion of several signaling molecules that are necessary for correct endothelial development.

Titel:Tumour angiogenesis in organotypic brain slices - the vogim procedure

Autoren: Savaskan N.(1), Hock S.(2), Buchfelder M.(2), Eyüpoglu I.(2),

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## Abstract:

In the past, a variety of assays has been established to study angiogenesis, but very few allow investigations in a model with organotypic cellular and microenvironmental composition. Previously, we established an Organotypic Glioma Invasion Model (OGIM) which allows monitoring in real-time tumor invasion, metastasis and angiogenesis in an invitro experiment with in-vivo conditions. Therefore, we extended this ex-vivo system by tracking the distribution of genetically marked glioma cells (RFP, GFP) and vascular components. In this model, we found that the blood-vessel architecture is altered drastically in and around the tumor-bulk in comparison to normal brain tissue without tumor contact. Within the tumor, the blood vessels show ranging diameters with erratic alterations of vessel types and course. The peritumoral region is characterized by the absence of large diameter vessels, or metarterioles whereas unaffected brain regions display an even and regular distribution of vessels from capillaries, arterioles and metarterioles. We classified these alterations in physiological vessel architecture with large diameter vessels at the pial surface and smaller vessels radiating into the cortex. In contrast, vessels in the peritumoral area show a heterogeneous and diffuse architecture. Vessels in the tumor core range from "bigger than normal" to "capillary size" diameter and represent an altered architecture in terms of variations, diameter and distribution. Thus, our novel model system represents a versatile system to study tumor - brain - blood vessel interaction and thus represents a bridging assay for purely cell based in-vitro assays and in-vivo animal experiments.

Titel: EGFL7 ligates  $\alpha V\beta 3$  integrin to enhance vessel formation

Autoren: Nikolic I.(1), Dudvarski Stankovic N.(1), Bicker F.(1), Baumgart J.(1), Chavakis M.(2), Schäfer M.(3), Mittelbronn M.(4), Schmidt M.(1),

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## Abstract:

Angiogenesis, defined as blood vessel formation from a pre-existing vasculature, is governed by multiple signal cascades including integrin receptors, in particular integrin alphaVbeta3. Here we identify the endothelial cell (EC)-secreted factor epidermal growth factor-like protein 7 (EGFL7) as a novel specific ligand of integrin alphaVbeta3 thus providing mechanistic insight into its proangiogenic actions in vitro and in vivo. Specifically, EGFL7 attaches to the ECM and by its interaction with integrin alphaVbeta3 increases the motility of EC, which allows EC to move on a sticky underground during vessel remodeling. We provide evidence that the deregulation of EGFL7 in zebrafish embryos leads to a severe integrin-dependent malformation of the caudal venous plexus (CVP), pointing towards the significance of EGFL7 in vessel development. Last, we are able to show that the expression levels of EGFL7 in human specimens of diseases rely largely on the remodeling state of the existing vasculature but not on the origin of the disease as EGFL7 was upregulated in blood vessels of human brain pathologies and of the penumbra of stroke upon reversible mouse middle cerebral artery occlusion (MCAO). Our work sheds a novel light on the molecular mechanism EGFL7 engages to govern physiological and pathological angiogenesis.

Titel:The estate of Heinrich von Eggeling – an invaluable source for the history of the Anatomische Gesellschaft has "resurfaced"

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Abstract:

Heinrich von Eggeling (1869–1954) war Professor der Anatomie in Jena und Breslau. Er war außerdem nach Karl von Bardeleben (1849–1918) der zweite Schriftführer der Anatomischen Gesellschaft (AG), und zwar über 30 Jahre lang von 1918 bis zur Wiedergründung der Gesellschaft 1949. Seine Amtsführung fällt damit in zwei für die deutsche Wissenschaft schwierige Phasen, die Zeit der anfänglichen Isolierung nach dem Ersten Weltkrieg und die Zeit des Nationalsozialismus. Die Erforschung der Geschichte der AG litt bisher unter einer relativ schwierigen Quellenlage, da sich über die Kette der sechs Schriftführer keine vollständigen Akten aus der Zeit vor 1974 erhalten hatten. Es ist nun gelungen, den "wissenschaftlichen Nachlass" Heinrich von Eggelings aufzuspüren und für die AG zu sichern. Das Material hatte sich zeitweise im Adelsarchiv Marburg, zeitweise in Familienbesitz erhalten. Es handelt sich um den beruflichen Schriftverkehr von Eggelings von 1919 bis 1953 und umfasst zusätzlich Unterlagen, die er von seinem Vorgänger übernahm, darunter auch die Originale des Gründungsschriftwechsels von 1886. Nach einer ersten Sichtung enthält dieser Nachlass auch Informationen zu bisher weniger bekannten Kapiteln der Geschichte unserer Gesellschaft, so zum Beispiel zu den Vorkommnissen um Prof. Martin Heidenhain aus Tübingen, dem wegen seiner jüdischen Vorfahren ein Vortrag auf der Tagung 1934 verwehrt wurde, obwohl er noch amtierendes Vorstandsmitglied war. Auch die (vergeblichen) Versuche, 1942 bei einem "Anatomenlager" genannten Treffen in Tübingen eine separate "Deutsche Anatomenschaft" zu etablieren, lassen sich mit diesem Nachlass näher untersuchen. Nach der wissenschaftlichen Erschließung soll der Nachlass in das Archiv der AG beim jetzigen Schriftführer übergehen.

Titel:Enzyme histochemistry in colonic motility disorders of adults: reference values for morphometry

Autoren: Oppitz M.(1), Al-Haidary J.(2), Duschka L.(2), Fisseler-Eckhoff A.(1), Scheil-Betram S.(1),

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#### Abstract:

Histochemistry and morphometry of the enteric nervous system are essential for the diagnosis of neuronal dysfunction on slow-transit constipation. Reference values for the enteric nervous system are mainly based on data given for motility disorders in childhood based upon data from 15 µm thick cryosections. For reference values of aduts, 100 colonic segments from patients were investigated (median age at diagnosis 60 years; range 26 to 89 years; 15 normal, 21 desmosis, 18 hypoganglionosis, 42 with autolysis). Frozen specimens were assessed by morphometry of the myenteric (PM) and submucosal plexus (PS) based on histochemistry (NOS, AChE, LDH, SDH). There was no statistical difference for age diagnosis and length of the specimen. Significant differences were found for hypoganglionosis, desmosis, or segments without pathological findings concerning the diameter of PM neurons in µm (21,2+/-0,5 vs. 24,4+/-0,6; p=0,0001) or 24,5 +/- 0,5), number of PM neurons per ganglion (10,5+/-0,3 vs. 11,9+/-0,3; p=0,004 or 11,6 +/- 0,3; p = 0,001) and area of PM neuron in µm2 (199,7+/-9,5 vs. 251,5+/-10,4; p=0,0001 or 266,2 +/- 9,5; p= 0,0004). No significant difference was observed in the number of PS neurons (3, 4 + 2). Autolysis could be easily distinguished from hypoganglionosis by cell size (153,5 +/- 6,3 vs. 199.7+/-9.5; p= 0.0001). For differential diagnosis in colonic motility disorders the PM neuron diameter can be assessed using a minimum count of 40 neurons.

Titel:Alterations of intestinal smooth muscle in patients with diverticulosis

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## Abstract:

Background & aims: Diverticular disease (DD) is associated an enteric neuromuscular pathology. However, studies on muscular alterations are limited to patients with diverticulitis characterized by previous inflammatory episodes. To assess whether myopathic lesions represent a primary event in the pathogenesis of DD unrelated to inflammation-induced secondary changes, the enteric musculature was assessed in patients with diverticulosis ("cold diverticula"). Material & methods: Full-thickness sigmoid specimens from patients with diverticulosis (n=10) and controls (n=17, colorectal neoplasia) were processed for morphological and molecularbiological studies. Morphometric analysis was performed to evaluate the thickness and connective tissue index (CTI) of circular and longitudinal muscle. Structural alterations were determined by light and electron microscopy. mRNA profiles of components of the contractile smooth muscle apparatus including smooth muscle ?-actin (?-SMA), smoothelin (SM), histone deacetylase 8 (HDAC8), smooth muscle myosin heavy chain (SMMHC), caldesmon (CALD1) and tropomysion (TPM3) were assessed by qPCR. Results: Compared to controls patients with diverticulosis showed mRNA down-regulation of smooth muscle markers similar to previous data obtained for diverticulitis. Whereas moderate structural muscular alterations were detectable, both the thickness and CTI of circular and longitudinal muscle layers were unaltered in patients with diverticulosis. Conclusions: Diverticulosis is associated with a significant down-regulation of essential components of the contractile smooth muscle apparatus and moderate structural alterations. The data give evidence that muscular alterations are present at mRNA level during early stages of diverticula formation and thus support the hypothesis that an enteric myopathy may contribute to the pathogenesis of DD independent from inflammatory events.

Titel:Contracting enteric muscle cells in vitro – interplay between initiators and effectors of intestinal peristalsis

Autoren: Böttner M.(1),Oxmann D.(2),Frandsen O.(2),Harde J.(2),Barrenschee M.(2),Hellwig I.(2),Ziaeian S.(3),Schmidtmayer J.(3),Wedel T.(2),

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#### Abstract:

Background & amp; aims:

Regulation of intestinal motility depends on an undisturbed communication between the initiators of motility (enteric neurons) and the effectors (enteric smooth muscle cells). This interplay is mediated by neurotransmitters and requires both morphological and physiological coupling of these components. In vitro models to analyze the interaction between enteric nerve cells and smooth muscle cells, however, need to be established yet.

#### Material & amp; methods:

Co-cultures of rat postnatal enteric neurons and smooth muscle cells were established and characterized morphologically by immunocytochemistry for the pan-neuronal marker PGP 9.5 and the smooth muscle contractile filament smooth muscle alpha-actin. Neurons associated topographically with contracting smooth muscle aggregates underwent patch clamp analysis. The influence of enteric nerves on muscular contraction pattern was assessed by application of the sodium channel blocker lidocain.

#### **Results:**

Co-cultures of enteric nerve and smooth muscle cells led to the formation of cellular aggregates that displayed rhythmic contractions in vitro. The predominant cell type identified in these aggregates corresponded to smooth muscle cell clusters surrounded by neuronal networks. Patch clamp analysis of these aggregate-associated nerve cells revealed the presence of sodium currents and action potentials characterizing these neurons as functionally active. Application of lidocain to the co-cultures led to a decrease in the frequency of contractions.

#### Conclusions:

The established co-cultures could serve as an in vitro model to study functional enteric nerve-muscle interactions and to monitor the impact of pharmacological agents, neurotransmitter systems and neurotrophic factors on both the functional and morphological differentation of the enteric neuromuscular network.

Titel:Pelvic belt effects on sacroiliac joint motion - a numerical analysis

Autoren:Sichting F.(1),Rossol J.(2),Soisson O.(2),Milani T.(1),Bechmann I.(2),Hammer N.(2),

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## Abstract:

Introduction: The sacroiliac joint is a widely described source of low back pain. Therapeutic approaches to relieve pain encompass the application of pelvic belts. However, the effects of pelvic belts on the sacroiliac joint ligaments being potential pain generators are mostly unknown. The aim of our study was to analyze the influence of pelvic belts on sacroiliac joint ligament load by means of an experimental computer study. Material and Methods: A numerical model of the human pelvis was created, comprising bones, ligaments and cartilage. Detailed geometries, material properties of ligaments and in-vivo pressure distribution patterns of a pelvic belt were implemented. The effects of pelvic belts on ligament strain were computed in the double-leg stance. Results: Pelvic belts increase sacroiliac joint motion around the sagittal axis but decrease motion around the transverse axis. Due to pelvic belt application, most of the strained sacroiliac joint ligaments were relieved, especially the sacrospinous, sacrotuberous and the interosseous sacroiliac joint ligaments. Sacroiliac joint motion and ligament strains were minute. However, these results agree with validation data from other studies. Discussion: Pelvic belts alter sacroiliac joint motion and cause partial relief of ligament strain to a minimal absolute but marked relative extent. These findings confirm theories that besides being mechanical stabilizers, the sacroiliac joint ligaments are likely involved in neuromuscular feedback mechanisms. Conclusively, the numerical model helps unraveling the therapeutic effects of pelvic belts.

Titel:Thigh muscle anatomcial cross-sectional areas and strength in knees with early vs no radiographic osteoarthritis – a cross sectional and longitudinal between-knee within-person comparison in osteoarthritis initiative participants

Autoren: Ruhdorfer A.(1), Dannhauer T.(1), Wirth W.(1), Roemer F.(2), Nevitt M.(3), Eckstein F.(1),

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#### Abstract:

Background: Thigh muscle weakness is commonly observed in patients with knee osteoarthritis (KOA). However, this association may be indirect and confounded by factors that cause both KOA and muscle weakness. The current study therefore directly compares muscle status in limbs with early KOA vs. contra-lateral limbs without KOA (between-knee, within-person comparison). Methods: 55 of 4796 Osteoarthritis Initiative participants fulfilled the inclusion criteria of a) one limb with early radiographic KOA (i.e. definite osteophytes and no radiographic joint space narrowing [JSN]) at baseline; b) the other (contra-lateral) limb without radiographic KOA. Axial MRIs were used to compare anatomical cross-sectional areas (ACSAs) of the thigh muscles, and isometric testing to compare maximal extensor/flexor strength between both limbs, at baseline and 2-year follow-up Results: At baseline, no significant side differences were observed between limbs with early KOA vs. those without KOA: This applied to quadriceps ACSAs (-0.1±8%), hamstring and adductor ACSAs (?7.3%), and isometric strength and specific strength [strength/ACSA] (?7.8%; p?0.44; paired t-test). The longitudinal 2-year reduction in extensor specific strength was greater (p=0.03; paired t-test) in limbs with early KOA (-14±27%) than in the contra-lateral limbs without KOA (-9±22%). No significant differences in longitudinal change were observed in other measures. Conclusion: This study provides no evidence of baseline differences in muscle status between limbs with early KOA vs. contra-lateral ones without KOA. The longitudinal decline in specific strength may be greater in limbs with early than in those without KOA, potentially because of somewhat greater pain levels in limbs with early KOA.

Titel:Is the risk of incident radiographic knee OA related to severity of contra-lateral radiographic knee status? -Data from the osteoarthritis initiative

Autoren:Cotofana S.(1), Wirth W.(1), Kwoh K.(2), Hunter D.(3), Duryea J.(4), Eckstein F.(1),

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## Abstract:

Purpose: Radiographic knee osteoarthritis (ROA) is frequently a bilateral disease. The aim of this study was to investigate the incidence rate of ROA and changes in joint space width (JSW) in radiographically normal knees in relationship to the contralateral (CL) ROA knee status. Methods: Participants were selected from the Osteoarthritis Initiative (OAI) based on radiographic readings (according to Kellgren and Lawrence [KLG] classification) of both knees using following criteria: one knee with KLG0 and the CL knee KLG0+1 (no CL ROA), KLG2 (moderate ROA) or KLG3+4 (severe CL ROA). We determined the percentage of incident ROA and changes in JSW (in a subset of the sample) at 2 and 4-yrs follow-up. Results: 1618 participants were included (892 women, BMI: 27.2 +-4.3kgm<sup>2</sup>, age: 59.6+-9.1yrs, 757 right knees). Of these 1142 had CL no ROA, 253 moderate ROA and 146 had severe CL ROA. Incidence rates were 0.7%, 2.4% (RR 2.7 [0.93-7.8]) and 3.4% (RR 3.7 [1.3-10.8]) at 2-yrs and 1.6%, 4.4% (RR 2.4 [1.1-5.1]) and 10.35% (RR 5.7 [2.8-11.5]) at 4yrs. Changes in JSW (n=425) were -148µm, -84µm (p=0.22) and -286 (p=0.17) at 2-yrs and -365µm, -340µm (p=0.67) and -622µm (0.03) at 4-yrs. Conclusions: The findings support the concept that idiopathic OA is a bilateral disease, and that the risk of incident ROA and structural change in radiographically normal knees is strongly related to the severity of the CL ROA status.

Titel:Bacterial endotoxins as a causative agent in rheumatoid arthritis

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#### Abstract:

Objective: It has been previously reported from our group that bacterial endotoxins derived from damp wall extracts have sever implications for the development of Rheumatoid Arthritis (RA). The aim of the presented study was to investigate in more detail how bacterial endotoxins interact with the cartilage matrix and influence inflammatory processes related to RA. Methods: The effects of LPS-mediated inflammatory signaling on primary human chondorcytes were evaluated in vitro in monolayer and high density culture. Results: Physical interaction of LPS with collagen type II in the chondrogenic matrix and degradation of cartilage matrix was demonstrated with immuno-electron and electron microscopy. LPS induced NF-kappaB which correlated with activation of IkappaB? kinase, IkappaB? phosphorylation, IkappaB? degradation, p65 phosphorylation and p65 nuclear translocation. Inhibition of IKK (as upstream kinase of NF-kappaB pathway signalling) with BMS-345541, or inhibition of the PI-3K/Akt with wortmannin, or the combinational treatment significantly inhibited LPS-induced degradation of ECM and apoptosis in chondrocytes. Treatment with BMS-345541 or/and wortmannin markedly reduced LPS-induced up-regulation of catabolic enzymes that mediate ECM degradation (matrix metalloproteinases-9 and -13), cyclooxygenase-2 and apoptosis (activated caspase-3). Further, LPS induced the expression of TLR4 in chondrocytes and bound with TLR4, indicating that LPS acts through TLR4. Conclusion: Our results demonstrate for the first time a LPS/TLR4/collagen association in chondrocytes in an in vitro model of RA and LPS induced up-regulation of NF-kappaB/PI3K signalling pathways, indicating these as targets for novel therapeutic approaches.

Titel: Primordial Germ Cells (PGCs) of the rabbit embryo and the expression of the chemokine SDF1 and its receptor CXCR4

Autoren: Hassan W.(1), Viebahn C.(1), Pueschel B.(1),

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Abstract:

PGCs present a good model for directed cellular migration since they originate during gastrulation distant from the prospective gonad anlagen. PGC migration has been studied in different species including mammalian. However, despite the variability amongst mammals, research projects are mostly carried out in mouse. Recently, few studies analyzed the rabbit PGCs in early embryonic stages considering, also, the rabbit's increasing importance in medical research. Here, the PGC distribution was further analyzed in the rabbit embryo at later stages, i.e. until they are found in the genital ridge. In addition, the SDF1/CXCR4 is investigated as an important molecular pathway for directed cellular migration in several species.

As amnion and endoderm fold at day 8.5 post coitum (dpc), rabbit PGCs disperse along the crescent-shaped posterior border in mesoderm still in close contact to the open endoderm. At 9-9.5 dpc PGCs occupy the ventral mesoderm of the closing gut between the allantoic diverticulum posteriorly and yolk sac anteriorly. After 10 dpc the dorsal gut mesentery extends and PGCs scatter along it until the genital ridge. At this latter stage SDF1, the expected directing cue, is detected in the proximal mesentery and genital ridge suggesting that it acts as a PGC guide in the rabbit, too. CXCR4 shows spotted expression in the endothelium, gut and in different embryonic cells including some PGCs. Laser microscopy analysis could define the spot-like expression of CXCR4 in PGCs at this stage and other stages; while mRNA expression could exclude the possibility of short half-life of CXCR4 protein.

Titel:Sulfated steroids and steroid sulfatase in the human testis - is there a sulfatase pathway?

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## Abstract:

Sulfated steroid hormones were thought to be inactive metabolites. Detection of specific membrane transporters such as sodium dependent organic anion transporter (SOAT) and others in the testis raised the question of a functional sulfatase pathway and opened a new field of reproduction research. Expression and localization of membrane transporters and steroid sulfatase (StS) were analysed on mRNA level performing quantitative (gPCR, testis homogenate), gualitative RT-PCR (single cell populations following laser assisted cellpicking) and in situ hybridization as well as by immunohistochemistry and Western blotting on protein level. For functional analyses, stably transfected HEK293 cells were generated. SOAT mRNA is expressed in pachytene spermatocytes and quantitatively reduced in hypospermatogenesis and maturation arrest. SOAT protein was detected in spermatocytes and spermatids. SOAT shows transport ability for various sulfated steroids such as estrone-3-sulfate and estradiol-3-sulfate in stably transfected HEK293 cells. StS mRNA and protein are localized in spermatocytes, but can also be found in Sertoli and Leydig cells. We conclude, that sulfated steroids are not inactive and may play a role in local supply of steroid hormones and the regulation of spermatogenesis. The generation of a Soat-knockout mouse will explore the consequence of a complete loss of Soat for reproduction. Further studies have to reveal the connection between the sulfatase pathway and other receptor and transporter systems as estrogen receptors (expressed in spermatocytes) and ABC transporters (expressed in Sertoli cells). The latter may play an important role in guiding sulfated steroids through the the blood-testis barrier. (DFG FOR 1369, "Sulfated steroids in Reproduction")

Titel:Slo1 is the principal potassium channel of human sperm

Autoren: Mannowetz N.(1), Naidoo N.(1), Choo S.(1), Smith J.(2), Wennemuth G.(3), Lishko P.(1),

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#### Abstract:

To become competent for fertilization, sperm cells have to undergo a maturating process in the female genital tract, termed capacitation. This multistep process includes – amongst others - the induction of intracellular alkalinization and membrane potential hyperpolarization of which the latter is evoked by the efflux of potassium ions. In murine sperm, potassium currents mainly arise from KSper/mSlo3. This channel is activated upon intracellular alkalinization. However, in human spermatozoa, the identity of the principal potassium channel remains elusive. In order to characterize human the KSper (hKSper) channel, we used the patch-clamp technique to record potassium currents from human ejaculated spermatozoa. Our data shows that (i) the hKSper current derives from the flagellum and is outward rectifying. (ii) hKSper is not activated by intracellular alkalinization. (iii) hKSper is sensitive to intracellular calcium, (iv) hKSper currents are blocked by the Slo1 inhibitor charybdotoxin (ChTX) and (v) hKSper is blocked dose-dependently by progesterone, a known activator of the sperm-specific calcium channel CatSper. In contrast, potassium currents from mouse epididymal sperm are insensitive to ChTX and progesterone. The unique biophysical and pharmacological properties of hKSper suggest that the Slo1 channel constitutes the main potassium channel in human spermatozoa. The Slo1 protein is localized to the principle piece of the sperm tail where other ion channels (Hv1 and CatSper) reside. Its inhibition by progesterone, a physiological activator of CatSper, favors the idea that Slo1 depolarizes the sperm membrane to fully open CatSper thus initiating calcium-dependent processes and contributing to a successful fertilization.

Titel:3d culture system for trophoblast-endometrial interaction

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Abstract:

Endometrial epithelial cells (EECs) form the first barrier for the invading trophoblast in early human implantation. A redistribution of adhering junctions - recently observed on glandular EECs during the menstrual cycle - points to a change in EEC polarity that may facilitate trophoblast invasion.

In this study a new 3D in vitro system was established to investigate whether and how trophoblast-EEC interactions depend on the degree of glandular EECs' polarity. Three differently polarized endometrial adenocarcinoma cell lines were used to mimic different degrees of EEC polarity in vitro: RL95-2 (poorly polarized), Ishikawa (moderately polarized) and HEC-1-A (highly polarized). Gland-like morphology was achieved by culturing EECs in Matrigel. To induce trophoblast-EEC interaction in vitro the trophoblast cell line AC-1M88 - a fusion cell line of primary extravillous trophoblast cells and JEG-3 cells - was added to the multicellular EEC spheroids.

Confocal microscopy revealed a basal localization of alpha-6-integrin for all EEC lines. Luminal ZO-1 staining was only found in Ishikawa and HEC-1-A spheroids. The desmosomal plaque protein desmoplakin was differently distributed: all over the lateral membranes in RL95-2 spheroids, but concentrated towards the inner lumen of HEC-1-A spheroids. Ishikawa spheroids showed an intermediate state of desmoplakin distribution. In confrontation experiments desmoplakin-positive adhesion sites were detected between AC-1M88 trophoblast cells and EEC spheroids. The invasiveness of AC-1M88 cells was inversely correlated to the degree of EEC polarity.

In conclusion, the established 3D culture system provides a suitable tool for studies on trophoblast-endometrial interaction depending on the grade of epithelial differentiation.

Titel:Decidualization in human ectopic endometrial lesions in vivo is induced by HCG

Autoren: Grümmer R.(1), Kimmig R.(2), Koch Y.(1),

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## Abstract:

Endometriosis is a common gynaecological disease associated with pelvic pain and infertility. Current medical treatment inducing a hypoestrogenic state still is associated with undesirable side effects and high recurrence rates. Terminal differentiation of the endometrial stromal cells by inducing decidualization may be a promising target for novel therapeutical approaches. Previous in vitro studies showed that the combination of progestins with cAMP acts synergistically in enhancing decidualization of human endometrial stromal cells. In the present study, decidualization of ectopic endometrial tissue

is induced in a humanized endometriosis mouse model in vivo by activating cAMP signaling. Human endometrium of the proliferative phase of the menstrual cycle was transmplanted into the peritoneal cavity of NOD-SCID mice and mice were treated for seven days with progesterone alone or with progesterone in combination with the intracellular cAMPenhancing compounds forskolin or hCG.

Compared to the treatment with progesterone alone, combined treatment with forskolin or hCG led to an increase in decidualization of the ectopic human endometrial tissue as shown by morphology as well as by expression of marker genes of decidualization. HCG revealed a stronger effect than forskolin. Only after treatment with progesterone and hCG transcription of the decidualization markers prolactin (PRL) and insulin-like growth factor-binding protein 1 (IGFBP-1) was significantly increased, whereas transcription of forkhead box protein O1 (FOXO-1) was upregulated by both compounds, forskolin as well as hCG.

Taken together, a combined treatment with progesterone and hCG induces decidualization and thus terminal differentiation of stromal cells of ectopic endometrial lesions in vivo.

Titel:Exploring novel pathways of neural reprogramming by instructive factors and pharmacological intervention

Autoren: Edenhofer F.(1), Bung R.(2), Lakes YB.(2), Seiferling D.(2), Wörsdörfer P.(2)

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## Abstract:

Transcription factor-driven reprogramming of fibroblast cells has been shown to yield neurons, cardiomyocytes, neural as well as hepatocyte progenitors, demonstrating that lineage reprogramming of somatic cells developed into a new paradigm for both, regenerative medicine and disease modeling. Recently, we demonstrated the direct derivation of neural stem (NS) cells from mouse fibroblasts employing a modified Yamanakatype reprogramming paradigm. Retroviral transduction of Sox2, Klf4, c-Myc and timely restricted activation of Oct4 was used to initiate dedifferentiation of fibroblast cells and 19 days post infection we observed neurosphere-like colonies that could be readily isolated and clonally expanded both as sphere and adherent cultures. Such induced NS (iNS) cells are able to differentiate into all three neural lineages, neurons, astrocytes as well as oligodendrocytes. Fibroblast-derived iNS cells exhibit clonal growth and maintain their marker expression profile and differentiation capability over prolonged expansion (>50 passages). Here we show that transduction of neurogenic transcription factors as well as the application of small molecule inhibitors can be used to induce alternative pathways of targeted lineage reprogramming. We generated alternative neural progenitor populations with modified developmental plasticity. Putative mechanisms and therapeutic value of reprogrammed cells will be discussed. We expect directly converted somatic stem cells such as iNS cells to provide a safe and robust, virtually unlimited source of patient-specific cells for future applications in regenerative medicine and disease modeling.

Titel:The chondroitin sulfate code hypothesis: does the sulfation pattern in the neural stem cell niche direct proliferation and neurogenesis of cortical neural stem cells?

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Abstract:

Chondroitin sulfate proteoglycans (CSPGs) and their sulfation by chondroitinsulfotransferases (Chsts) appear to play a crucial role for the behaviour of neural stem cells (NSCs) in the embryonic neural stem cell niche during mouse forebrain development. It has been shown that the inhibition of the sulfation by sodium chlorate or the degradation of the CSPG glycosaminoglycans by chondroitinase ABC leads to less proliferation and altered cell fate decisions of the NSCs (Sirko, von Holst et al. 2007; Akita, von Holst et al. 2008; Sirko, von Holst et al. 2010). The proliferation and differentiation of cortical neural stem cells from E13.5 mouse embryos upon forced expression of distinct Chst-EGFP constructs as well as the knockdown of one specific Chst was examined by neurosphere forming/proliferation assay and differentiation assay in vitro. Furthermore, the overexpression and knockdown experiments will be performed by in utero electroporation. The overexpression of distinct Chsts in the NSCs was functional as revealed by an increased signal for the complex sulfated CS-epitope detected by the monoclonal antibody 473HD. In the differentiation assay a significant increase in neurogenesis at the expense of gliogenesis was observed. In consistence with previous observations, the sulfation of the CSPGs plays a role in the commitment of the NSCs within the neural stem cell niche and could function as a possible communication platform between the NSCs and their extracellular surrounding in the neural stem cell niche.

Titel:Rhythmicity in cell proliferation in the median eminence and pituitary of adult mice

Autoren:Fredrich M.(1), Christ E.(1), Derouiche A.(1), Korf H.-W.(1),

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### Abstract:

Cell proliferation in the adult hypothalamic-pituitary system is required to adjust neuroendocrine circuits to environmental stimuli such as the photoperiod. We investigated whether cell proliferation in the median eminence (ME), the hypophysial pars tuberalis (PT) and pars distalis (PD) of adult mice follows a diurnal rhythm. Melatonin-proficient C3H mice were adapted to a 12h-light-12h-dark cycle and sacrificed at ZT00, 06, 12 and 18. Immunohistochemistry for Ki67 served to measure cell proliferation. In all regions analyzed, we found ongoing cell proliferation, but the PT showed no rhythm. On average, 1% of the cell nuclei in the PT, labeled by Hoechst were Ki67 immunoreactive. In the ME, Ki67 immunoreactive cell nuclei accounted for about 2.2% of the total number of cell nuclei, except at ZT12, when the number of proliferating cells increased significantly. In the PD, the percentage of Ki67 immunoreactive cell nuclei decreased by about 40% at ZT6 as compared to ZT00, 12 and 18. The total number of cell nuclei did not change in the PT, ME or PD. In summary, our results show that cell proliferation in the ME and PD underlies a distinct day/night rhythm while the total number of all cell nuclei does not change significantly. We suggest that proliferation is accompanied by apoptosis to ensure maintenance and function of the PT, ME and PD. As a next step we shall identify the type(s) of proliferating cells. According to the size and shape of their cell nuclei numerous proliferating cells may belong to folliculo-stellate or endocrine cells.

Titel:Phosphorylation of cyclic AMP-response element-binding protein (CREB) and calcium signaling components are influenced by melatonin in rat pancreatic beta-cells

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## Abstract:

The pineal hormone melatonin inhibits insulin secretion through activation of specific membrane receptor isoforms (MT1 and MT2), which are expressed in rat pancreatic islets as well as in the rat insulinoma beta-cell line INS-1. Melatonin's influence on insulin release is receptor-mediated and coupled to the cAMP cascade via inhibitory G-proteins (Gi). Changes in the cAMP level can be translated into changes in insulin secretion and in cAMP-response element-binding protein (CREB) phosphorylation. Pharmaceuticals were used to increase intracellular cAMP levels and to raise insulin release in INS-1 cells after different incubation times. However, concomitant incubation with melatonin significantly decreased insulin levels. In the same cell batches, phosphorylation of CREB (pCREB) in INS-1 cell nuclei, which was strongly increased through pharmacological treatments, was significantly reduced through concomitant application of melatonin. Melatonin receptor antagonists abolished the melatonin-mediated, reductive effect on pCREB. The use of transfected INS-1 cells, overexpressing the human MT2 receptor, showed a crucial influence of melatonin receptor density on pCREB formation. Transcript analysis of INS-1 cells revealed increased mRNA levels of calcium/calmodulin-dependent kinase (Camk) isoforms (Camk2d and CamkIV) after forskolin or IBMX incubation. However, co-incubation with melatonin significantly reduced these levels. Thus, the results provide evidence that the phosphorylation level of CREB is modulated through melatonin in pancreatic beta-cells. In summary, melatonin, possibly via CREB, regulates the expression of genes containing a functional CRE sequence (e.g. Camks); these play an important functional role in the regulation of beta-cell signaling pathways and, subsequently, insulin secretion.

Titel:A critical period of structural plasticity for axon initial segment maturation during visual cortex development

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## Abstract:

Throughout cortical development, neuronal networks are shaped by sensory experience during critical periods, for which significant cellular plasticity is a hallmark. On a cellular level, plasticity has been documented for dendritic spines, neurochemical phenotypes, axonal boutons, soma size and others. A system frequently studied in this context is the visual system. A neuronal microdomain that has not yet been studied in visual system plasticity is the axon initial segment (AIS), which is the site of action potential generation. Recent studies showed that it can be dynamically regulated in vitro and in vivo. We therefore hypothesized that the AIS shows equally dynamic regulation during development and maturation of the visual system. A morphological analysis of AIS development in mouse visual cortex from E12.5 to adulthood showed that AIS length increases over the postnatal period with a peak between P10-P15. With eye-opening and the onset of sensory-driven activity, a significant shortening of the AIS was observed. These findings suggest a dynamic maturation period of this axonal microdomain which coincides with eye-opening and thus the onset of sensorydriven activity. To determine whether sensory activity is required for normal AIS maturation, external visual input was diminished for various time points crucial for visual cortex development. Strikingly, sensory deprivation from the beginning through the peak of the critical period (P0-P28) resulted in a significant AIS length increase, mimicking the developmental peak of juvenile AIS. Our study demonstrates that visual input influences structural characteristics of cortical AIS and hence possibly alters neuronal excitability in vivo.

Titel:Reelin promotes microtubule dynamics in processes of developing neurons

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Abstract:

The extracellular matrix protein reelin controls radial migration and layer formation of cortical neurons by modulating cytoskeletal dynamics. A stabilizing effect of reelin on the actin cytoskeleton has been shown recently. However, it is poorly understood how reelin modulates microtubule dynamics. Here, we provide evidence that reelin increases microtubule assembly. This effect is mediated, at least in part, by promoting microtubule plus end dynamics in processes of developing neurons. Thus, we treated primary neuronal cultures with nocodazole to disrupt microtubules. After nocodazole washout, we found microtubule reassembly to be accelerated in the presence of reelin. Moreover, reelin treatment promoted the formation of microtubule plus end binding protein 3 (EB3) comets in developing dendrites, and EB3 immunostaining in the developing wild-type neocortex was most intense in the reelin-rich marginal zone where leading processes of radially migrating neurons project to. This characteristic EB3 staining pattern was absent in reeler. Also reassembly of nocodazole-dispersed dendritic Golgi vesicles, which are closely associated to microtubules, was accelerated by reelin treatment, though with a substantially slower time course when compared to microtubule reassembly. Taken together, our results suggest that reelin promotes microtubule assembly, at least in part, by increasing microtubule plus end dynamics. Supported by DFG (FO 223/6-1 to EF).

Reference: Meseke M, Cavus E., Förster E (2013). Reelin promotes microtubule dynamics in processes of developing neurons. Histochem Cell Biol;139(2):283-97.

Titel:Molecular mechanisms of active zone assembly

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Abstract:

Active Zones (AZs) are regions at the presynaptic nerve terminal that mediate neurotransmitter release. They are located exactly opposite postsynaptic specializations and composed of a dense scaffold of proteins called the cytomatrix at the active zone (CAZ). In order to understand how the CAZ is assembled, we aim to characterize the localization of AZ proteins and their transport vesicles in developing neuronal cultures. To test this we use high-resolution microscopy techniques e.g. STED. Here, we show that two-color STED microscopy reveals Bassoon, Piccolo and Munc13-1 precursor vesicles at Golgi subcompartments with different vesicle or vesicle cluster sizes. Blocking vesicle exit from the Golgi apparatus leads to a trans-Golgi network specific localization of Bassoon and Piccolo and an exclusive cis-Golgi localization of Munc13-1. Electron microscopy and DABphotoconversion show that AZ proteins are loaded on clear core vesicles with a diameter of 50-60 nm which migrate in clusters from the soma into the axon initial segment. In proximal and distal axonal segments of immature neurons AZ proteins display high colocalization frequencies. These data currently suggest that AZ proteins are transported to a high degree together on transport carriers within axons but loading and sorting of these proteins at the Golgi apparatus on precursor transport vesicles is more complex.
Titel:The role of synaptopodin and the spine apparatus in the regulation of denervationinduced homeostatic synaptic plasticity

Autoren: Vlachos A.(1), Ikenberg B.(1), Lenz M.(1), Becker D.(1), Reifenberg K.(2), Deller T.(1),

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### Abstract:

Synaptopodin (SP) is an essential component of the spine apparatus (SA), an enigmatic cellular organelle composed of stacked endoplasmic reticulum which has been linked to synaptic plasticity. However, SP/SA-mediated synaptic plasticity remains incompletely understood. To study the role of SP/SA in homeostatic synaptic plasticity we used denervation-induced synaptic scaling of mouse dentate granule cells as a model system. In entorhino-hippocampal slice cultures prepared from SP-deficient mice, which lack the SA, a compensatory increase in excitatory synaptic strength was not observed after partial deafferentation. By crossing SP-deficient mice with a transgenic mouse strain that expresses GFP-tagged SP under the control of the Thy1.2 promoter, the ability of dentate granule cells to form the SA and to homeostatically strengthen excitatory synapses was rescued. Interestingly, homeostatic synaptic strengthening was accompanied by a compensatory increase in SP-cluster sizes/stability and SA-stack number, suggesting that activitydependent SP/SA remodeling could be part of the homeostatic mechanism which adjusts the strength of excitatory synapses to persisting changes in network activity. Thus, our results disclose a novel role for SP/SA in homeostatic synaptic plasticity. (Supported by DFG; CRC1080).

Titel:Reelin promotes dendritic golgi translocation via the Cdc42/Rac1-specific guanine nucleotide exchange factor alphaPIX/Arhgef6

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#### Abstract:

Neuronal migration defects in reeler have been studied intensively, but it is poorly understood how misoriented neuronal apical dendrites are related to reelin deficiency. In wildtype, the Golgi apparatus transiently translocates into the leading process of radially migrating neurons. Translocation of the Golgi apparatus into one process defines this process to become the largest dendrite. In the reeler mutant, lacking reelin expression, the Golgi apparatus translocates into dendrites that are randomly orientated. We and others recently found that recombinant reelin promotes dendritic Golgi translocation in vitro. Next, to address the mechanisms that underlie dendritic Golgi translocation, we transfected developing neurons in vitro with constructs expressing the Cdc42/Rac1 specific guanine nucleotide exchange factor (GEF) alphaPIX/Arhgef6 or GEF-deficient alphaPIX. We found that alphaPIX, but not the closely related GEF-betaPIX, promoted dendritic Golgi translocation. This effect of alphaPIX was further enhanced in the presence of recombinant reelin. GEF-deficient alphaPIX reduced dendritic Golgi translocation. Similarly, inhibition of Cdc42/Rac1-activity in neurons transfected with dominant-negative Cdc42- or Rac1 constructs impaired dendritic Golgi positioning. Our findings suggest that the Cdc42/Rac1 specific GEF-alphaPIX is part of a reelin triggered signalling pathway that is involved in dendritic Golgi translocation in neurons (Meseke et al., 2013). Reference: Meseke et al., 2013. Eur J Neurosci. 37(9):1404-12. Supported by DFG (FO 223/6-1 to EF).

Titel:The cell adhesion molecule neuroligin-1 is essential for intact excitatory synaptic transmission at glutamatergic perforant path synapses

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#### Abstract:

Neuroligins are transmembrane cell adhesion proteins with a key role in the regulation of excitatory and inhibitory synapses. Based on previous in vitro and ex vivo studies, neuroligin-1 (NL1) has been suggested to play a selective role in the function of glutamatergic synapses. However, the role of NL1 has not vet been investigated in the brain of live animals. We studied the effects of NL1-deficiency on synaptic transmission in the hippocampal dentate gyrus using field potential recordings evoked by perforant path stimulation in urethane-anesthetized NL1 knockout (KO) mice. We report that in NL1 KOs the activation of glutamatergic perforant path - granule cell inputs resulted in reduced synaptic responses. In addition, NL1 KOs displayed impairment in long-term potentiation (LTP). Furthermore, field EPSP-population spike (E-S) coupling was greater in NL1 KO than WT mice and paired pulse inhibition was reduced, indicating a compensatory rise of excitability in NL1 KO granule cells. Consistent with changes in excitatory transmission, NL1 KOs showed a significant reduction in hippocampal synaptosomal expression levels of the AMPA receptor subunit GluA2 and NMDA receptor subunit GluN1. Taken together, we provide first evidence that NL1 is essential for normal excitatory transmission and long-term synaptic plasticity in the hippocampus of intact animals. Our data provide insights into synaptic and circuit mechanisms of neuropsychiatric abnormalities such

as learning deficits and autism. (supported by NeFF, LOEWE-Schwerpunktprogramm)

Titel:Local estradiol synthesis controls spine synapse density in the hippocampus of female but not of male animals

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Abstract:

Hippocampal neurons express aromatase, the final enzyme of estradiol synthesis, and actually synthesize and secrete estradiol. Our recent data (Vierk et al., 2012) showed that inhibition of local estradiol synthesis by phosphorylation of aromatase in these neurons resulted in loss of spine synapses in female but not in male mice. Since inhibition of aromatase results in elevated levels of testosterone the question arised whether testosterone may be necessary for the maintenance of spine synapses in the male hippocampus. Stereological counts of spine synapses revealed a significant increase in spine synapse number in male hippocampal slice cultures in response to treatment with 5 alphadihydrotestosterone (DHT), a non-aromatizable androgen. Consistently, treatment with DHT together with letrozole had an additive effect regarding the increase in synapse density. In contrast, in female hippocampal slice cultures treatment with DHT had no effect on the density of spine synapses. These in vitro results point to a role of testosterone in males and to a role of estradiol in females in the maintenance of hippocampal synapses. In animals, this sexual dimorphism is maintained by GnRH, enhancing estradiol synthesis in the female hippocampus while blocking estradiol synthesis in the male hippocampus. As a consequence, GnRH increases synapse density in females by stimulating estradiol synthesis in hippocampal neurons and in males by testosterone, since blockade of estradiol synthesis results in elevated testosterone levels. Taken together, the maintenance of hippocampal synapses depend on estradiol in females and on testosterone in males.

Titel:Translational aspects in ProSAP/Shank mutant mice

Autoren: Schmeisser M.(1),Grabrucker S.(2),Halbedl S.(1),Reim D.(1),Mett C.(1),Beth N.(1),Bockmann J.(1),Ey E.(3),Bourgeron T.(3),Grabrucker A.(2),Boeckers T.(1),

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### Abstract:

The ProSAP/Shank family comprises three essential postsynaptic scaffold proteins of excitatory synapses in the mammalian brain: Shank1, ProSAP1/Shank2 and ProSAP2/Shank3. Importantly, over recent years, ProSAP/Shank malfunction has been associated with various neuropsychiatric disorders including autism spectrum disorders (ASD). In this context, we generated ProSAP/Shank mutant mice and have started to analyze neurobiological and neurobehavioral phenotypes. One of the hallmarks in ProSAP/Shank mutants is dysfunction of the glutamatergic system, most probably varying throughout brain regions. To uncover the molecular mechanisms and origins of the abnormal behaviors seen in our mutants, we are now using different kinds of methodological approaches including the Cre-lox system, cross-breeding of mutants and detailed neuroanatomical investigation of distinct brain regions. We are further focussing on the characterization of group I metabotropic glutamate receptor (mGluR) signaling in our mutants, a key molecular pathway in the pathogenesis of ASD. Taken together, the results from our ongoing studies should further develop the framework for translational medicine approaches in ProSAP/Shank mutant mice.

Titel:Control of early neuronal activity by synaptic phospholipids governs connectivity and memory

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Abstract:

Early neuronal activity prior to experience-dependent input is essential for proper brain connectivity. Synchronized neuronal activity occurring over large groups of neurons influences developmental processes from correct formation of neuronal circuits up to transmitter specification. However, the molecular mechanisms synchronizing neuronal activity are not known. Bioactive phospholipids induce Ca2+-transients in neuronal stem cells, and synaptic phospholipids modulate neuronal excitability in the juvenile brain suggesting an important role for bioactive lipids in the regulation of neuronal activity. Here we show that proper onset of early synchronous neuronal activity depends on intact synaptic bioactive lipid signaling. Development of entorhinal neuronal networks which are essential for memory capabilities proceeds explorative behavior for several days prior to the input derived from external stimuli. During this period, typical synchronous activity occurs in a time-locked developmental fashion. Alteration of synaptic bioactive lipid signaling induced a premature onset of synchronous activity during a critical postnatal phase. Premature onset of synchronized neuronal activity by only 2 days resulted in structural reduction of the entorhinal-hippocampal perforant path leading to memory deficits later in life. While it is widely accepted that delayed onset of neuronal activity can result in the disturbance of proper brain development, our data show that increased and premature synchronization of neuronal networks may be equal detrimental. Our findings indicate that homeostatic regulation of neuronal activity

Titel:Tumor necrosis factor-alpha maintains denervation-induced homeostatic synaptic plasticity of dentate granule cells in mouse entorhino-hippocampal slice cultures

Autoren: Becker D.(1), Lenz M.(1), Zahn N.(1), Deller T.(1), Vlachos A.(1),

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### Abstract:

Homeostatic synaptic plasticity is a cellular mechanism which allows neurons to adjust their synaptic strength to perturbations in network activity. It aims at keeping the firing of neurons in a physiological range. Hence, in response to a prolonged reduction in afferent activity neurons strengthen ("scale-up") their excitatory synapses in a compensatory manner. Using the entorhinal cortex in vitro model we have recently shown that homeostatic synaptic plasticity is induced in neurons a consequence of partial denervation. This observation is of considerable interest in the context of neurological diseases, which are accompanied by the loss of neurons and a subsequent deafferentation of connected brain regions. However, the molecular mechanisms of denervation-induced homeostatic synaptic plasticity remain not well understood. Here, we studied the role of tumor necrosis factor alpha (TNFalpha) in denervation-induced synaptic strengthening of dentate granule cells in mouse slice cultures containing the entorhinal cortex and the hippocampus. By employing pharmacological and genetic approaches we demonstrate that TNFalpha is required for the maintenance (at 3 - 4 days post lesion) but not the induction (1 - 2 days post lesion) of a compensatory increase in excitatory synaptic strength following denervation. These results disclose an important role for TNFalpha-signaling in stabilizing the activity of denervated neuronal networks by maintaining the homeostatic increase in excitatory synaptic strength of neurons. (Supported by DFG; CRC1080).

Titel:Development of an in vivo photoactivated gene expression method

Autoren: Dr. Cambridge S.(1), Dr. Dubos A.(2),

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#### Abstract:

Current transgene technologies do not provide the desired temporal and spatial resolution necessary for many research applications. We therefore developed a method that allows long-term genetic manipulation of cells via precise irradiation with light, called photoactivated transgene expression. This method is based on the inducible tetracycline (Tet) system and the reversible inhibition of the tetracycline analogue doxycycline by conjugation with a photolabile protection compound ("caging"). Inactive, caged doxycycline is membranepermeant and upon irradiation, the release of active doxycycline induces transcription in targeted cells by binding to a tetracycline-dependent transcription factor. Using UV or 2photon light, the method provides flexible, single cell resolution of transgene expression and was successfully demonstrated in various eukaryotic systems including plant leafs, brain slices, Xenopus tadpoles, and mouse embryos. We are now in the process of establishing the photoactivated transgene expression method in nervous tissue of living animals. The goal is to use this method for two different projects: first, for genetic silencing of individual neurons in the cortex to study the network effects on synaptic morphology and transmission. Second, for precise local blockade of angiogenesis in a mouse model of pathologic neovascularisation to develop improved, personalized therapies for retinopathy diseases. Our method will allow excellent correlation of transgene expression to changes in cells and tissues because the targeted area can be extensively analyzed before and after transgene expression. We believe that photoactivated gene expression will be a very powerful and versatile method that can nicely complement the currently available optogenetic tools.

Titel:Stromal cell-derived factor-1 alpha (SDF-1alpha) improves neural recovery after spinal cord contusion in rats

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Abstract:

Stromal cell-derived factor-1 alpha (SDF-1alpha) is an important cytokine, implicated in the control of stem cell trafficking and bone marrow-derived stem cell mobilization. Generally, SDF-1alpha regulates multiple physiological processes such as embryonic development and organ homeostasis. There is additional evidence that SDF-1alpha and its receptor CXCR4 are key regulators of neurorepair processes and network restoration after brain ischemia and spinal cord injury. In the present study, we investigated the influence of chronic intra-thecal delivery of SDF-1alpha after spinal cord contusion on neuronal damage and behavioral performance. After spinal cord injury, male Wistar rats at the age of 12 weeks were exposed to SDF-1alpha at different dosages (100, 500 and 1000 ng/ml) through an intra-thecal catheter using an osmotic pump for 28 days. Thereafter, animals were subjected to an open field locomotor test. Behavioral scores were significantly higher in SDF-1alpha treated animals compared to placebo-treated groups. In addition, we evaluated histopathological changes in the spinal cord in the presence or absence of SDF-1alpha. Chronic delivery of SDF-1alpha decreased the numbers of apoptotic cells, boosted astroglia and microglia responses, induced angiogenesis, and potentiated the number of proliferating cells in a dosedependent manner. These results clearly indicate an improved functional neural long-term recovery after focal spinal cord injury. The behavioral restoration was paralleled by a reduction of apoptosis and changes in neuroinflammatory cells.

Titel:An in vitro model for scar formation to study the mechanisms of scar-reducing treatments used in spinal cord injury

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### Abstract:

Spinal cord injury leads to permanent damage of axon tracts and impairment of sensory and motor functions. Lesion-induced fibrous scarring is a major impediment for regeneration of injured axons in the CNS. The collagen-rich scar contains axon growth inhibitory factors. In the molecular neurobiology laboratory a pharmacological treatment was

developed, transiently suppressing fibrous scarring (Klapka et al, 2005). This "anti-scarring treatment" (AST) consists of local application of an iron chelator and cyclic AMP, inhibiting collagen synthesis by invading fibroblasts. AST treatment stimulated regeneration of various axon tracts, leading to improvements in locomotor recovery.

In order to study the molecular mechanisms of AST we used an in vitro model for scar formation. In this model, fibroblasts and astrocytes in co-culture form scar-like clusters after addition of TGF-beta1. After characterization of scarring mechanisms and axon growth-inhibitory properties, we used the model to study various putative scar-reducing treatments. We found significant differences in the scar-reducing and growth-promoting properties of the treatments and were able to select a more suitable treatment strategy for scar-reduction after spinal cord injury.

Titel:Amyotrophic lateral sclerosis: a matter of gender and hormones?

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### Abstract:

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease leading to the degeneration of upper and lower motoneurons in the brain and spinal cord. A plethora of possible genetic and non-genetic risk factors are associated with ALS including cellular stress parameters such as exitotoxecity, oxidative stress, and mitochondrial dysfunction. Age and gender are equally established risk factors. Epidemiological studies show gender differences in ALS with a male predominance and a gender ratio of approx. 1:3 (male:female). Furthermore, gender influences the age of onset, site of onset, and clinical characteristics of ALS. Higher disease frequency in male indicates the involvement of hormones, such as estrogens and androgens, for the development and progression of ALS. Pre-clinical studies propose either adverse effects of androgens or protective effects of estrogens. In the present study, we have analyzed gender-specific differences in mitochondrial dynamics and function, growth factor expression and neurotransmitter metabolism in female and male Wild-Type and ALS-Mice. Furthermore, we have investigated the impact of 17beta-estradiol and testosterone treatment on neuronal and astroglial spinal cord cell cultures to unravel cell type specific effects of the hormones. Our study revealed differences referring to gender and hormonal treatment in the expression of growth factors, cholinergic marker genes and mitochondrial biogenesis. In conclusion, our data contribute to a better understanding of gender differences and hormone action in the spinal cord under physiological and pathophysiological circumstances.

Titel:Calretinin as a marker for upgaze pathways in the oculomotor system

Autoren: Zeeh C.(1), Ahlfeld J.(1), Mustari M.(2), Horn-Bochtler A.(1),

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### Abstract:

Different eye movement types are controlled by anatomically separated premotor pathways, which may be affected selectively in certain eye movement disorders. The occurrence of isolated upgaze or downgaze palsy or selective upbeat or downbeat nystagmus in patients indicates that up- and downgaze pathways differ in their organization or histochemical properties.

With combined tract-tracing and immunofluorescence the afferent terminals to motoneurons of vertically pulling eye muscles in monkey were investigated for the presence of the GABAsynthetizing enzyme Glutamate decarboxylase (GAD), Glycine Transporter 2 (GlyT2) and the calcium-binding protein calretinin (CR). Whereas GAD was equally found around motoneurons of all vertically pulling eye muscles, GlyT2 is absent from these neurons. CR was specifically found in terminals contacting superior rectus, inferior obligue and levator palpebrae motoneurons in the oculomotor nucleus complex. All these muscles participate in upward eye movements. The sources of CR inputs to upgaze motoneurons were studied by tracer-injections into the monkey nIII and combined CR-immunostaining. Tracer-labeled CRimmunoreactive neurons were found in the y-group, in the rostral interstitial nucleus of the medial longitudinal fasciculus and the interstitial nucleus of Cajal - the latter representing saccadic burst and burst-tonic neurons, respectively. The lack of GAD-immunoreactivity in CR-positive premotor terminals indicates an excitatory action on upgaze motoneurons. In conclusion the present results indicate histochemical differences for premotor pathways subserving up-versus downgaze and may be the cause for a selective vulnerability for both systems and may explain the isolated vertical gaze deficits observed in several diseases.

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Titel:Using the premotor network for eye movements as tool for the investigation of the progression line in progressive supranuclear palsy (PSP)

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### Abstract:

Progressive supranuclear palsy (PSP) is a tauopathy characterized by accumulations of hyperphospharylated tau-immunoreactive inclusions in different brain regions, including pons, pallidum, subthalamus and substantia nigra. A hallmark of clinical symptoms is the disturbance of vertical eye movements, initially present as slowing of vertical saccades, later as vertical gaze palsy accompanied by horizontal eye movement deficits, which may end up in a complete gaze palsy. Based on monkey tract-tracing studies with subsequent immunostaining for histochemical markers, considerable progress has been made in the identification of functional cell groups and their transmitters in the oculomotor system in human. In eight post-mortem cases with PSP we studied, whether observed eye movement deficits can be correlated with tau-pathology in the following eye-movement-related neuron groups: the vertical and horizontal premotor gaze centers, the glutaminergic rostral interstitial nucleus of the medial longitudinal fascicle (RIMLF) and paramedian pontine reticular formation (PPRF), respectively, the glycinergic saccadic omnipause neurons (OPN) and the cholinergic motonuclei of extraocular muscles. Since the vestibulo-ocular reflex is rather preserved until late in the disease, an analysis of the vestibular nuclei was included in the study. In all PSP-cases taupathology is present in RIMLF, PPRF and OPNs, later in motonuclei, affecting neuronal and glial cells. The taupathology in premotor areas preceds the involvement of motor nuclei, and the vestibular nuclei are relatively spared. In conclusion, the findings support a hypothesis that the pathology in PSP progresses along neuronal pathways in an anterograde fashion and is not confined to specific transmitters. BMBF (IFB-01EO0901, Brain-Net-01GI0505)

Titel:Myocilin-deficient mice are protected from neuronal damage in the retina

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### Abstract:

Purpose: To investigate the role of myocilin in the mouse retina. Myocilin is a secreted glycoprotein of the olfactomedin family whose biological function(s) are still largely unclear.

Methods: Myocilin-deficient mice (Myoc-/-) and Myoc-/-; ?B1-Crystallin-Myocilin mice with ocular overexpression of myocilin were characterized and analyzed by real-time RT-PCR, semithin sectioning and electroretinography (ERG). Apoptosis of retinal neurons was visualized by TUNEL-labeling and quantified. Western blotting was used to investigate different signaling pathways. Apoptosis of RGC was induced by NMDA-injection and excitotoxicity, while apoptosis of photoreceptors was induced by light damage.

Results: During postnatal synaptogenesis, apoptotic death of retinal neurons was significantly decreased in Myoc-/- pups. The decrease resulted in a significantly higher number of retinal ganglion cell (RGC) perikarya and their axons in the optic nerve, as well as in an increased thickness of outer and inner nuclear layer in adult Myoc-/- mice compared to wild-types. In contrast, myocilin-deficient mice with simultaneous ectopic overexpression of myocilin from the lens (Myoc-/-; ?B1-Crystallin-Myocilin) did not show differences in retinal structure or developmental apoptosis compared to wild-type mice. In adult mice, apoptotic death of photoreceptors following light damage was markedly reduced in Myoc-/- mice. Similarly, apoptosis of retinal ganglion cells after excitotoxic damage following an intravitreal injection of NMDA was attenuated in Myoc-/- mice. Both effects were rescued upon simultaneous overexpression of myocilin.

Conclusions: Myocilin modulates programmed cell death during retinal development and after retinal injury.

Titel:Distinct thresholds of prg3 amplify ras-dependent oncogenesis in brain tumors

Autoren: Rummel P.(1), Eyüpoglu I.(2), Fan Z.(3), Buchfelder M.(3), Savaskan N.(3),

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### Abstract:

Malignant gliomas are one of the most devastating primary brain tumors in humans. One characteristic hallmark of malignant gliomas is their cellular heterogeneity with frequent genetic lesions and altered gene expression levels conferring selective growth advantage. Here, we report on the neuronal-associated growth promoting gene PRG3 executing oncogenic transformation in gliomas. We have identified perturbed PRG3 levels in human brain tumors displaying either elevated or down-regulated PRG3 levels compared to nontransformed specimens. We hypothesized that imbalances of PRG3 levels bear the capacity to transform cells by facilitating similar downstream effects. To test this we analyzed wildtype gliomas and gliomas with distinct PRG3 levels. Perturbation of PRG3 levels in glioma cells accelerates anchor-independent proliferation and migration, indicating amplified oncogenic signaling. In vivo disequilibrated PRG3 gliomas show aggravated proliferation, invasion, and deteriorate clinical outcome, whereas tumor angiogenesis remained unaffected. Hence, PRG3 interacts with RasGEF1 and activates oncogenic Ras and disrupts the lipid second messenger phosphatidylinositol-(4,5)-bisphosphate (PIP2) from the plasma membrane. Restoration of PIP2 levels via phosphatidylinositol 4-phosphate 5-kinase (PIP5K) attenuated PRG3-induced transformation and reverted the phenotype. In conclusion, these results show that PRG3 acts context-dependent in brain cells, and interference with the PRG3 homeostasis amplifies oncogenic signaling events.ns, diameter and distribution.

Titel:The role of the antimicrobial peptide CRAMP in glial cell activation after bacterial stimulation

Autoren: Brandenburg L.(1), Kress E.(2), Höß J.(2), Merres J.(2), Pufe T.(2), Tauber S.(3),

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### Abstract:

Antimicrobial peptides (AP) are important components of the innate immune system, yet little are known about their expression and function in the brain. Our previous work revealed a higher mortality rate and up-regulation of proinflammatory gene expression as well as glial cell activation in cathelicidin-related antimicrobial peptide (CRAMP)-deficient mice after bacterial meningitis. However, the consequence of CRAMP deficiency for the glial cell function and their involvement in inflammation after bacterial meningitis remains unknown. Therefore, we used CRAMP-deficient and wildtype glial cells to investigate the role of antimicrobial peptide CRAMP in glial cell viability, inflammation and glial cell activation after bacterial stimulation. CRAMP-deficiency was associated with a stronger morphological change of glial cells, whereas the microglial cells showed a decreased viability after bacterial stimulation. The analysis of inflammatory response revealed increased expression of different proinflammatory cytokines by CRAMP-deficient glial cells using realtime RT-PCR after bacterial treatment. CRAMP-deficient glial cells displayed a higher degree of glial cell activation that was accompanied by a stronger translocation of the transcription factor nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NFêB) after bacterial stimulation and endogen activation of the extracellular signal-regulated kinase 1/2 (ERK1/2) in glial cells.

Taken together, this work provides insight into the important role of CRAMP as part of the innate immune defense against pathogens and their involvement in glial cell function.

Titel:Brain-intrinsic inflammation triggers peripheral cell recruitment

Autoren: Kipp M.(1), Beyer C.(2),

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Abstract:

Multiple sclerosis (MS) is considered to be an autoimmune, inflammatory disease of the central nervous system. Although many facets of MS pathology may appear consistent with a primary autoimmune disease, questions have been raised as to whether inflammation and/or autoimmunity are really primary events during MS lesion formation. Results from several laboratories suggest that brain intrinsic inflammatory processes precede immune-cell infiltration, with lymphocyte recruitment reflecting secondary, albeit very important, reactions. To test for this hypothesis, we induced brain-intrinsic inflammation by systemic cuprizone intoxication and subsequently provoked a myelin-specific immune response by immunizing animals with MOG-peptide diluted in complete freund's adjuvant and pertussis toxin (i.e. classical active EAE induction in mice). Inflammatory reactions were followed by immunohistochemistry. As expected, administration of cuprizone induced profound oligodendrocyte loss paralleled by demyelination as well as astrocyte and microglia activation in various brain regions, among the corpus callosum. No lymphocyte infiltration was evident. In MOG-immunized animals, severe inflammation occurred in the spinal cord and cerebellum, whereas the corpus callosum and cortex region were virtually sparred. Interestingly, cuprizone-induced neuroinflammation was sufficient to trigger extensive monocyte and lymphocyte recruitment into EAE-resistant brain regions. Early peripheral cell recruitment was most pronounced around the ventricles, the choroid plexus and in the perivascular space. Furthermore, our results suggest that brain resident microglia and/or antigen presenting cells regulate immune cell entry into the brain. We argue that MS may be an 'immunological convolution' between an underlying primary degenerative disorder and the host's aberrant immune response.

Titel:The neuroprotective role of the L-type calcium channel antagonist nimodipine in experimental autoimmune encephalomyelitis

Autoren: Rottlaender A.(1), Volovitch O.(1), Kuerten S.(2)

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#### Abstract:

The neuroprotective role of the L- type calcium channel antagonist Nimodipine in experimental autoimmune encephalomyelitis Andrea Rottlaender 1, Oleg Volovitch 1 and Stefanie Kuerten 2 1 Department of Anatomy I, University of Cologne, Germany. 2 Department of Anatomy and Cell Biology, University of Wuerzburg, Germany. Common therapeutic strategies in multiple sclerosis (MS) research aim at attenuating the immuneresponse, but do not provide persistent prevention of neurodegeneration. To gain insight into the pathomechanisms during neurodegeneration experimental autoimmune encephalomyelitis (EAE) - the most widely used animal model of MS - was used in this study. As it is well known that increased levels of intracellular calcium mediate neurotoxicity we decided to examine the effects of calcium on the pathology of relapsing-remitting EAE. To this end MP4-immunized SJL/J mice were treated daily with s.c. injections of Nimodipine for a duration of 45-50 days. By this, we were able to show that the clinical course of disease was significantly reduced in Nimodipine treated mice. To evaluate whether this observation was reflected by the histopathology, immunohistochemical, semi-thin and ultra-structural analyses were performed. Our results indicate that the pattern of inflammation was independent of treatment whereas demyelination and axonal pathology were reduced in Nimodipine treated mice. In addition, treatment with Nimodipine increased the number of remyelinating nerve fibers. Taken together, these results indicate a potential neuroprotective effect of Nimodipine and confirm the need for neuroprotective agents as addendum to common therapeutic strategies.

Titel:TGF-beta1 protects mDA neurons from IFNgamma-mediated neurotoxicity by attenuation of microglia IFNgamma signalling

Autoren:Spittau B.(1),Zhou X.(1),Krieglstein K.(1),

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### Abstract:

TGF-betas have been described as potent regulators of microglial activation states inhibiting LPS-induced classical (M1) and promoting IL4-induced alternative (M2) activation of microglia. Since LPS is a rather artificial M1-activator, IFNgamma is one of the endogenous M1-activators which has been identified to drive neurotoxic responses in several neurodegenerative animal models. In the course of the MPTP-model for Parkinson's disease (PD), IFNgamma has been shown to be a major player in mediating microglia-driven neurotoxicity. Interestingly, a rapid upregulation of TGF-beta1 is also detectable after MPTP intoxication of mice. Here, we show that TGF-beta1 is able to protect midbrain dopaminergic (mDA) neurons during IFNgamma-induced neurotoxicity in mixed neuron-glia cultures. Further, we demonstrate that TGF-beta1 blocks the IFNgamma-mediated microglia activation characterised by the release of neurotoxic molecules such as TNF? and nitric oxide (NO). Microarray studies revealed that TGF-beta1 downregulated a plethora of genes related to microglia IFNgamma signalling, among them the essential signalling components IFNgammaR? and the downstream mediator STAT1. Moreover, TGF-beta1 treatment resulted in significant reduction of STAT1 phosphorylation in primary microglia. Together, our data indicate that TGF-beta1 is potent inhibitor of IFNgamma-induced microglia activation by interfering with the IFNgamma signalling cascade. Therefore, TGF-beta1 might have important functions as an endogenous regulator of IFNgamma-induced microglia activation and microglia-mediated degeneration of mDA neurons.

Titel:Neuroimaging and preclinical applications in non-human primate models of parkinson's disease and multiple sclerosis

Autoren: Garea-Rodríguez E.(1), Schlumbohm C.(2), Helms G.(3), König J.(4), Meller B.(5), Meller J.(5), Krieglstein K.(6), Fuchs E.(4),

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### Abstract:

Single photon emission computed tomography (SPECT) imaging and magnet resonance imaging (MRI) play an increasing role in animal models of neurological disorders. To overcome the limitations of rodent studies regarding complex brain function and drug safety, non-human primates after often used in preclinical research. Here we present in vivo imaging methods and their application in common marmoset monkey models of Parkinson's disease and multiple sclerosis. The first study aimed to establish a protocol for imaging the dopamine transporter (DAT) in common marmosets. Serial SPECT and structural MRI were performed on an upgraded clinical scanner to determine the distribution kinetics of 123I-N-?fluoropropyl-2?-carbomethoxy-3?-{4-iodophenyl}nortropane (123I-FP-CIT) and the underlying brain anatomy. In the 6-hydroxydopamine (6-OHDA) model of Parkinson's disease, complete loss of striatal DAT binding in combination with behavioral deficits was observed. The second study focussed on a new targeted experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis in marmosets. Lesions were induced by intracerebral injections of pro-inflammatory cytokines in animals that were subclinically immunized with myelin oligodendrocyte glycoprotein (MOG). Lesion development was monitored using a clinical MRI scanner. Signs of inflammation were visible on T1- and T2weighted images four days after intracerebral cytokine injection. The presented studies demonstrate that 123I-FP-CIT SPECT and MRI are suitable tools to investigate neurodegeneration and neuroinflammation in common marmosets. Supported by the DFG Research Center of Molecular Physiology of the Brain (CMPB) and GE Healthcare (Munich, Germany). EGR was funded by EU ERA-Net NEURON.

Titel:The temporal and spatial dynamics of glyoxalase 1 following neuronal injury

Autoren: Pieroh P.(1),Koch M.(2),Wagner D.(3),Boltze J.(3),Ehrlich A.(2),Ghadban C.(1),Hobusch C.(2),Birkenmeier G.(4),Dehghani F.(1),

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### Abstract:

During enhanced glycolysis elevated levels of the toxic dicarbonyl compound methylglyoxal (MG) are formed and lead to the loss of protein-function, metabolic imbalance and cell death. Neurons showed high susceptibility to MG toxicity. Glyoxalase 1 (Glo1) as an ubiquitous endogenous detoxification system catabolises MG. Especially, astrocytes presented high Glo1 levels compared to neurons. After inhibition of astrocytic Glo1 and MG treatment cell survival was rapidly decreased. Furthermore, Glo-1 underlies changes in ageing and neurodegeneration.

However, data about the role of Glo-1 in hypoxic insult are still rare.

In the present study, we analysed the temporal dynamics of Glo1 distribution and expression by immunohistochemistry and Western Blot analysis. Organotypic hippocampal slice cultures were excitotoxically (N-methyl-D-aspartate,  $50\mu$ M for 4 hours) lesioned in vitro (5 minutes to 72 hours) and permanent middle cerebral artery occlusion was performed (75 minutes to 60 days). The possible involvement of Glo1 on astrogliosis was investigated in the scratch-wound model of astrocyte monolayers which were treated with the Glo1 inhibitor ethyl pyruvate (EP;  $0.84\mu$ M,  $8.4\mu$ M,  $84\mu$ M).

We found i) the predominant localisation of Glo1 in endothelial cells in non-lesioned brains ii) a time-dependent up-regulation and re-distribution of Glo1 in neurons and astrocytes iii) a strong increase in Glo1 dimers after injury (24h-72h) when compared to monomers of the protein during hypoxic neuronal damage and a reduced astrocytic scar formation after EP treatment.

In conclusion, the temporal and spatial dynamics following the process of neuronal injury may present Glo1 as important player in the process of secondary neuronal injury.

Titel:Uncoupling protein (UCP) 2-dependent regulation of reactive oxygen species (ROS) in hypothalamic neurons determines metabolic effects of cannabinoids

Autoren: Koch M.(1),Kim J.(2),Varela L.(2),Bechmann I.(3),Dietrich M.(4),Diano S.(5),Horvath T.(2),

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### Abstract:

The gut-derived hormone ghrelin and central endocannabinoids are mainly associated with their ability to adjust eating behavior and energy expenditure by targeting hypothalamic G protein-coupled receptors (Koch and Horvath, Biol. Psych., 2012). During fasting, ghrelin induces food intake by activation of hypothalamic NPY/AgRP neurons. Ghrelin switches mitochondrial respiration and triggers UCP2-dependent scavenging of ROS, which finally increases firing of NPY/AgRP neurons and suppresses satiety-promoting POMC neurons (Andrews et al., Nature, 2008).

The cannabinoid receptor 1 (CB1R) is responsible for most of the behavioral effects of endocannabinoids, but the precise mechanisms behind hypothalamic CB1R activation in order to regulate feeding and energy expenditure remain enigmatic. Since CB1R was addressed to reduce mitochondrial respiration in hippocampal neurons (Benard et al., Nat. Neurosci., 2012), we asked whether metabolic effects of endocannabinoids are driven by mitochondrial respiration and UCP2-dependent control of ROS in hypothalamic NPY/AgRP and POMC neurons. In mice, we observed that CB1R-mediated regulation of mitochondrial respiration differs between hippocampal and hypothalamic neurons and depends on animal's nutritional status. Pharmacological experiments revealed that endocannabinoids differentially regulate ROS in NPY/AgRP and POMC neurons. Transgenic mouse models that all feature altered NPY/AgRP neuronal circuit integrity and function (UCP2-/-, AgRP-Sirt1-/-, AgRPDTR) as well as transgenic POMCCre mice transfected with inhibitory DREADD (designer receptors exclusively activated by designer drugs) by use of Cre-recombinase-dependent adeno-associated virus (AAV) showed impaired CB1R-associated feeding behaviors. Taken together, our results support the idea that metabolic effects of endocannabinoids are transduced by mitochondrial respiration and UCP2-dependent ROS regulation in hypothalamic neurons.

Helmholtz-Alliance ICEMED (to Horvath and Bechmann)

Titel:N-arachidonoyl – dopamine (NADA) is a novel neuroprotective endocannabinoid

Autoren:Grabiec U.(1),Koch M.(2),Kraft R.(3),Hill K.(4),Merkwitz C.(2),Ghadban C.(1),Lutz B.(5),Straiker A.(6),Dehghani F.(1),

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### Abstract:

Endocannabinoids exert numerous effects in the CNS under physiological and pathological conditions. The aim of the present study was to examine whether the novel endocannabinoid N-arachidonoyldopamine (NADA) may protect neurons in excitotoxically lesioned organotypic hippocampal slice cultures (OHSC). OHSC were lesioned by the application of N-methyl-Daspartate (NMDA, 50  $\mu$ M) for 4 h and subsequently treated with different NADA concentrations (0.1 pM – 50  $\mu$ M) alone or in combination with cannabinoid receptor antagonists. NADA protected the dentate gyrus granule cells and caused a faint reduction in the number of microglial cells. The number of degenerated neurons significantly decreased between 100 pM and 10 µM concentrations of NADA. NADA (1 nM) mediated neuroprotection was CB1 receptor dependent. O-1918, 6-iodonordihydrocapsaicin and HC-030031 as specific antagonists of abnormal cannabidiol, TRPV1 and TRPA1 receptors showed no effects at all used NADA concentrations. In the next step we looked at the MAP kinase signal transduction pathway activation after stimulation with NADA. For this purpose neuronal hippocampal cell line HT22, microglial cell line BV2 and primary astrocytes were used. NADA did not influence the activation p38 and p44/42 MAPK in the analyzed cells after 30 min, 2 h, 6 h, 24 h. Our findings demonstrate that NADA protects dentate gyrus granule cells by acting upon CB1. NADA reduced the number of microglial cells at distinct concentrations. TRPV1 was not involved in NADA mediated neuroprotection. Thus, our data implicate that NADA-mediated activation of neuronal CB1 receptor may serve as a novel pharmacological target to mitigate symptoms of neuronal damage.

Rubrik: Methoden/Unterricht

Titel: Teaching professionalism during the dissection course

Autoren: Gahlen S.(1), di Santo A.(2), Hirt B.(1), Shiozawa T.(1).

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### Abstract:

BACKGROUND The dissection course is often named the most impressive and defining experience during medical studies. Death, the vulnerability of the human body, the first entrusted 'patient' – these are mighty impressions which naturally foster professional competencies. To value these experiences we offered a structured seminar on professionalism in winter term 2012/13. The aim was to initiate a first reflection about borderline situations in medical daily routine.

METHODS Two voluntary seminars were offered, at the course start and during dissection of the head. Small groups of 10 students were moderated by student tutors, who were supported by anatomists, doctors and healthcare chaplains. Specific interview guides helped to address various topics for reflection and discussion. The seminar was evaluated using a self-designed questionnaire (5 items with 5-point Likert scale, 4 free text questions).

RESULTS About 120 students voluntarily took part, though the demand was far higher. We faced a severe dropout towards the second date, so the return rate of the questionnaires was about 30%. The evaluation states that the seminar helped the students to cope with the special situation of the dissection course, to reflect their coping mechanisms, and made them think of new ways how to approach difficult situations in medical daily routine.

DISCUSSION The new seminar on professionalism in the dissection course was very well accepted and positively evaluated by the students. The impressions from the group discussions and the free text comments may support the conclusion that we attained our learning objective, however it will be challenging to measure the learning outcome.

Rubrik: Methoden/Unterricht

Titel: 3D illustrations in anatomy teaching

Autoren: Kipp M.(1), Frank S.(1),

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Abstract:

It has been shown already that it is difficult to improve the understanding of complex anatomical situations using regular two-dimensional (2D) images. Understanding of the complex topography of various anatomical regions, including neuroanatomical sites, is one of the outstanding reasons to conduct human dissections courses during the medical education. However, most teaching books present 2D images, making it complicated for the students to study complex anatomical structures at home our outside of the dissection room. The possible advantages of 3D presentations in learning about and understanding of complex spatial interactions have been described and are well appreciated. This work addresses the construction of an atlas of the cranial nerves and base of the skull. High resolution illustrations of various neuroanatomical regions were acquired and can be used for lectures and seminars or for the students to study anatomy at home. The use of 3D anaglyph glasses allows the visualization of the 3D images independent of any specific technical equipment. The presented high resolution illustrations are potentially useful for anatomy browsing, user self testing, automatic student assessment, preparing materials, and localization in clinical and paraclinical neurology.

Rubrik: Methoden/Unterricht

Titel:Structured oral exams are more reliable in assessment of anatomy knowledge compared with widely used unstructured exams.

Autoren:Shiraz P.(1),Wehrenberg U.(2),Horling K.(1),Schreiner J.(3),Sehner S.(4),Lüers G.(1),

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Abstract:

Oral exams are commonly used in medical faculties for assessment of students' knowledge of human anatomy. At the University Medical Centre in Hamburg-Eppendorf, unstructured oral exams have been used for decades and the student's performance was generally rated as pass or fail. The reliability of these exams has never been analyzed systematically and has been questioned by students several times. In order to judge the competence of students who passed the exams in a more differentiated manner, we have developed a new kind of structured oral exams that allows for rating the students' performance on a scale from 0-10. To measure the reliability of the assessment formats we have compared the results of structured oral exams, unstructured oral exams and written tests with multiple choice questions that all cover equivalent anatomical topics in a randomized crossover study. We could show that structured oral exams were more reliable than unstructured ones when compared to multiple choice tests. Furthermore, an evaluation of the student's opinion revealed that the influence on the grade by the examiner appeared less in structured oral exams compared with unstructured ones.

Rubrik: Methoden/Unterricht

Titel:Microporation is superior to liposomal and non-liposomal transfection reagents for siRNA-induced knockdown of the PEX5 gene expression in human hepatoma cells (HepG2)

Autoren:Ahlemeyer, B.(1),Vogt, J.-F.(1),Michel, V.(1),Hahn-Kohlberger, P.(1),Baumgart-Vogt, E.(1),

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### Abstract:

Peroxisomal biogenesis disorders (PBDs) are caused by mutations of PEX genes leading to developmental and metabolic disturbances predominantly in the liver and brain. The clinical phenotype of PBD patients varies widely with no clear correlation between the severity of the symptoms and the type of mutation in different PEX genes. To study in more detail the pathogenesis of this group of diseases, several knockout mouse models have been generated. We wanted to analyze the functional consequences of a knockdown of PEX5, since in a knockdown, the peroxisomal biogenesis is not completely lost mimicking some milder form of PBDs. For this purpose, we tested several different transfection methods to obtain an optimal reduction of the PEX5 mRNA in HepG2 cells as a model system for human hepatocytes. Using commercially available human PEX5 siRNA in combination with different transfections reagents (e.g. FuGENE6, Lipofectamine 2000, INTERFERin), we were able to reduce PEX5 protein up to a maximum of 50% with FuGENE6, however, with a broad variation between the experiments. Microporation (Neon transfection system) was found to be even more efficient than FuGENE6 with respect to the transfection rate and the amount of intracellular Cy3-labelled PEX5 siRNA; the PEX5 mRNA level was reduced with a high reproducibility and for long time periods (72 h). Reduction of the PEX5 protein resulted in a mistargeting of catalase into the cytosol and to increased cellular levels of reactive oxygen species independent of the transfection method.

Rubrik: Klinische Anatomie/Makroskopie

Titel:Correlations between morphometrical, structural and haemodynamic aspects within the renal arterial system

Autoren: Sapte E.(1), Sava A.(2),

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#### Abstract:

The proper morphological characteristics of both renal arteries, together with the structural peculiarities represent the factors that contribute to an almost equal amount of supply to both kidneys. Starting from the fact that the parenchymatous element induces and organize the vascular one, we examined, in parallel with the proper morphological characteristics (length and caliber of the vessels) some vascular parameters (systolic velocity, diastolic velocity, resistivity index) by imaging of the renal arterial system (renal artery, interlobar arteries). The decision upon the two types of vessels which we measured was based both on practical reasons - they are accessible to ultrasound measurements - and also on hemodynamic considerations. When arrived at the cortical level, the blood has the pressure required to achieve normal physiological processes. Histological sections of the arterial wall were made using Van Gieson's and Masson staining methods. Keywords: kidney, morphometrical study, renal artery, hemodynamic parameters

Rubrik: Klinische Anatomie/Makroskopie

Titel:The characterization of the inferior alveolar nerve with special reference to an anterior loop of the mental nerve in the mental canal

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### Abstract:

The course of the mandibular canal and variations in its path are very important factors for surgical procedures in the mandibular region. The aim of this study was to examine and describe the neurovascular bundle in the mandibular canal in edentulous and dentate human mandibles and to obtain significant informations about the presence of an anterior loop of the mental nerve.

For the present study, 24 hemi-mandibles were prepared macroscopically. The documentation was done with a digital camera and an electronic caliper. For analyzing the intrabony course of the neurovascular bundle we stained histological sections according to routine staining protocols.

For the immunocytochemical characterization of the mental neurovascular bundle, we used antibodies against S100 and tyrosine hydroxylase.

In 54 % of the mandibles, the mandibular as well as the incisive canal possessed welldefined bony walls. In 33 % of the mandibles we observed semicanals, and in 12 % the neurovascular bundle ran without bony covering. In 12.5 % of cases we found accessory mandibular canals.

In 70 % of dentate mandibles, the mental foramen was located between the first and the second premolar. In 43 % of the edentulous mandibles, the position of the mental foramen was shifted to the height of the alveolar ridge.

Since we found only in two cases of all examined hemimandibles an anterior loop of the mental canal, the dentist must considered the possibility that an anterior loop of the mental nerve may be present prior to preparing an osteotomy mesial to the mental foramen.

Rubrik: Klinische Anatomie/Makroskopie

Titel:Anatomical evaluation of the incisura dextra of gans and accessory incisures at the visceral surface of the liver as important structures for inflow control in hemihepatectomy

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#### Abstract:

Background: A precise evaluation of the incisura dextra of Gans as well as a description of other incisures, especially a left one on the visceral surface of the liver is required and useful as a guide through hemihepatectomy to control vascular inflow.

Material and Methods: The examination included 107 livers of human cadavers, without obvious signs of interventions and pathologies in the area of interest and embalmed with Thiel's method. The main points of data collection included markable additional periportal incisures, especially the incisura dextra of Gans, a left incisura and accessory incisures at the visceral surface of the liver as well as their dimensions and the position of the portal trias at the bottom of them.

Results: In 64 cases (60%) a clearly visible incisura dextra of Gans as well as a portal trias directly at the bottom were identified. In the other 40% without this incisura a prominent lobus caudatus with tissue bridging was identified in 28 cases (65%) instead. A left incisura was found in 22% of cases with the portal trias at the bottom in all cases. Furthermore additional incisuras appeared in 49 % and within these we demonstrated a portal trias in 87%.

Conclusion: If there is no prominent lobus caudatus, an incisura dextra of Gans can be identified in 85% and in all cases there was a portal trias found in the deep. A prominent bridge coming from the lobus caudatus excludes an appearance of the incisura on the right.

Rubrik: Klinische Anatomie/Makroskopie

Titel:Posterior tibial perforator veins - macroscopic and clinical anatomy

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### Abstract:

Perforator leg veins or Cockett posterior tibial veins that provides communication between superficial network and deep veins perforate the muscle fascia. We conducted the study of the perforator veins of the saphenous field on 50 adult human cadavers, 24 males and 26 females, dissected bilaterally, following the lower limb veins. Anatomical parts were dissected on the superficial plan, putting into evidence the entire superficial venous network, highlighting the fascial penetration of the perforator veins. Crural fascial perforation site varies in height: from 4 cm to Cockett I vein, 15 cm Cockett II vein, 4 cm Cockett III vein, 4 cm Cockett IV vein (perforated at 24 cm). Among the Cockett perforators we have found the most common Cockett II variant. While Cockett I perforators were located retromaleolar, perforators Cockett II were located at a variable height of 7-9 cm, superior to the tip of the medial malleolus. The most commonly were found Cockett II perforators at 9 cm above the medial malleolus (42%). Cockett III perforators were placed at a height of 10-12 cm above the medial malleolus to the tip. Of these, the most commonly were found at the location of 12 cm (39%). "24 cm" perforators (so called Cockett IV) were found vertically placed, from 18 to 22 cm, superior to the tip of the medial malleolus. The most frequent we found them at 20 cm superior to the medial malleolus tip.

Keywords: perforator veins, medial malleolus, lower limb

Rubrik: Klinische Anatomie/Makroskopie

Titel:Holoprosencephaly with cyclopia – case presentation

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#### Abstract:

Cyclopia is a congenital malformation of the face characterized by the fusion on the midline of the 2 orbits of the eye, resulting in a single orbit. The eye ball may be absent, other times rudimentary, apparently normal or duplicated. The nose can be nonexistent or substituted by a tubular proboscis appendix, situated on the superior part of the orbit.

Cyclopia represents the extreme form of hypotelorism being the most serious form of facial malformation of all malformative spectrum with a very rare incidence of 1/100.000 births. Holoprosencephaly is a serious developmental disorder (HPE) of the cephalic pole where the prosencephalon (forebrain of the embryo) fails to separate into two cerebral hemispheres during the 5th-6th week of pregnancy in humans.

The present case is that of a 22 week male fetus, weighing 1000 grams, plurimalformed from a diabetic parturient. The cyclopia shocks from the first inspection with arinia and proboscis. During the necroptic examinations we found a cerebral substance with a flaccid immature aspect, with a presence of a cystic occipital intracranial formation .The fetus presents bilateral hypoplasia, atelectatic lung, the heart presents hypoplasia of the left ventricle and dilatation of the right atrium with a common arterial trunk with an orifice in the left ventricle. Also the fetus presents hepatomegaly, liver stasis and ascites with 50 ml serous citrine fluid. The placenta and the umbilical cord were examined macroscopically and microscopically that brought valuable information regarding vascular changes that led to hypoxia. Key words: congenital malformation, cyclopia, feto-placental unit.

Rubrik: Klinische Anatomie/Makroskopie

Titel:Patterns of Knee Cartilage Thickness Loss after Posterior Crucial Ligament Injury

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### Abstract:

Objective: The magnitude and pattern of knee cartilage thickness change after posterior cruciate ligament (PCL) injury and reconstruction has not been previously reported. Detailed knowledge on such changes may, however, be potentially useful in monitoring the success of therapeutic intervention by surgery, medication, and/or physiotherapy.

Methods: 20 participants who had sustained PCL injury and consecutive reconstructive surgery had MR images acquired at baseline, i.e. 3-38 months after PCL surgery. To date, 9 participants had one-year follow-up imaging on the same scanner (6 men, 3 women; age 37±10 yrs.). Changes in cartilage thickness between baseline and one year follow-up were computed after manual segmentation of the cartilage and bone interfaces in the femorotibial and femoropatellar compartments.

Results: Cartilage thinning was observed in all femorotibial cartilage plates, with a greater annual percent change in the medial tibia (-4.7% [95% confidence interval -8.9;-0.5%]) and medial femur (-5.1% [-10.2; 0.0%]) than in the lateral tibia (-1.2% [-3.4; +0.9%]) and lateral femur (-1.8% [-3.3; -0.3%]). Cartilage thinning also occurred in the femoropatellar compartment, with a greater observed annual percent change in the patella (-3.5% [-5.4;-1.7%]) than in the femoral trochlea (-0.9% [-2.2; +0.3%]).

Conclusion: The rates of cartilage thinning observed after PCL rupture and reconstruction were greatest in the medial femorotibial compartment and in the patella, and exceed those typically seen in primary knee osteoarthritis. They also differ from those in patients with anterior cruciate ligament rupture (and repair), in whom cartilage thickening was observed after injury.

Rubrik: Klinische Anatomie/Makroskopie

Titel:Splanchnic nerves - topography and clinical anatomy

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Abstract:

Greater splanchnic nerve penetrates into the abdominal cavity through a foramen located posterior to the diaphragmatic pillars. We dissected 20 formalised human bodies of different age and gender, on which we followed the splanchnic nerves trajectory. Regarding the splanchnic nerve transcrural passages we obtained the following results: on 20 specimens (100%) greater splanchnic nerve crosses through a gap defined between the inner and middle pillars of the diaphragm: lesser and imus splanchnic nerves were among the fibers of the middle pillar in the absence of an obvious muscular space (40%) or in the gap between the external and middle pillars. The gaps are present on the pillar diaphragmatic passages, being a communication between lower thoracic paravertebral space (below endothoracic fascia of Luschka) and coeliac region. The presence of splanchnic nerves and sympathetic laterovertebral chains in these diaphragmatic gaps is a way to broadcast anatomical collections fluid (whether pathological or anaesthetics) from lower paravertebral thoracic space to the coeliac region. The specimens which we dissected have the greater splanchnic nerves crossing the gap between the inner and middle pillars of the diaphragm on that side. Half of the specimens (10, dissected bilaterally) showed lesser and imus splanchnic nerves with transcrural trajectory (the middle pillar) and the rest (50%) of the paths through the gap between the middle and outer pillars. Keywords: greater splanchnic nerve, pillar, diaphragm.

Rubrik: Klinische Anatomie/Makroskopie

Titel:The effects of time point, location and dosage of VEGF-c application in the regeneration and reconnection of lymph node fragments in rats

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Wiederherstellungschirurgie|Hannover|Deutschland; (4)Physiologisches Institut|Tierärztliche Hochschule Hannover|Hannover|Deutschland

### Abstract:

After breast cancer treatment, up to 30% of the patients develop secondary lymphedema. Currently, there is no long-term surgical therapy for this condition. Therefore, the purpose of this study was to improve the application pattern of VEGF-C to achieve a higher regeneration rate of autologous transplanted lymph node fragments. We focused on the time point, location and injected dosage. Inguinal lymph nodes of adult, healthy female Lewis rats (~200g) were remove. Three nodes were fragmented and transplanted back subcutaneously. Lymphatic-transplant-reconnection was investigated by Patent Blue and regeneration was analysed by B-and T-lymphocyte-, HEV- and lymph endothelial cell- distribution (fluorescent immunohistochemistry). An early application (day 1,2,3 post OP) and the application in the medial side of the thigh, have significant influence (53%, control:15%) on reconnection, whereas application into the abdominal wall showed only a statistical tendency (50%) and a late time point (day 14,15,16 post OP) revealed no statistical effect (35%). Time point and location do not have a statistical effect on lymphatic regeneration either. A higher dosage (13.34µg/rat) results in 95% regenerated lymph node fragments whereas the control group ends up with only 70%. The reconnection also benefits from a high VEGF-C dose: with an outcome of 80% compared to 15% in the control group, the reconnection rate is extremely significantly enhanced. Supported by Deutsche Forschungsgemeinschaft and Gesellschaft der Freunde der MHH.

Rubrik: Klinische Anatomie/Makroskopie

Titel:A CT-supported analysis of the radial head and comparison with a preexisting prosthesis

Autoren: Puchwein P.(1), Tesch N.(2), Pichler W.(1),

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### Abstract:

Objectives: Fractures of the head and the neck of the radius are one of the commonest fractures about the elbow comprising some 33% of all elbow fractures and 1.5-4% of all fractures in adults. The purpose of this study was to investigate the three-dimensional morphology of the proximal radius using a modern technique and to the compare the results to morphology of the radial head prosthesis currently available. Methods: CT-scans of thirty cadaveric elbows and a 3D-reconstruction software was used to analyze the morphometry of the proximal radius, results were compared with manufactors' data of several prostheses. Results: The mean maximum diameter of the radial head was 22.79 mm (19.55 mm to 25.76mm), the mean diameter at the proximal articular surface between the articular lips amounted 18.81 mm (15.90 mm to 22.01 mm), the diameter at the level of the head – neck interface was 15.08 mm (12.30 mm to 17.88 mm) and the mean radial head length was 11.80 mm (10.14 mm to 15.18 mm). Statistically significant gender differences in the maximum diameter of the radial head (p<0.01), the diameter at the level of the head – neck junction (p=0.01) and the radial head length (p<0.01) could be found. Conclusion: Currently available radial head prostheses cover the range of sizes encountered. Products with choice of head and stem sizes in any combination are preferable. Especially in unstable elbow fractures correct implant size is an important factor to avoid subluxation of the radial head (Mason type IV fractures).
Rubrik: Klinische Anatomie/Makroskopie

Titel:Stereological assessment of effects of ex vivo lung perfusion in porcine lungs

Autoren:Steinmeyer, J.(1),Becker, S.(1),Avsar, M.(2),Warnecke, G.(2),Ochs, M.(1,3,4),Schnapper, A.(1),

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### Abstract:

In lung transplantation, ischemia-reperfusion injury (I/R injury) is one of the most dreaded complications. To recondition injured (marginal) donor lungs and thus to avoid manifestation of I/R injury the technique of ex vivo lung perfusion (EVLP) has been developed. The aim of our study was to detect and quantify structural damage typical for I/R injury in lungs submitted to two different techniques of EVLP treatment after an ischemic insult. After explantation, pig lungs (n=16) were assigned to 4 experimental groups: 0-control, ischemia control (24h cold ischemia), aEVLP (24h cold ischemia, 12h EVLP, perfusion with acellular SteenTM solution), eEVLP (as aEVLP, plus addition of 13% erythrocytes to perfusion solution). All lungs were perfusion-fixed in an inflated state. Histological samples were obtained by systematic uniform random sampling and analyzed using stereological methods. All lungs presented with intact parenchyma on a light microscopical level. In all groups a small amount of atelectatic parenchyma was detected (group means 0.4-6.2% of lung volume, corresponding to 10-87 ml). The lungs in the EVLP groups showed a trend towards less atelectases. Hardly any edema was detectable in the control groups (group means 0.2 and 0.5% of lung volume, representing 3 and 8 ml of edema fluid, resp.). In the EVLP groups edema amounted to 2.6 and 2.4% of lung volume, resp. (60 and 51 ml, resp.). In conclusion, EVLP treatment was able to prevent a reperfusion injury almost completely even after a severe ischemic insult. No significant difference was detected between the acellular and ervthocytic EVLP technique.

Rubrik: Klinische Anatomie/Makroskopie

Titel:Histomorphometric analysis of the lymphatic and blood vascular system of the glottis of adult pigs

Autoren: Reinhard H.(1),Koch R.(1),Gasse H.(1),

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Abstract:

Introduction: The porcine glottis has been proposed as an experimental model in human phoniatry, however, certain differences must be considered, e.g. there are two folds - cranial (CraF) and caudal (CauF) – in the pig's glottis. The CraF (presumably the main oscillator) corresponds only in a topographical sense to the vestibular fold in the human. As the stratigraphical organisation, and the systems of interstitial-fluid drainage, have an impact on phoniatric properties, the microvessels are described in this study. Methods: Paraffin cross sections of the glottis (8 minipigs, female, adult, aged 11-14 months) were stained with polyclonal anti-von-Willebrand-Factor or with polyclonal anti-Smooth-Muscle-Actin to distinguish lymph and blood microvessel. The slides were subsequently digitized (Scan Scope), and studied morphometrically. Results: The vascular system was heterogeneous. but a common pattern existed in CraF and CauF. (A) A superficial, 100-300 µm wide Zone A comprised two rows of vascular profiles, i.e. blood capillaries in Row 1, and arterioles plus venoles in Row 2; lymph capillaries and precollectors were mainly distributed within Row 2, i.e. 10-30 µm away from the epithelium. (B) Underneath, in Zone B, the vascular density continuously decreased downwards in the CauF. In the CraF, Zone B displayed a homogenously low vascular density, with some profiles of larger calibre in deeper regions. At the crest of CauF and CraF, there was a distinct a-vascular area in Zone A, located away from the laryngeal ventricle. Discussion: The vascular distribution and the a-vascular areas are discussed with regard to Reinke's Space in humans.

Rubrik: Klinische Anatomie/Makroskopie

Titel:The elastic system of the porcine glottis: a model for human phoniatry? A histomorphometric study of age-related changes in pigs

Autoren: Lang A.(1),Koch R.(1),Gasse H.(1),

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### Abstract:

Introduction: Elastic fibre amounts and their local distribution impact on the phoniatrical characteristics of the human vocal fold. The pig is an established model in human phoniatry, however, the functional homology of both its cranial and caudal vocal fold (CraF, CauF) to the human vocal fold has not been evaluated until now, particularly in terms of age-related changes. Methods: 23 female minipigs ("young": 2-3 months; "adult": 11-27 months; "old": 54-84 months) were examined histomorphometrically by use of a semi-automated system (Adobe Photoshop; paraffin sections; resorcin-fuchsin stain). Results: The amounts per area of elastic fibres (apa.elast) increased with age in all layers of the lamina propria. The apa.elast was always highest in the thin subepithelial layer (SEL) in CauF and in CraF. The greatest fibre increase with age occurred in the SEL. In the CraF, another area of high values was the intermediate layer (IL) in the young animals. Discussion: The structure of the elastic fibre apparatus in the human vocal fold (data from literature) is similar only with the elastic system of the CraF of the young minipigs. All the other porcine specimens were markedly or gradually different. We therefore suggest that in terms of elastic fibres, the CraF - which is presumed to be the main oscillator - in young minipigs is best suited as a model for human phoniatry.

Rubrik: Klinische Anatomie/Makroskopie

Titel:Variations in the flexor forearm muscles under clinical aspects

Autoren: Claassen H.(1), Schmitt O.(2), Schulze M.(2), Wree A.(2),

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#### Abstract:

Variations of the forearm muscles are not uncommon and can result in nerve compressions when running through carpal canal or Guyon's canal. Among 50 arms, evaluated during the gross anatomy course of Rostock Anatomical Institute in summer term 2013, variants in the flexor forearm muscles were detected in 5 specimen (10%). Case 1: A variant muscle characterized by a digastric like configuration with a central tendon connecting the two bellies, originated superficially in the muscle mass of the flexor carpi ulnaris. It inserted with its distal muscle belly on the medial rim of the abductor digiti minimi. The distal muscle belly traversed Guyon's canal with two thirds of its final length. Case 2: A variant muscle took its origin from the flexor digitorum superficialis, just upon its ring finger part. It inserted at the hook of hamate and at the flexor retinaculum. Since the palmaris longus as the most variable forearm muscle was present, the variant muscle in case 1 can be interpreted as an accessory flexor digiti minimi. The distal belly of this muscle which traverses the Guyon's canal is likely to have compressed the ulnar nerve. The variant muscle in case 2 might be a variant palmaris longus which was not directly connected with the palmar aponeurosis. From the viewpoint of its insertion it can be considered as an accessory flexor of the wrist. On its way to the hook of hamate the muscle enters the carpal canal and therefore is supposed to have compressed the median nerve.

Rubrik: Klinische Anatomie/Makroskopie

Titel:Median nerve fascicular anatomy as a basis for distal neural prostheses

Autoren: Planitzer U.(1), Steinke H.(1), Meixensberger J.(2), Bechmann I.(1), Hammer N.(1), Winkler D.(2),

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#### Abstract:

Introduction: Spinal cord injury can result in partial to complete loss of motor and sensory nerve function below the injury. Functional electrical stimulation (FES) serves as a possible therapy to restore missing motor functions of peripheral nerves by means of cuff electrodes. FES is established for improving lower limb function. Transferring this method to the upper extremity is complex, due to lack of anatomical data of nerve fascicles. Our study's aim was to provide an anatomical basis for FES of the median nerve in the distal forearm and hand. Methods: We investigated 21 distal median nerves of 12 body donors. The peripheral fascicles were traced back by removing the external and interfascicular epineurium and then assigned to four quadrants. Results: A distinct motor and sensory distribution was observed. The fascicles innervating the thenar eminence and the first lumbrical muscle originated from the nerves' radial parts in 82%. The fascicle supplying the second lumbrical muscle originated from ulnar in 78%. No macroscopically visible plexus formation was observed for the median nerve in the forearm. Discussion: Based on the distribution pattern, it can be concluded that selective median nerve stimulation may be accomplished successfully. Multicontact cuff electrodes may reduce sensory irritations caused as side-effects of overstimulation. Knowing the sensory distribution, FES could also be used for the treatment of regional pain syndromes.

Rubrik: Klinische Anatomie/Makroskopie

Titel: Uncemented Glenoid Metalback fixation : position of the superior implant screw in relationship to suprascapular nerve and scapular notch analysed in an anatomical cadaver dissection study

Autoren: Burkhard M.(1)\*, Mathews S.(2)\*, Link K.(2), Franke I.(1), Rühli F.-J.(2), Harper G.(3), Quereshi F.(4), Bloch H.(5), Ullrich O.(1), Eppler E.(1) \* Shared first authorship

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Abstract:

Anatomic Total and Reverse Total Shoulder Arthroplasty is a common treatment option for primary and secondary osteoarthritis of the shoulder joint. Surgical placement of the superior glenoid baseplate screw might endanger the suprascapular nerve after its passage through the scapular bone towards the scapular notch which could cause chronic postoperative pain and weakness. Despite this well-known complication, only a few studies have been performed so far to investigate the distance between the screw entry point and the scapular notch. In a cadaver dissection study of 17 cadavers from the institutional body donation programme (8 female, 9 male, average age 78 yrs, range 63-99 yrs) we documented the size (ave. 34.8 mm x 26.5 mm) and inclination angle (0-40°) of the glenoid. Furthermore, we measured the distance from the superior screw entry point to the scapular notch both from outside and through the screw canal, the latter revealing an average canal length of 30.3 mm. Additionally, the distance from the supraglenoid tubercle to the scapular notch was measured: 33.2 mm. The majority of dissected joints presented a degenerative illness, so in addition, pathologies such as primary and secondary osteoarthritis (cuff arthropathy) were documented and the relationship of the suprascapular nerve to the adjacent tissue including the suprascapular vessels. Data are discussed in light of recommendations for safe screw placement and secure distance to the nerve.

Rubrik: Neuroanatomie/Neurobiologie

Titel:The neuronal connectivity between the optic tectum and the dorsal thalamus of the salamander (plethodon shermani)

Autoren:Wetjen O.(1), Dicke U.(1), Coors(1),

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#### Abstract:

Amphibian brains possess a lower number of neurons and a lower degree of morphological differentiation compared to other vertebrates. Therefore, amphibians are especially qualified for investigations on anatomical structures and basic physiological processing. Prey-catching behaviour in salamanders is predominantly guided by visual perception. The midbrain tectum is the main centre for visual processing and initiates prey-catching behaviour. Recent investigations have revealed that the dorsal thalamus is critically involved in the selection of visual objects. Lesion of the dorsal thalamus results in inhibition of orienting behaviour and in disinhibition of spike pattern of tectal visual neurons. The inhibition of behaviour leads to the incapability of a salamander to select one out of two different prey-objects presented simultaneously on a screen. The goal of the present study is to analyze the neuronal connectivity between the optic tectum and the dorsal thalamus. Therefore, the connectivity is studied by tracing experiments using biocytin and/ or TMR. The neurotransmitters involved in these connections are visualized immunohistochemically. The investigations yield reciprocal connections between the dorsal thalamus and the tectum. A direct tecto-thalamic loop is postulated and the relationship to the dis/ inhibition phenomena is discussed.

Rubrik: Neuroanatomie/Neurobiologie

Titel:Ectonucleotidases and purinergic signaling in the hypophysis

Autoren: Homola M.(1), Fischer C.(1), Meissl H.(2), Zimmermann H.(3), Korf H-W.(1),

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### Abstract:

Adenosine is an important neuromodulator, which has been shown to stimulate prolactin production in the hypophysial pars distalis (PD). Adenosine receptors are expressed in the hypophysial pars tuberalis (PT) and PD. Production of adenosine can occur via the degradation of extracellular ATP by ectonucleoside triphosphate diphosphohydrolases (E-NTPDases) and ecto-5'-nucleotidase (ecto-5'). To investigate whether adenosine is produced from ATP in the PT and PD we examined the activity and expression of these enzymes. Real-time measurements with biosensors demonstrated the conversion of exogenously applied ATP to adenosine and its derivatives in PT and PD living tissue, implying the ability to produce extracellular adenosine from ATP in these regions. By means of lead phosphate enzyme histochemistry, ATPase and ADPase catalytic activity was observed in the PT and PD. AMPase activity was not detected in the PD and only moderately in the PT. By use of radioactive in situ hybridization in brain and PD of C3H (melatoninproficient) and C57BI (melatonin-deficient) mice, sacrificed at four different time points, mRNA expression of E-NTPDase 1, 2, 3 and ecto-5' was located to specific brain regions and to the hypophysial lobes. All enzymes excluding NTPDase2 were expressed in the PD with NTPDase3 being the dominant form. In the PT only NTPDase1 was detected, showing higher expression in the C3H mice PT at CT18 and lower expression in the C57BI mice PT at CT12. Since ecto-5' is not expressed in the PT, the conversion of AMP to adenosine presumably occurs via enzymes located in its close proximity.

Rubrik: Neuroanatomie/Neurobiologie

Titel:Annexin A1, a signaling molecule from folliculo-stellate cells in the pituitary, is regulated by endocannabinoids

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### Abstract:

Endocannabinoids are signaling molecules from the hypophysial pars tuberalis (PT) which influence the hormonal output of the pars distalis (PD) via not yet clarified mechanisms. Preliminary results suggest that endocannabinoids may target folliculo-stellate (FS) cells of the PD, which express cannabinoid receptors of the CB1 type. The FS cells are located adjacent to endocrine cells of the PD (e.g., lactotrophs) and often ensheath them. The FS cells produce various signaling molecules including NO and annexin A1. Therefore we hypothesized that the PT-derived endocannabinoids may indirectly influence endocrine cells in the PD via acting upon FS cells. To corroborate this hypothesis we investigated the rodent PD and immortalized cell lines (for FS cells: TtT/GF and Tpit/F1, for corticotrophs: AtT-20/D16-F2, for lactotrophs: GH4/C1) by means of immunocytochemistry, immunoblotting and in situ hybridization. FS cells in the PD and FS cell lines expressed the CB1 receptor and annexin A1. Immunoblotting showed that stimulation of the FS cell lines with 2arachidonoylglycerol resulted in a significant and dose-dependent increase in annexin A1 protein levels. Annexin A1 binding sites (Fpr-rs1) were detected by means of in situ hybridization in distinct PD cells and in the corticotroph and the lactotroph cell lines. Our data provide further evidence that PT-derived endocannabinoids elicit their function via acting upon FS cells, in which they influence the annexin A1 levels. As a next step we shall investigate the impact of annexin A1 on production and secretion of prolactin in GH4/C1.

Rubrik: Neuroanatomie/Neurobiologie

Titel:Impact of mitochondrial DNA variations on dendritic spines in a mouse model of betaamyloidosis

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### Abstract:

In order to investigate the impact of conplastic mitochondrial DNA variations on the pathology of beta-amyloidosis as seen in Alzheimer's disease (AD), APP-transgenic mice (APP/PS1, referred to as AD-BL6) were crossed with different mitochondrial conplastic mouse lines with C57BL/6 genomic background (mtAKR/J, mtFVB/N and mtNOD/LtJ) to obtain APP-transgenic mice that differ only in th

eir mitochondrial DNA (conplastic mtDNA mice). Aside from the typical amyloid pathology, we observed a dramatic loss of hippocampal dendritic spines of about 70% in AD-BL6xmtAKR/J at an age of 250 days as compared to age-matched AD-BL6 control mice. However, young (125 days old) AD-BL6xmtAKR/J did not show reductions in spine densities. To detect whether the reduction seen in aged AD-BL6xmtAKR/J mice is only related to this special mouse line, we analyzed hippocampal spine densities also in AKR/J and C57BL/6xmtAKR. The results obtained so far, indicate that only AD-BL6xmtAKR/J mice display such a remarkable loss of spines in the dentate gyrus and the CA1-region. In future experiments we will analyze whether this dramatic loss of dendritic spines may be compensated by the addition of shaft synapses and we will analyze whether the spine loss seen in 250 days old AD-BL6xmtAKR/J translates into physiological changes (e.g. LTP) or behavioural changes (open field, hole board, dark-light box, Morris water maze). Moreover, we intend to use electron microscopy to study spines in young and old AD-BL6xmtAKR/J mice as compared to control mice to see whether ultrastructural differences can even be found before light-microscopic changes have emerged.

Rubrik: Neuroanatomie/Neurobiologie

Titel:Deletion of the p75 neurotrophin receptor: effects on the hippocampal formation in aged and adult mice

Autoren: Dokter M.(1),Busch R.(1),von Bohlen und Halbach V.(1),Poser R.(1),Vogt M.(2),Gass P.(2),von Bohlen und Halbach O.(1),

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#### Abstract:

The p75 receptor binds all neurotrophins with low affinity and depending on co-signaling with the high affinity trk-receptor and the availability of neurotrophins, p75 can signal survival, differentiation but also cell death. Since the p75 receptor is highly expressed in the dentate gyrus (DG) and since the DG is a structure capable of spinogenesis and adult neurogenesis, we tried to identify possible roles of the p75 receptor in these processes. Concerning a possible importance of the p75 in apoptosis, we used active-caspase3 as antigen. Since the hippocampus is highly innervated by the cholinergic system, we evaluated the involvement of p75 on the density of the cholinergic fibers in the hippocampus. To determine possible alterations of the gross morphology we analyzed the volume and the thickness of the layer of the hippocampus. To evaluate whether there is there is an impact of aging in one of the mentioned processes we compared adult (6 month) and aged (20 month) p75 mice and there respective controls. To investigate whether p75 deficiency has an impact upon behavior, we designed a series of behavioral tests, including Open Field, T-Maze and Morris Water Maze. Together, we could show that deficiency for p75 affects some hippocampus dependent behavioral tasks and induces specific morphological alterations in the hippocampus. Moreover we could demonstrate that some of these alterations vanish during aging, while some do not.

Rubrik: Neuroanatomie/Neurobiologie

Titel:Calcium activated potassium channel expression during human IPS cell-derived neurogenesis

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### Abstract:

The family of calcium activated potassium channels of low and intermediate conductance, known as SK channels, consists of four members (SK1-4). These channels are broadly expressed throughout the organism and are involved in various cellular processes, such as the afterhyperpolarization in excitable cells but also in differentiation processes of different tissues. To date, the role of SK channels in developmental processes remains marginally investigated, although it is well accepted that cell differentiation and maturation affect the expression patterns of certain ion channels. Recently, several studies delineated the influence of SK channel expression and their respective activity on cytoskeletal reorganization in neural and pluripotent stem cells and regulation of cell fate determination towards the cardiac lineage in human and mouse pluripotent stem cells. Herein, we have now analyzed SK channel expression patterns and distribution in various stages of human induced pluripotent stem cell-derived neurogenesis particularly focusing on undifferentiated iPS cells, neural progenitors and mature neurons. All family members could be detected starting already at the iPS cell level and were differentially expressed during the subsequent maturation process. The abundance and specific regulation of SK channel expression during iPS cell differentiation indicates distinct roles of these ion channels not only for the cardiac but also for neuronal cell differentiation and in vitro neurogenesis.

Rubrik: Neuroanatomie/Neurobiologie

Titel:Sex steroid hormones regulate astroglia and microglia function after hypoxia

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Abstract:

Hypoxia causes a stepwise cascade of neuropathological processes including neuroinflammation and oxidative stress which provoke neuronal damage and subsequent neurological deficits. 17B-estradiol (E) and progesterone (P) have a neuroprotective function in acute brain ischemia. Using a transient occlusion model of the middle cerebral artery, we have shown that E and P reduce the infarct area by 60% in a long-term survival study and prevent behavioural deficits. An intriguing aspect was that gonadal steroids reduced the number of microglia and microglia-related markers in the penumbra. Besides microglia, penumbral astroglia are coevally regulated by E and P. To clarify whether both glia types are direct steroid targets, we established an in vitro model consisting of a hypoxia chamber flooded with inert nitrogen to replace oxygen. The murine microglia-like cell line BV-2 and primary rat microglia/astroglia were maintained for 3h under hypoxia. All cell types expressed different sets of classical and membrane steroid receptors. Oxygen deprivation increased the expression of pro-inflammatory markers (iNOS, Hif1?, COX2) and chemoattractant molecules (IL-6, CCL2/5) in microglia and astroglia, respectively. These effects were predominately inhibited by sex steroids. Hypoxia reduced the phagocytotic activity of microglia which was reversed by E and P resulting in a switch from a pro-inflammatory to an anti-inflammatory phenotype. Our data suggest that microglia attraction-activation and astroglia regulation occur side by side and that hypoxia-related neuroinflammation is dampened by E and P. Anti-inflammatory effects of gonadal steroids might thus directly be mediated through hormone-microglia interactions but coevally require the interaction with astroglia.

Rubrik: Neuroanatomie/Neurobiologie

Titel:Magnetic resonance imaging of the human brainstem in vivo at 7 Tesla

Autoren: Deistung A.(1), Biedermann U.(2), Schäfer A.(3), Schweser F.(1), Güllmar D.(1), Trampel R.(3), Turner R.(3), Reichenbach J.(1),

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### Abstract:

The human brainstem plays an important functional role in the human brain and is composed of a multitude of axonal nerve fibers and nerve nuclei. We explored the potential of highresolution magnetic resonance imaging for depicting the intricate anatomy of the human brainstem in vivo. To this end, multiple image contrasts including nuclear relaxation, magnetic susceptibility, and molecular diffusion were acquired and created by using a 7 T MRI system: T2-weighted images, quantitative maps of longitudinal relaxation (R1-maps) and effective transverse relaxation (R2\*-maps), magnetic susceptibility maps, as well as direction-encoded track density images. These images and maps were compared with histological stains and anatomical atlases to identify nerve nuclei and nerve fibers. Among the investigated contrasts susceptibility maps displayed most structures of the brainstem. The contrasts of the R1-maps and T2-weighted images were rather homogeneous compared to R2\*, magnetic susceptibility and track density images. R2\*, susceptibility and track density images clearly displayed a multitude of smaller and larger fiber bundles. Furthermore, several brainstem nuclei were identifiable in sections covering the pons and medulla oblongata: the spinal trigeminal nucleus (V) and the reticulotegmental nucleus based on magnetic susceptibility maps, as well as the inferior olive on R1, R2\*, and susceptibility maps. The substantia nigra and red nuclei were visible on all investigated image contrasts. High-resolution, multi-contrast MR imaging of the brainstem at 7 Tesla is a versatile tool to non-invasively assess the individual anatomy and tissue composition of the human brainstem.

Rubrik: Neuroanatomie/Neurobiologie

Titel:Strong upregluation of small heat shock proteins in the ischemic rat brain

Autoren: Bartelt-Kirbach B.(1), Beyer C.(2), Dang J.(2), Golenhofen N.(1),

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### Abstract:

Small heat shock proteins (sHsps) are molecular chaperones protecting cells from various adverse conditions as heat shock or ischemia. HspB1/Hsp27 and HspB5/alpha-B-crystallin are known to be cytoprotective after brain ischemia. However, little is known about the other nine members of the sHsp family. Thus, we were interested which of the sHsps are upregulated in the rat brain after cerebral ischemia. We investigated the mRNA expression of all eleven sHsps in penumbra tissue of the cerebral cortex using a transient middle cerebral artery occlusion model (1h occlusion and 23h recovery) via real-time RT-PCR and compared it to the contralateral hemisphere. The expression level in healthy cortex was in the same range as measured by us previously. HspB1, B5, B6 and HspB8 showed expression levels between 0.8 and 13.6 percent of the reference genes whereas HspB3, B7, B9 and B11 were expressed at a very low level (below 0.3 percent). HspB2, B4 and B10 were not expressed. 23 hours after MCAO HspB1 was upregulated 79-fold, HspB5 4.8-fold and HspB8 6.3-fold. The sHsps expressed at a low level, HspB3, B7 and B9, showed an induction around 3-fold. Interestingly, HspB4, whose expression level was below threshold in untreated brain tissue, was increased 44.4-fold after ischemia. mRNA levels of HspB2, B6, B10 and B11 did not change. This shows that not only HspB1 and HspB5 but several other sHsps seem to be involved in cytoprotection after ischemia.

Rubrik: Neuroanatomie/Neurobiologie

Titel:CNTF and LIF promote the growth of adult neural stem cells in mitogen-free cultures

Autoren: Reiser J.(1), Chakrapani B.(1), Hofmann H.(1), Kirsch M.(1),

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Abstract:

So called neurosphere cultures, prepared from the SVZ of mouse brains, represent the standard in vitro model for neural stem cells (NSCs) that is widely used to study mechanisms controlling adult neurogenesis. However, the spheres, requiring the presence of mitogens (EGF and/or bFGF) for growth, are heterogeneous in their cellular composition. Containing a small fraction of true stem cells (around 1%) and an excess of rapidly proliferating progenitors as well as neural and glial precursors, they appear to reproduce to some extend the in vivo neurogenic/gliogenic cell lineage. Since the cytokines ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF) have been shown to promote the maintenance and/or self renewal of NSCs, we wanted to examine if the cytokines could be instrumental to establish more homogeneous NSC cultures. For this, cells dissociated from normal neurospheres were grown in culture medium lacking the mitogens EGF and bFGF and the effects of CNTF and LIF were monitored. In the absence of the mitogens, a very small population of cells survived in a cytokine-dependent manner. These surviving cells showed stem cell characteristics. They proliferated very slowly and formed neurospheres only after long culture periods. After re-adding EGF and bFGF they were able to restore normal neurosphere cultures. As compared to normal neurosphere, the expression of the immunocytochemical stem cell marker CD15 and of mRNAs for typical stem cell transcription factors, like Sox2, c-Myc, Nanog, Klf4 and others, was massively upregulated. The results demonstrate the existence of cytokine-dependent cells with stem cell characteristics in SVZderived NSC cultures and indicate the possibility to establish a culture system of "true" neural stem cells.

Rubrik: Neuroanatomie/Neurobiologie

Titel:Differentiated NSC-34 motoneuron-like cells as experimental model for cholinergic neurodegeneration

Autoren: Böhm J.(1), Maier O.(1), Brück S.(1), Dahm M.(1), Beyer C.(1), Johann S.(1),

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#### Abstract:

Alpha-motoneurons are mainly affected in amyotrophic lateral sclerosis (ALS). Morphological and physiological degeneration of this neuronal phenotype is characterized by a marked decrease of neuronal and cholinergic markers. The motoneuron-like cell line NSC-34 is a hybrid cell line produced by the fusion of neuroblastoma cells with mouse motoneuronenriched primary spinal cord cells and regarded as a valid model to study motoneuron degeneration. In order to effectively employ NSC-34 cells for a prospective study of cholinergic neurodegeneration, a reliable differentiation protocol was established by serum deprivation and treatment with all-trans retinoic acid. Maturation was characterized by neurite growth and expression of general neuronal and specific cholinergic markers, including MAP2, GAP-43, and ChAT. Subsequently, we used differentiated NSC-34 cells to analyze early degenerative responses following exposure to various neurotoxins (H2O2, TNF-alpha, glutamate). Susceptibility to toxin-induced cell death was determined by means of morphological changes, expression of neuronal markers, and the ratio of pro-(Bax) to anti-(Bcl-2) apoptotic proteins. After differentiation, NSC-34 cultures were composed of a stable mixture of differentiated and undifferentiated cells. These cultures responded to low doses of neurotoxins with increased cell death rates mainly affecting undifferentiated cells. In contrast, differentiated cells preponderantly tolerated these treatments and showed no obvious signs of degeneration. The unequal vulnerability of differentiated and undifferentiated NSC-34 cells to neurotoxins is a key characteristic of NSC-34 cells and needs to be considered in neurotoxic studies. Nonetheless, differentiated NSC-34 cells provide a suitable model to investigate molecular events of neurodegeneration.

Rubrik: Neuroanatomie/Neurobiologie

Titel:Developmental expression of synaptopodin in the mouse visual system

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### Abstract:

Synaptopodin (synpo) is an actin-associated protein, whose short isoform is expressed in the spine apparatus in dendritic spines of cortical and hippocampal neurons. Synpo-deficient mice develop no spine apparatus and show a reduction of synaptic plasticity, indicating shortcomings in learning and memory. Synpo is also an essential component of the cisternal organelle, located in the axon initial segment (AIS). Both, the spine apparatus and the cisternal organelle are composed of stacked smooth endoplasmic reticulum. A functional role of synpo in Ca2+-trafficking and in denervation-induced homeostatic synaptic plasticity has been implied.

In this study, we investigated developmental expression of synpo in visual cortex and retinal neurons from mouse embryonic to adult stages by immunohistochemistry, qPCR and Western Blot.

Synpo protein is not expressed in dendritic spines or the AIS during embryonic and early postnatal stages. It first appears in dendritic spines at P7 and in the AIS at P10, and is then maintained throughout adulthood. In the retina, synpo RNA is expressed much earlier beginning at E16.5 and is dynamically regulated with a 2.5-fold expression at P1 and P14 compared to embryonic expression. Levels from P21 until adult stages show then a continuous decrease. Confocal microscopic analysis of synpo protein expression in the retina showed an initially location to retinal ganglion cells somata during late embryonic and early postnatal stages. Synpo is then redistributed to the nerve fiber layer at P21, where it resides throughout adulthood. Taken together, we demonstrate a dynamically expression of synpo in the mouse visual system.

Rubrik: Neuroanatomie/Neurobiologie

Titel:Intracerebral microinjection of lysophosphatidylcholine as a model to study early microglia/macrophage activation

Autoren: Hoeflich K.(1), Nurtaeva K.(1), Beyer C.(1), Kipp M.(1),

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### Abstract:

Experimental demyelination due to the injection of gliotoxic agents into the CNS has provided powerful models for studying the biology of remyelination. Intracerebral microinjection of the membrane detergent lysophosphatidylcholine (LPC) results in a rapid recruitment of peripheral immune cells with subsequent activation of microglia/macrophages and demyelination. This demyelination is closely followed by remyelination. Activated microglia and recruited monocytes play a critical role in removal of myelin debris through phagocytosis activity and, thus, are pivotal for effective myelin repair. During phagocytosis, however, microglia should survive under the harmful condition of self-producing ROS and pro-inflammatory mediators.

In the current project we aim to analyze the extent of lesion formation as well as it's three dimensional expansion and to correlate these parameters to the temporal dynamics of microglia/macrophage activation and recruitment. Furthermore, we address the relevance of the unfolded protein stress response (UPR) for macrophage function.

2 days after stereotactic LPC application into the corpus callosum, intralaminar myelin edema was paralleled by early microglia/macrophage mobilization and oligodendrocyte dystrophy. 5 days p.i. lesions displayed clear characteristics of active demyelination, namely the presence of foamy macrophages, early myelin degradation products and myelin loss. Interestingly, early activation of an UPR was characteristic for microglia/macrophages during both, the recruitment and effector phase. Effects of the UPR-modulator salubrinal on microglia/macrophage activation are currently analyzed.

We are clearly able to show that focal intracerebral LPC-injection serves as a convenient and straight forward model to study dynamics of microglia/macrophage recruitment.

Rubrik: Neuroanatomie/Neurobiologie

Titel:Histone modifications during cerebral cortex development

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### Abstract:

Recent advances in neuroscience support the importance of epigenetic modifications, such as methylation, acetylation and ubiquitylation of histones, for function and development of the central nervous system. Histone modifications are known to regulate gene transcription and they can be inherited to subsequent cell generations.

In this context our aim is to determine histone modification marks that are associated with restricted cell fate of neuronal progenitors in the developing cerebral cortex of the mouse. We speculate that regional as well as temporal identities of progenitors are correlated with specific patterns of modified histones and that these patterns are necessary for proper specification of cellular identity within a certain cortical area.

To decide upon which histone modification we are using for ChIP-analyses, we studied different histone methylation marks for spatio-temporal differences using Western Blots. Thus far, we detected expression changes between rostral and caudal cortex for H3K4 trimethylation (H3K4me3), a modification linked to transcriptional activation, and for H3K27me3, a mark implicated with transcriptional repression of target genes. Furthermore, we observed altered Kmt2d mRNA expression levels between the cortical regions. KMT2D is a member of a large methyltransferase complex known to methylate H3K4.

Therefore we started our analyses with isolation of rostral and caudal cortices of E14.5 embryonic mouse brains and performed ChIP-Seq with regard to different H3K4me3 patterns expected comparing the two regions of the murine cerebral cortex.

Rubrik: Neuroanatomie/Neurobiologie

Titel:Organotypic slice cultures of human glioblastoma show individual susceptibility to radioand chemotherapeutic treatment

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### Abstract:

In this preliminary study, we describe how human WHO grade IV GBM specimens obtained from resected material can be processed into organotypic slice cultures. These cultures allow for direct observation, application of temozolomide (TMZ), and irradiation in a time frame of up to two weeks. Differently treated slice cultures were processed for histological analysis including HE- staining, detection of proliferation (Ki-67), apoptosis/cell death (activated Caspase 3, PI), DNA double-strand breaks (DSBs, gammaH2AX), and neural subpopulations. First clinical trials employ irradiation with carbon ions for the treatment of GBM patients, but most effective treatment combinations remain to be identified. Therefore, we developed an approach to expose GBM slice cultures to 12C and X-rays. Treatments resulted in activation of Cas3, inhibition of proliferation, cell loss and induction of DSBs. In line with clinical observations, individual tumors differed significantly in their susceptibility to TMZ independent from MGMT promoter methylation status. GBM slice cultures provide a unique tool to explore susceptibility of individual tumors for specific therapies, thus potentially allowing more personalized treatments and helping explore mechanisms of tumor resistance and strategies to overcome them.

This study is funded by BMBF

Rubrik: Neuroanatomie/Neurobiologie

Titel:Deficiency of the mitochondrial rhomboid protease Parl causes a secondary lamination defect of the outer retina

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### Abstract:

Presenilin - associated rhomboid – like protease (PARL) is an integral enzyme of the inner mitochondrial membrane situated at the crista neck region. PARL deficiency causes dysregulation of crista junction diameter, augmenting a net flow of pro-apoptotic factors towards intermembrane space and cytosol, and increasing apoptosis predominantly in lymphatic tissues. Major aspects of PARL function may be exerted via an interaction with OPA1. OPA1- inactivating mutations are responsible for dominant optic atrophy in humans, characterized by a selective loss of ganglion cells.

In PARL – deficient mice, retinal morphology is normal during early postnatal life. Photoreceptor ectopia develop predominantly in the outer plexiform layer, starting around the 8th postnatal week, while only rarely cells migrate towards the subretinal space. At the same time, photoreceptors feature abated outer segments and an altered topography of connecting cilia. We do not see increased apoptosis in any of the retinal layers. Most notably, ganglion cell layer and optic nerve develop only limited defects, arguing against a simple upstream action of PARL on OPA1 in the eye.

Taken together, our data show that PARL deficiency causes a novel type of adult-onset retina degeneration distinct from that caused by OPA1 inactivation, focussing on photoreceptor positioning rather than ganglion cell survival.

Rubrik: Neuroanatomie/Neurobiologie

Titel: Cytokin storm induced by superagonistic anti-CD28 in organotypic slice cultures of human tonsils mimics in vivo effects

Autoren: Kallendrusch S.(1), Semmler L.(2), Bartholomaeus P.(2), Hünig T.(3), Dietz A.(4), Bertolini J.(5), Kalinke U.(2), Bechmann I.(1),

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Abstract:

Application of the superagonistic anti-CD28 monoclonal antibody (TGN1412) caused severe toxic shock syndrome induced by a "cytokine storm" in all six study participants upon firsttime delivery of the drug. Since TGN1412 was well tolerated in primates, the "London tragedy" highlighted the relevance of species differences and the need for human test systems to evaluate and predict effects of novel biological drugs. Here, we established slice cultures derived from tonsillectomy as a tool to investigate immune cells in their organotypic environment. The follicular structure of tonsils and the proliferative activity was maintained in culture up to day five. CD3 positive T-cells were located perifollicularly while CD20 positive B-lymphocytes were observed in the germinal centers of the follicles. To investigate the relevance of tonsil slice cultures for drug approval we applied TGN1412 to tonsil slice cultures. TGN1412 induced T cell proliferation and cytokine production (IL-17, TNF-, IFNcompared to control cultures similar to conditions as observed in the six volunteers. Cell culture experiments from tonsillectomy derived immune cells and blood derived PBMC of the same donors further clarified that TGN1412 together with B lymphocytes (low affinity FcgRIIB) stimulated T cell reactivity, whereas monocytes expressing the high affinity FcgRIII CD64 were less potent to do so. The obtained results demonstrate that tonsillectomy derived slice cultures serve as an adequate model to study the effect of novel biological drugs and therewith to predict potential risks for clinical studies. Funded by BMBF (to I.B. & U.K.)

Rubrik: Neuroanatomie/Neurobiologie

Titel:The master scaffold ankyrin G in neuronal polarity and axon initial segment maturation

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### Abstract:

Neurons are highly polarized cells, as reflected by the anatomical distinction between their somatodendritic and axonal domains. Neuronal polarity, which is a key feature not only for normally functioning neurons, also plays an important role in human neurodegenerative disease. In mature neurons, the somatodendritic compartment borders on the axon initial segment (AIS). Several studies indicate a role of the AIS as a physical barrier supporting structural and functional distinction between the cellular compartments. At the molecular level, the AIS is composed of a protein complex consisting of the spectrin/actin-membranecytoskeleton and its binding partner, the membrane scaffold ankyrinG (ankG). AnkG recruits a variety of proteins including voltage-gated sodium channels to the AIS and is therefore essential for AIS formation. Intriguingly, the developmental localization of ankG and establishment of the AIS in-vivo remains elusive. Therefore, we initiated a comprehensive immunohistochemical, confocal study aiming to determine the precise timecourse of AIS formation and maturation. We show that ankG is expressed in cortical neurons during embryonic development beginning at E12.5. An early ubiguitous, cytoplasmatic expression changes to an exclusive axonal expression in the early postnatal phase. Interestingly, migrating Cajal-Retzius cells in the marginal zone of E14.5-E20.5 mice exhibit somatic ankG immunoreactivity and clearly distinguishable AIS. In order to provide a comprehensive characterization of AIS maturation during the development of the cortex and cerebellum, we now aim at combining qualitative imaging data with PCR and Western blot assays. The insights gained from this study should help to further our understanding of neuronal polarity.

Rubrik: Neuroanatomie/Neurobiologie

Titel:Morphological analysis of pyramidal neurons in the lateral and basolateral nucleus of the amygdala in tryptophan hydroxylase 2 (TPH2) deficient mice

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### Abstract:

Serotonin (5-HT) plays a central role in brain development and in the modulation of emotional behavior. Tph2 is the rate-limiting enzyme for neuronal 5-HT synthesis. Mice deficient for Tph2 (Tph2-/-) are viable and morphologically normal but exhibit delayed development. Adult Tph2-/- mice display extremely low levels of brain 5-HT and preliminary studies indicated differences in emotional behavior.

Functional magnetic resonance imaging (fMRI) documented significantly lower perfusion measured by continuous arterial spin labeling (CASL) representing decreased baseline activity in all analysed brain regions including the amygdala in Tph2-/- compared to wildtype (WT) mice.

After fMRI analyses, the brains of the tested animals were subjected to Golgi impregnation, and morphological studies were carried out on pyramidal neurons in the lateral (La) and basolateral (BL) amygdaloid nuclei. Quantitative analyses documented that the length of dendritic material and the total number of spines on apical dendrites of La pyramids was significantly lower in Tph2-/- compared to WT animals. Detailed analyses of the entire apical dendritic trees of these neurons showed significantly decreased spine densities on 4th order branches. Genotype differences were lacking for branching points of La pyramidal apical dendrites, and for all analysed parameters of basal dendrites and of BL pyramidal neurons. The data indicate that low or absent 5-HT during development and in adulthood causes subtle morphological differences of projection neurons of the input region of the amygdala which may be associated with altered amygdalar activity and reactivity.

Rubrik: Neuroanatomie/Neurobiologie

Titel:The role of receptor tyrosine kinase inhibitors in neuroprotection and anti-tumor signaling

Autoren: Hatipoglu G.(1),Hock S.(1),Weiss R.(1),Buchfelder M.(1),Eyüpoglu I.(1),Savaskan N.(1),

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### Abstract:

Sunitinib (SU011248; Sutent) is a rationally designed small molecule inhibitor in clinical use directed against tumor angiogenesis. Sunitinib inhibits various receptor tyrosine kinases (panb RTK inhibitor) including the vascular endothelial growth factor receptors (VEGFRs) types 1 and 2 (FLT1 and FLK1/KDR), platelet-derived growth factor receptors (PDGFR- $\alpha$  and PDGFR-B), the stem cell factor receptor c-KIT, and FLT3 and RET kinases. Inhibition of these RTKs block many VEGFA/B signaling and thereby affecting many processes involved in tumor growth, progression, metastasis, and angiogenesis. We demonstrate that Sunitinib operates in addition as a neuroprotective agent. We established a cell death assay and monitored both sunitinib and glutamate as a neurotoxic agent. Firstly, cells were stressed with high-dose glutamate ( $50\mu$ M) and then treated with sunitinib. In addition to that, we analyzed whether sunitinib can interfere with glutamate-induced cell death. Cells were stressed with glutamate at 10 mM concentration and subsequently treated with sunitinib. We found that sunitinib alleviated glutamate-induced neuronal cell death. In conclusion we could show that Sunitinib is toxic to glioma cells and at the same time prevents neuronal cell death in vitro. Further investigations focuses now on ex vivo brain slice experimentations and its application as a chemotherapeutic agent against malignant brain tumors.

Rubrik: Neuroanatomie/Neurobiologie

Titel:Prosapip1 - a novel candidate gene for autism?

Autoren: Weis T.(1), Schmeisser M.(1), Grabrucker A.(1), Boeckers T.(1),

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Abstract:

Many genes implicated in autism might converge on a single pathway present at excitatory glutamatergic synapses, among them, for example, interaction partners centered around synaptic ProSAP/Shank scaffolds such as Neurexin-Neuroligin protein complexes. In line with this, ProSAP-interacting protein1 (ProSAPiP1) was found to be deleted or mutated in several patients suffering from Autism Spectrum Disorders (ASD). As previously reported by our workgroup, ProSAPiP1 is a post-synaptic density protein that belongs to the Fezzin family of proteins and that links SPAR to the scaffolding protein ProSAP2/Shank3. Here, we performed immunohistochemistry experiments in primary rat hippocampal neurons using a viral system to investigate the effects of ProSAPiP1 and two different ProSAPiP1 point mutations on dendritogenesis and synaptogenesis. The results show that these point mutations that are located within a protein-protein interaction domain, seem to generate a more pronounced phenotype when compared to wildtype ProSAPiP1. We therefore propose a gain-of-function-effect for these mutations that might be at the basis of the pathomechanisms triggered in those individuals with corresponding mutations.

Rubrik: Neuroanatomie/Neurobiologie

Titel:Gabaergic innervation of the ciliary ganglion in rats

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### Abstract:

In vertebrates the ciliary ganglion (CG) contains postganglionic parasympathetic neurons that mediate pupillary constriction and lens accommodation by their projections to the sphincter and ciliary muscle, respectively. The CG neurons are activated from cholinergic preganglionic neurons in the Edinger-Westphal nucleus (EWpg). Recent studies in monkey and avian identified a CG neuron subpopulation that is in addition contacted by GABAergic terminals originating from the supraoculomotor area in monkey.

To characterize the GABA-recipient postganglionic CG neurons by their innervation targets, two pigmented rats received tracer-injections (non-toxic choleratoxin subunit B or wheat germ agglutinin) into the anterior (iris sphincter) and posterior (ciliary muscle) eye chamber, respectively. The analysis revealed that virtually all CG neurons were tracer-labelled after posterior chamber injections compared to at least 75% after anterior chamber injections. Double-immunofluorescence revealed that terminals containing the GABA-synthesizing enzyme, glutamate decarboxylase (GAD), were associated with both, tracer-labelled and non-labelled neurons.

In a parallel study the GABA-recipient CG neuron populations of three pigmented wildtype and three albino rats were compared, since albinos are known to exhibit accommodation deficits. The quantitative analysis revealed that approximately 59,1 % of all CG neurons in wildtype, but only 12,7 % in albino rats receive a dense supply of GAD-positive terminals. These results did not reveal a specific association of GABAergic input to either pupillary constriction or ciliary muscle related CG neurons. Nonetheless the small population of GABA-recipient CG neurons in the albino rat suggests that the GABAergic CG input may mainly contribute to pathways for lens accommodation.

Support: Graduiertenkolleg 1091

Rubrik: Neuroanatomie/Neurobiologie

Titel:Oligodendrocyte stress in the cuprizone model is paralleled by selective expression of ATF3 and CHOP

Autoren: van Heuvel Y.(1), Clarner T.(1), Victor M.(1), Goldberg J.(1), Gerritsen W.(1), Beyer C.(1), Kipp M.(1)

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### Abstract:

Oligodendrocyte loss is a hallmark of Multiple Sclerosis (MS) lesions. Such inflammatory foci are characterized by proliferation and invasion of microglia and astrocytes. Why oligodendrocytes die, whereas other glia cells proliferate, is unknown. A unique characteristic of oligodendrocytes is the high protein synthesis rate. In case protein misfolding occurs, cells activate an "unfolded protein response" (UPR). Key players of the UPR are the transcription factors CHOP and ATF3. We recently identified these factors to be highly induced in the cuprizone model of MS. I investigated the cellular source of CHOP and ATF3 in short term cuprizone fed mice.

Immunofluorescence stainings against cellular markers Iba1, APC, GFAP and S100 beta were performed. Anti-Caspase 3 staining was used to identify apoptotic cells. Double stainings against cellular markers and either Caspase 3, CHOP or ATF3 were used to identify apoptotic cells and to characterise the cellular source and location of the transcription factors. Cuprizone treatment induced oligodendrocyte apoptosis and microgliosis within the corpus callosum, whereas astrocyte numbers and morphology was unchanged.CHOP and ATF3 were found to be exclusively expressed by oligodendrocytes. Both transcription factors were located in the nucleus, indicating that they are functionally active. Our data indicate a critical role for UPR in selective oligodendrocyte loss. Further studies will have to show the functional role of ATF3 and CHOP in lesion development and progression.

Rubrik: Neuroanatomie/Neurobiologie

Titel:µ-opioid receptor (MOR) localization in peripheral sensory axons: a novel target for regional anesthesia

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### Abstract:

Selective targeting of peripheral nociceptive neurons in regional anesthesia is a challenging clinical goal. Perisciatic injections of high concentrations of the lipophilic MOR agonist fentanyl moderately increase nociceptive thresholds. The analgesic effect can be blocked by MOR antagonists and is significantly enhanced using coinjections of low concentrations of fentanyl or of the hydrophilic MOR agonist [D-Ala2, N-MePhe4, Gly-ol]-enkephalin (DAMGO) together with hypertonic saline (HTS) or metalloproteinase 9 hemopexin domain (MMP9-PEX). These findings indicate the necessity of an "enhancing" treatment to open perineural barriers and/or to increase axonal pools and membrane localization of functional MOR. This study was designed to analyze subcellular MOR localization in identified sciatic nerve fibers under normal conditions and to assess whether perisciatic injections of HTS or MMP9-PEX lead to alterations in axonal MOR content and/or subcellular localization. Multiple immunofluorescence labeling using various MOR antisera provided evidence for colocalization of MOR with markers for small sensory unmyelinated fibers in sciatic nerve specimens. Preimbedding immunogold electronmicroscopy indicated specific intraaxonal and plasmamembrane localization of MOR in unmyelinated fibers. Western blots of nerve extracts showed significantly higher MOR levels after perisciatic HTS injections and indicated increased membrane localization of MOR after HTS and MMP9 PEX treatments. The results confirm presence of MOR in peripheral axons of nociceptive neurons and suggest increased synthesis/axonal transport/membrane localization after treatment. Mechanisms of these alterations, effects of other enhancers and consequences for a possible regional opioid anesthesia need to be explored in the future.

Rubrik: Neuroanatomie/Neurobiologie

Titel: Similarities and differences in the neuronal wiring of the cerebellar nuclei of rodents and primates

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#### Abstract:

The deep cerebellar nuclei (DCN) undergo marked changes during the evolution of mammals and especially in primates. It is little known whether and how these morphological changes relate to changes in the cellular architecture of the DCN and also how these changes may lead to an added vulnerability in humans leading to neurodegenerative diseases such as Friedreich's ataxia. Addressing this question has been hindered by the limited scope of methods so far to compare the neuropil in phylogenetically diverse species. In this study we quantified the components of the neuropil of the rhesus monkey's deep cerebellar nuclei (DCN) to compare it to the results we have previously obtained in rats. We obtained 3D immunostained samples that were segmented, reconstructed and quantified with a 3D quantitative immunohistochemical method. The method was sufficiently fast enough to cover the different subregions of the DCN. The neuropil was either stained with an antibody against dendrites (microtubule associated proteins, MAP2a, b) or against the Purkinje cell axons (PCP2 antibody). We obtained 512 probes sampling from a total volume of 163.8mm3. We observed systematic differences in fiber length density, average fiber diameters and volume fraction within different parts of the DCN. In the rat the dendritic and axonal (PCP2) fiber diameters were highest in the phylogenetically older medial and anterior interposed nuclei (MN and AIN), whereas the fiber density was higher in the newer posterior interposed and lateral nuclei (PIN and NL). In the rhesus monkey a similar picture emerged with the exception that the dendritic diameters within the PIN were as large as those in the AIN and NM. This could indicate that the PIN neurons are scaled up in the larger rhesus monkey cerebellum. In contrast, this effect was not seen in the NL. This observation could point towards a cellular mechanism that leads to the gyral folding observed within the primate NL. A detailed mapping on the NL surface will allow us to further test this hypothesis.

Rubrik: Neuroanatomie/Neurobiologie

Titel: The evolution of the hominoid brain from a cerebellar perspective

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### Abstract:

Comparative anatomy of the cerebellum has yielded important insights into its organization and function. Recent studies have focused on the output structures of the cerebellum, the deep cerebellar nuclei (DCN) and their connections with the cerebral cortex. One of these nuclei is the dentate nucleus, so called because of its heavily folded appearance in humans. The dentate nucleus is also the largest single structure linking the cerebellum to the rest of the brain. The peculiar shape and large size of the human dentate nucleus has sparked a number of theories about the role of the cerebellum in human evolution and its contribution to non-motor functions. Detailed 3D exploration of the hominoid dentate shows that the dentate complexity already emerged in the arboreal and brachiating Gibbons and Orang-Utan. In view of recent studies proposing the cerebellum to be a state-estimator or sensory predictor, I propose that this helped these early apes to occupy a special niche: arboreal life style with a frugal energy metabolism. The gibbons and the Orang-Utan predecessor maximized arboreal skills to minimize energy expenditure. In these early stages of hominoid evolution (~15Mya) the challenge on the nervous system was likely more to develop skilled lowenergy-smooth upper-arm movements than social and executive prefrontal skills.

Rubrik: Neuroanatomie/Neurobiologie

Titel:Ligand and receptor are differentially sorted in human glioma cells

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### Abstract:

Fibroblast growth factor receptors (FGFR1-4) and their ligands (FGFs) are key regulators during development, regeneration and tumorigenesis. In human glioma cells, ligand binding activates intracellular signaling cascades promoting cell proliferation. This is followed by endocytosis of the receptor/ligand complex and shuttling of receptor and ligand to the degradation and recycling compartment. In this study, we overexpressed fluorescentlytagged FGFR1 in human glioma cells (U373), and treated the cells with naïve and fluorescently-labelled FGF2. The time course for activation of the Erk pathway was documented. The highest level of pERK was observed after 30 min corresponding to the highest level of FGF2 internalization. Exogeneously added FGF2-Cy5 was internalized into endosomal vesicles but translocated to the nucleus as well. Triple colocalization analysis of FGF2, FGFR1 and phosphorylated FGFR1 revealed a maximum of colocalization 1 h after treatment. After 2 h, 25% of FGFR1 containing vesicles represented early endosomes, 15% recycling endosomes and 40% of colocalized with the late endosomal compartment. Both, FGFR1 and FGF2, colocalized with transferrin, suggesting that not only the receptor is recycled back to the cell surface, but also the ligand in separate vesicles. TIRF experiments are currently performed to assess exocytosis events of receptor and/or ligand. Furthermore, we want to influence FGFR1/FGF2 trafficking by blocking recycling and promoting degradation. This gives us a better understanding of the dynamics of receptor/ligand compartmentalization and helps us to provide a basis for the development of new treatment strategies to reduce RTK-mediated cell proliferation and tumor growth (supported by ÖKH).

Rubrik: Neuroanatomie/Neurobiologie

Titel:Evidence for a role of neuregulin-1 and its signaling system ErbB2/ErbB3 in diverticular disease

Autoren:Barrenschee M.(1),Wedel T.(1),Hellwig I.(1),Harde J.(1),Egberts J.(2),Becker T.(2),Böttner M.(1),

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### Abstract:

Neuregulin-1 (NRG-1), an EGF-like growth and differentiation factor, is suggested to promote the survival/maintenance of the enteric nerve system (ENS), since deficiency in its receptor complex ErbB2/ErbB3 leads to colonic aganglionosis. As diverticular disease (DD) is associated with intestinal hypoganglionosis, the NRG-1-ErbB2/ErbB3 system was studied in patients with DD and controls. Localization of NRG-1, ErbB2 and ErbB3 was determined by dual-label-immunohistochemistry in colonic control specimens using the pan-neural marker PGP 9.5. The tunica muscularis from controls and patients with DD was assessed for mRNA expression levels of NRG-1. Site-specific gene expression of the NRG-1-ErbB2/3 system was determined in laser-microdissected (LMD) control samples harvested from circular/ longitudinal muscle and myenteric ganglia. Based on these results, site-specific mRNA expression levels of NRG-1 and ErbB3 were assessed in LMD samples from patients with DD. NRG-1, ErbB2 and ErbB3 immunoreactivity was observed in both muscle layers and in submucosal/myenteric plexus. Site-specific mRNA expression analysis localized the main source of NRG-1 and ErbB3 within myenteric ganglia. NRG-1 mRNA expression was downregulated in the tunica muscularis of patients with DD. In myenteric ganglia of patients with DD, NRG-1 and ErbB3 mRNA expression was decreased compared to controls. The findings provide evidence that NRG-1 and its receptors ErbB2/ErbB3 are expressed in the human ENS. The down-regulation of NRG-1 and ErbB3 in myenteric ganglia of patients with DD further support the hypothesis that intestinal hypoganglionosis previously reported in DD may be attributed to a lack of neurotrophic factors.

Rubrik: Neuroanatomie/Neurobiologie

Titel:Underlying mechanisms of selective oligodendrocyte loss in an MS animal model

Autoren: Clarner T.(1), Victor M.(1), Goldberg J.(1), Gerritsen W.(1), Beyer C.(1), Kipp M.(1),

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### Abstract:

Demyelination is a histopathological hallmark of Multiple Sclerosis (MS) lesions. Such inflammatory foci are characterized by proliferation and invasion of astrocytes and microglia, whereas oligodendrocytes die. The selective vulnerability of oligodendrocytes could be linked to the extraordinarily high protein synthesis rate of this cell type. Disrupted protein synthesis triggers a cellular response called "endoplasmatic-reticulum (ER) stress response" which aims to either cope with the stress or to eliminate the cells via apoptosis. A key player in this scenario is thought to be the transcription factor CHOP. In the cuprizone model of MS, we recently showed that an ER-stress response is active.

In this study, we aimed to elucidate the functional role of CHOP in selective oligodendrocyte loss. Furthermore, we investigated a possible crosslink of mitochondrial dysfunction and triggering of ER-stress in oligodendrocytes.

Gene expression studies, western blot and immunohistochemistry were performed in cuprizone-fed wt and CHOP-deficient animals. In separate experiments, wt animals were injected with salubrinal, a chemical modulator of ER-stress. An in vitro approach was conducted to analyse a possible crosslink between mitochondrial dysfunction and ER-stress responses.

Cuprizone fed CHOP-deficient animals displayed reduced numbers of apoptotic cells when compared to wt animals. Reduced expression of CHOP and lower apoptosis rate was observed in wt-mice treated with salubrinal.

Furthermore, we could show that the inhibition of mitochondrial functions leads to an induction of CHOP expression in cultured oligodendrocytes. Further studies will have to identify the signalling mechanisms involved in ER-mitochondria crosstalk.

Rubrik: 3.Neuroanatomie/Neurobiologie

Titel:XCT-mediated glutamate release promotes tumour angiogenesis

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### Abstract:

Malignant brain tumors are hallmarked by the induction of brain edema, neurodegeneration and pathological vasculature. Interference with the glutamate transporter xCT alleviates tumor-associated brain edema and reduces neuronal cell death. However, the interrelation of tumor-induced edema and angiogenesis has yet to be unraveled. Here we show that diminished glutamate secretion by xCT silencing normalizes tumor vasculature and gliomainduced angiogenesis. Consistent with this, xCT overexpressing gliomas corroborate this finding showing enhanced tumor vessels and increased cell death. Further, we demonstrate that glioma-derived product glutamate acts directly on endothelial cells via NMDA receptor activation. Glutamate induces endothelial sprouting and angiogenesis independently of vascular endothelial growth factor receptor 2. Thus, our data reveal that tumor-derived glutamate operates on endothelial cells as a VEGF-independent cue, thereby promoting angiogenesis and brain edema. Further, the functional tropism of glutamate signaling marks its NMDA-receptors as a therapeutic target for brain tumor angiogenesis and neurodegeneration.
Rubrik: Zellbiologie

Titel:Curcumin enhances chemosensitization of human colon cancer stem cells to 5-FU in high density cultures

Autoren: Buhrmann C.(1), Kraehe P.(1), Goel A.(2), Shakibaei M.(1),

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Abstract:

Objective: Colorectal cancer (CRC) is the world third most common cancer with up to 60% recurrence rates. Additionally, more than 15% of patients are resistant to well established treatment regimens with 5-Fluorouracil (5-FU). Plant polyphenols might offer novel avenues for enhancing chemosensitization especially targeting cancer stem cells (CSC). In this study we investigated the effects of curcumin and 5-FU therapy on DNA MMR-deficient/proficient CRC and CSC activity. Methods: Human colon cancer cell lines HCT116, HCT116+ch3 (complemented with chromosome 3) and their corresponding isogenic 5-FU-chemo-resistant derivative clones (HCT116R and HCT116+ch3R) were either cultured in monolayer or high density culture and evaluated macroscopically, ultrastructurally and with westernblotting. Results: Curcumin markedly enhanced chemosensitization of all four CRC cell lines to 5-FU treatment decrasing colonoshere size, enhancing colonosphere disintegration and enhancing apoptosis. DNA MMR system proficient cell lines HCT116+ch3 and HCT116+ch3R were more sensitive to treatment with curcumin and/or 5-FU compared to HCT116 and HCT116R cells. Furthermore, as shown with immunoblotting and immunofluorescence, combinational treatment of curucmin and 5-FU specifically targeted colon CSC by significantly decreasing the number of colon CSC marker positive cells (ALDH1, CD44 and CD133). Conclusion: Our study demonstrates novel effects of curcumin in enhancing chemosensitization to 5-FUbased chemotherapy on DNA MMR-deficient/proficient cells and their resistant cells, targeting also and preferably the colon cancer CSC sub-population. As chemo-resistance is mediated by CSC, this indicates a possible new treatment approach.

Rubrik: Zellbiologie

Titel:ProSAPs/Shanks in early development of rat hippocampal neurons

Autoren: Halbedl S.(1), Schön(1), Böckers(1), Schmeisser(1),

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Abstract:

All three ProSAP/Shank family members (Shank1, ProSAP1/Shank2, ProSAP2/Shank3) are essential scaffold proteins of the postsynaptic density (PSD) of excitatory glutamatergic synapses. In the PSD, ProSAP/Shank proteins multimerize and build large molecular platforms thus providing multiple protein-protein-interaction sites. These platforms are linking postsynaptic receptors with their downstream signaling proteins and the actin cytoskeleton of dendritic spines. Over the last decade, mutations in those genes were found to play a central role in the pathogenesis of autism spectrum disorders and schizophrenia. Interestingly, two studies (Du et al., 1998 and Durand et al., 2012) already implicated a role of ProSAP/Shank in early development of primary hippocampal neurons by revealing ProSAP1/Shank2 and ProSAP2/Shank3 immunoreactivity in growth cones. For a more detailed analysis of ProSAPs/Shanks in early neuronal development, we examined hippocampal neurons at distinct early developmental stages. Furthermore we analyzed the expression dynamics of all ProSAPs/Shanks on mRNA level in developing hippocampal cultures. We could show that ProSAP1 and ProSAP2 are indeed found in early hippocampal neurons and that they exhibit a gradual increase in protein expression over time. Additionally, we could also detect Shank1 in early developing hippocampal growth cones. The findings implicate a new role of ProSAP/Shank proteins in early neuronal development compared to their well-known function during synaptogenesis and synaptic plasticity.

Rubrik: Zellbiologie

Titel:Embryonic and neural stem cell markers in human ectoderm-derived tumors

Autoren:Ardabili S.(1),Hattermann K.(1),Gebhardt H.(1),Held-Feindt J.(2),Schumacher U.(3),Lange T.(3),Mentlein R.(1),

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#### Abstract:

Recent investigations on the tumor stem cell hypothesis and tumor-initiation suggest that malignant cells may arise from stem or progenitor cells after few mutations. This contrasts the previous view that tumor cells de-differentiate during progression. Nevertheless, cells with stem-like properties play a pivotal role in tumor growth and progression expressing unique patterns of transcription factors and receptors for growth and chemotactic factors. Therefore, we investigated various tumor cell types derived from the ectoderm for occurrence of embryonic (Nanog, Klf4, Oct4, Sox2, c-Myc) and neural (Musashi, Sox2, CD133) stem-cell markers and chemokine receptors regulating the migration of stem cells (CXCR4, CXCR7). Using quantitative RT-PCR and immunocytochemistry, we detected Nanog and Oct4 in all neuroblastoma, melanoma, small cell lung cancer (SCLC) and epithelial breast cancer cell lines investigated. Klf4 was highly expressed in breast and lung cancer cells, variable in others. Sox2 was heterogeneously distributed; c-Myc was highly expressed in all tumor cells. The neural stem cell markers Musashi and CD133/prominin were prominent in neuroblastoma and SCLC cells, lower or absent in all others. Immunocytochemistry revealed a homogenous distribution, if present, among all cells of one cell line. The chemokine receptor CXCR4 was expressed in all tumor cells lines, CXCR7 was present in only few lines; both are targeted by CXCL12 / SDF-1. Stimulation with this ligand upregulated embryonic transcription factors, furthermore CXCR4 expression itself. In conclusion, established tumor cell lines derived from neural crest show signatures of embryonic and partly of neural stem cells that are regulated by the chemokine CXCL12.

#### Rubrik: Zellbiologie

Titel:Inverse signaling of the transmembrane chemokines CXCL16 and CX3CL1 in human meningiomas

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#### Abstract:

Meningiomas are slowly growing benign tumors, however, anaplastic meningiomas have an aggressive biological and clinical behavior. Since the molecular mechanisms involved in progression of meningiomas are not yet fully understood and recent investigations have suggested a possible role of chemokines in tumor biology, the aim of the study was to investigate the expression and functional role of CX3CL1/CX3CR1 and CXCL16/CXCR6 in human meningiomas. Quantitative PCR revealed a distinct expression in solid human meningioma samples, and double-immunostaining showed a predominant expression of the chemokine/-receptor pairs in the tumor cells themselves, in infiltrating microglia cells/macrophages and endothelial cells of blood vessels. Interestingly, cultured human meningioma cells were characterized only by the expression of the chemokine ligands -CXCL16 and CX3CL1, omitting the corresponding receptors. Nevertheless, cultured human meningiomas bound the soluble chemokines and responded after stimulation with CXCL16 or CX3CL1 by phosphorylation of the extracellular signal-regulated kinases (ERK) p42/44 and pAkt, as well as by translocation of the transcription factor NF kappaB into the nucleus. Same results were observed when using CXCL16 and CX3CL1-specific antibodies. Additionally, enhanced proliferation and rescue from apoptosis were measurable in human meningioma cells after stimulation with transmembrane chemokines. Since intracellular signaling effects were repressed after siCXCL16 / siCX3CL1 transfection of human meningioma cells, we concluded that the transmembrane ligands themselves act as receptors and generate auto- or paracrine signals ("inverse signaling"). In this view, our results provide an interesting basis for further investigations on the functional roles of chemokines and their receptors in human meningiomas.

Rubrik: Zellbiologie

Titel:Differentiation of THP-1 monocytes regulates expression of chemokine receptors CXCR4 and CXCR7 and effects of ligands

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#### Abstract:

After circulating in the blood, bone marrow-derived monocytes enter peripheral tissues and differentiate into a heterogeneous lineage of mononuclear phagocytes / macrophages. An established model for this differentiation is the non-adherent, human monocytic leukemia cell line THP-1 where differentiation into phagocytic, adherent macrophage-like cells can be induced by treatment with phorbol esters (phorbol-myristate-acetate, PMA). Several (pro-) inflammatory or tumoral-derived signals like cytokines, microbial products, or in particular chemokines can be involved in monocyte recruitment, differentiation and lineage restriction. CXCL12/SDF-1 (stromal cell-derived factor-1) is a widely produced chemokine that might regulate monocyte or macrophage functionality. CXCL12 binds to the receptors CXCR4 and CXCR7, the later binding with tenfold lower affinity also CXCL11/I-TAC (interferon-inducible T cell alpha chemoattractant) that is also a ligand for CXCR3. By quantitative RT-PCR, Western blot and immunocytochemistry we could show that both CXCL12-receptors, but negligible amounts of CXCR3 were expressed by non- and differentiated THP-1 cells. Remarkably, CXCR7, but not CXCR4, was about 50-fold upregulated upon exposure to PMA. Exposure of non-differentiated cells to chemokine ligands CXCL11 or CXCL12 induced proliferation that could be diminished by pre-incubation with selective non-peptide antagonists; differentiated cells failed to proliferate. Furthermore, phagocytosis of differentiated cells as measured by uptake of fluorescent latex particles was significantly enhanced by both chemokine ligands. Again, selective antagonists (AMD3100 for CXCR4, CCX733 for CXCR7) reduced chemokine-induced phagocytosis, but in a complex manner. These experiments show that chemokine exhibit functional effects on monocytes and/or macrophages and that their receptors are regulated during differentiation.

Rubrik: Zellbiologie

Titel:Embryonic stem cell markers in human glial tumors

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#### Abstract:

As early as in 1858 Rudolf Virchow assumed that cancer arises from embryo-like cells. In this later extended concept (embryonal rest theory) aggressive cancer cells share histological similarities to embryonic stem cells. As differentiated cells acquire embryonic pluripotency and self-renewal by transfection with transcription factors like Oct4, Sox2, Nanog, Klf4 and c-Myc, we investigated their occurrence and frequency in human astrocytomas and glioma cell lines. Gene transcription was analyzed by quantitative RT-PCR and protein expression by immunohistochemistry. Positive cells were counted by an unbiased person for single or multiple expressions. Tumor samples showed a moderate to high mRNA expression of all transcription factors, with Sox2 and c-Myc as most pronounced. Among astrocytomas of different WHO grades, expression of c-Myc, Oct 4 and Sox2 increases with malignancy. As in solid tumors, Sox2 and c-Myc were most abundantly expressed also in glioblastoma cells lines, Nanog and Klf4 were expressed only at low levels. Co-staining with glial-specific markers proved an expression of all transcription factors in astrocytoma cells in situ and in vitro. However, in solid tumors expression was restricted to a subpopulation of cells ranging from 5-10% for all factors. Furthermore, cells positive for one transcription factor frequently co-stained for another factor, but single positive cells were also found in different proportions. We conclude that human astrocytomas contain a low, but constant proportion of cells expressing embryonic and/or neural stem cell factors, some increasing with malignancy. Investigations are undergoing to relate them to further characteristics, e. g. receptors for growth and chemotaxis factors.

Rubrik: Zellbiologie

Titel:Enhanced desmoplakin-keratin association ameliorated pemphigus vulgaris autoantibody-mediated loss of cell cohesion

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#### Abstract:

Desmoplakin (DP) serves to anchor intermediate filaments in desmosomal adhesive complexes. Recent data suggest that a specific DP point mutation (S2849G) exhibits increased keratin filament anchorage and fosters the maturation of desmosomes in keratinocytes, presumably by rendering DP inaccessible for PKC phosphorylation. Previously, we have shown that depletion of the desmosomal adhesion molecule desmoglein 3 (Dsg3) induced by autoantibodies from patients with the blistering skin disease pemphigus vulgaris (PV-IgG) is reduced in maturated desmosomes and dependent on PKC signaling. Thus, we investigated the role of DP-S2849G for loss of cell cohesion mediated by PV-IgG. In cell dissociation assays, expression of DP-S2849G increased cell cohesion in two different human keratinocyte cell lines and ameliorated loss of cell adhesion induced by PV-IgG or treatment with AK23, a pathogenic Dsg3 antibody derived from a pemphigus mouse model. Depletion of Dsg3 was inhibited by DP-S2849G in the cytoskeletal (Triton X-100 insoluble) fraction and activation of p38MAPK, a signaling molecule well established in PV pathogenesis, was reduced. Furthermore, keratin filament retraction, a hallmark of PV, was efficiently blocked by DP-S2849G. Taken together, these data demonstrate the relevance of keratin filament anchorage in desmosomes for both cell adhesion and regulation of p38MAPK activity. Furthermore, PKC-mediated DP phosphorylation induced by autoantibody binding may be a central mechanism in PV pathogenesis.

Rubrik: Zellbiologie

Titel:The effect of OXLDL on JAB1/CSN5 expression and foam cell formation in human macrophages

Autoren:Schwarz A.(1),Bonaterra G.(1),Kinscherf R.(1),

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#### Abstract:

Atherosclerosis is an inflammatory disease involving recruitment of macrophages, which are generally the most abundant cell type in atherosclerotic plagues. Previous studies of Jab1/CSN5 (c-Jun activation domain binding protein-1) have shown a coexpression of macrophages migration inhibitory factor (MIF) in all stages of human plagues. Macrophages internalize oxLDL (oxidized low density lipoprotein), develop into foam cells and release diverse pro-inflammatory cytokines, phenomena which are constituents of vulnerable plaques. Thus, we were interested to investigate the role of Jab1/CSN5 during foam cell formation and release of pro-inflammatory cytokines. U937 cells were differentiated (24h; phorbol 12-myristate 13-acetate [PMA, 20nM]) to macrophages and afterwards incubated (4h; 24h) with human oxLDL (50µg/ml, 100µg/ml; 200µg/ml) to induce foam cell formation (confirmed with OilredO staining). Using real time RT-PCR (gRT-PCR) we measured the expression of Jab1/CSN5 and TNF-alpha. Additionally, we guantified the release of TNFalpha (ELISA) and NO (nitric oxide/nitrite; Griess method). We found a significant 33%-43% decrease of Jab1/CSN5 mRNA expression after 24h oxLDL treatment in comparison with the control. Moreover, in oxLDL stimulated macrophages we found a significant 34-/50-fold increase of TNF-alpha and a significant 6.6-300-fold increase of NO/Nitrite release in comparison with the control. Additionally, we found a significantly enhanced TNF-alpha mRNA expression, which was concentration- and time-dependent. Our results suggest an oxLDL-mediated inverse regulation of Jab1/CSN5 and inflammatory markers (NO, TNFalpha) in human macrophages, which may lead to an enhanced internalization of oxLDL in macrophages.

Rubrik: Zellbiologie

Titel:Ago-immunoprecipitation as an approach to identify new miRNA targets in prostate carcinoma

Autoren:Szczyrba J.(1),Sapich S.(2),Hart M.(3),Wennemuth G.(1),

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### Abstract:

The adenocarcinoma of the prostate is still the most commonly diagnosed cancer in males worldwide. Among hereditary genetic mutations and nutrient factors, a link between the deregulation of miRNA expression and the development of prostate carcinoma is assumed. MiRNAs are small non-coding RNAs which posttranscriptionally regulate gene expression and which are involved in tumor development and progression as oncogenes or tumor suppressors. Although many genes could be confirmed as targets for deregulated miRNAs, common target prediction algorithms provide inaccurate results whose majority is false positive. Our aim was to facilitate the selection of putative miRNA target genes by immunoprecipitation of the RISC (RNA-induced silencing complex) from normal prostate fibroblasts and prostatic carcinoma cells, using a monoclonal AGO2 antibody. This complex contains bound miRNAs as well as their corresponding target messenger RNAs, allowing to perform microarray analysis. The obtained data was combined with common target lists and mRNA expression profiles of prostate carcinoma to predict new miRNA targets involved in prostate tumorigenesis. In subsequent luciferase assays we could confirm several new target mRNAs for deregulated miRNAs, such as TP53IP1, a newly identified prognostic marker in PCa, DEDD and TNFRSF10B, both involved in apoptosis. Our results demonstrate the improvement of miRNA target prediction by combining additional data obtained by AGO2-IP with algorithm based target lists and mRNA expression profiles.

Rubrik: Zellbiologie

Titel:Desmoglein 3 regulates keratin cytoskeleton organization via p38MAPK

Autoren: Hartlieb E.(1), Roetzer V.(1), Spindler V.(1), Waschke J.(1),

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Abstract:

Desmosomal cadherins are transmembrane adhesion molecules that provide cell adhesion by interacting in the intercellular space of adjacent cells and are either organized in desmosomes or located extradesmosomal in the cell membrane. In keratinocytes, several desmoglein (Dsg1-4) and desmocollin isoforms are co-expressed. Beside their adhesive function some desmosomal cadherins are recognized as important intracellular signaling molecules and targeted by circulating antibodies such as Dsg1 and Dsg3 in the autoimmune blistering skin disease pemphigus. We have previously shown that Dsg2 is less important for keratinocyte cohesion compared to Dsg3 and that the latter forms a complex with p38 mitogen-activated protein kinase (p38MAPK). In the present study, we compared the involvement of Dsg2 and Dsg3 in p38MAPK-dependent regulation of keratinocyte cohesion and intermediate filament organization. We show that keratin filament retraction, a p38MAPK-dependent event, is induced by siRNA-mediated Dsg3-depletion and is ameliorated by specific p38MAPK inhibition. In contrast, depletion of Dsg2 did not change the cytokeratin network organization. Furthermore, immunoprecipitation demonstrated an interaction of activated p38MAPK with Dsg3 but not with Dsg2. Interestingly, this complex is located in both the cytoskeleton-unbound as well as in the cytoskeleton-anchored desmosome-containing pool. These results demonstrate a unique function of Dsg3, in contrast to Dsg2, in controlling keratin cytoskeleton organization via p38MAPK signaling in keratinocytes.

Rubrik: Zellbiologie

Titel:LPS impairs repair of the murine tracheal epithelium in vivo

Autoren: Döring K.(1), Knebel G.(1), König P.(1),

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Abstract:

Cell culture experiments indicate that bacterial components are beneficial for airway epithelial regeneration. To examine if this is true also in vivo we studied the effect of LPS on epithelial regeneration in the murine trachea in vivo. To define suitable intervention time points, we first studied the repair process of the tracheal epithelium after naphthalene application in vivo at various time points between 12 h and 15 d. Then we investigated the repair processes with a combination of naphthalene (300 mg/kg, intraperitoneal) and a single dose of LPS (5 mg/kg, intranasal) 48 h or 60 h after naphthalene application. Tracheae were explanted and examined using semi-thin sections, scanning electron microscopy, immunohistochemistry, real-time RT-PCR and high speed video microscopy. 48 h after naphthalene application ciliated and non-ciliated cells were destroyed. 60-72 h after naphthalene application, short cilia were observed, 15 d after application, repair of the epithelium was completed and expression of marker genes was normal. Application of LPS 48 h after naphthalene application prevented the closure of the epithelial barrier at 4 d and application of LPS after 60 h resulted in a closed epithelium but differentiation of cells was delayed at 4 d. LPS also inhibited epithelial proliferation at 4 d. Irrespective of the time point of LPS application, ciliated and non-ciliated cells were observed after 15 d. However, although ciliary beat frequency was normal after LPS application cilia-driven transport was impaired. In conclusion, a single dose of LPS during regeneration impairs airway epithelium repair in vivo.

Rubrik: Zellbiologie

Titel:Morphological and morphometric analysis of the endothelial glycocalyx using transmission electron and confocal laser scanning microscopy

Autoren: Wagner N.(1), Waldmüller H.(1), Asan E.(1), Ergün S.(1),

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### Abstract:

The endothelial cell surface is covered by an elaborate network of membrane-bound glycoproteins and proteoglycans building the glycocalyx (GCX). GCX composition and integrity are of central importance for endothelial barrier and its dysfunction, and mediate processes from inflammation to mechanotransduction. To assess its role in vascular biology and disease in detail, establishment of techniques enabling direct visualization of the GCX is crucial.

To date, different attempts were made to visualize the GCX using transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM). Conventional fixation and dehydration of specimens for TEM often leads to a significant reduction or collapse of the GCX, rendering morphometric analysis difficult. To circumvent this problem, CLSM was used to analyze the hydrated GCX. However, CLSM spatial resolution is limited to 200nm, and immunofluorescence visualization does not allow exact comparative analyses of GCX thickness.

In our study, we first compared different fixation methods to achieve the best preservation of the aortic endothelial GCX for visualization and morphometric analysis using TEM. In a second step, we used different fluorescence markers to analyze the GCX using CLSM. Finally, we applied a combination of TEM and CLSM to assess GCX thickness and structure of adjacent aortic sections, taking advantage of both visualization techniques with high spatial resolution provided by TEM and optimal preservation of the hydrated GCX using CLSM.

Our analysis exemplarily reveals on aortic endothelial GCX that appropriate vascular tissue preservation enables comparative morphological and morphometric analyses on endothelial GCX via TEM alone or in combination with CLSM.

Rubrik: Zellbiologie

Titel:Novel domains of murine Prominin-1 (CD133) localization in the kidney

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#### Abstract:

The mammalian kidney is particularly enriched in transcripts encoding for Prominin-1 (CD133), and the confinement of this cholesterol-binding membrane glycoprotein to metanephric proximal tubules was previously described. Its spatial compartmentalization, however, neither in adult kidney nor in the newborn (murine) organ with still ongoing nephrogenesis has been documented in detail.

Using non-radioactive ISH, (immuno)histochemistry and nephron markers Prominin-1 was detected in S-shaped bodies of the nephrogenic peripheral zone in newborns. It was also present in proximal straight portions [PST] of the differentiating nephrons but was excluded from proximal convoluted segments [PCT]. In addition, the differentiating renal medulla with developing loops of Henle was highly enriched in Prominin-1 transcripts. Analysis of adult kidney provided a refined picture as to the spatial compartmentalization of Prominin-1 along distinct nephron segments. Thus, Prominin-1 immunoreactivity was localized to [PST], while it was excluded from [PCT] reminiscent of the localization in newborns. Moreover, Prominin-1 was also detected in a more distal segment of the nephron - at apical membranes of epithelial cells of the thick ascending limb of Henle's loop [TAL] - in accordance with the medullary enrichment of Prominin-1 seen in newborns. At the same time, the absence of Prominin-1 from the collecting duct system [CD] and urothelium of the pelvis renalis was observed. Taken together, our results indicate that Prominin-1 is more broadly expressed in the kidney than it was previously documented and it is enriched in structures that are derivatives of the metanephric mesenchyme, but not in ureteric bud-derived structures of the metanephros.

#### Rubrik: Zellbiologie

Titel:Mitochondrial and inflammatory changes in triceps surae muscle from mice treated with repeated cold stress: a model of fibromyalgia

Autoren: Bonaterra G.(1), Then H.(1), Özel L.(2), Dill L.(1), Rempel W.(1), Ocker M.(2), di Fazio P.(2), Ocker M.(2), Kinscherf R.(1),

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#### Abstract:

Introduction: Fibromyalgia (FM) is a disorder, which concerns up to 5% of the general population worldwide. Mitochondrial dysfunction and inflammation are suggested to be involved in the pathophysiology of FM. Thus, we have investigated the possible relationship between mitochondrial dysfunction and inflammation in FM using an experimental model of fibromyalgia. Method: Therefore, we analyzed morphological changes of mitochondria and inflammatory components in the triceps surae muscle of mice treated with intermittent cold stress (3 days intermittent periods of 30 min at 4 °C and 22 °C [treated]). Samples of soleus and gastrocnemius muscles were fixed for transmission electron microscopy and cryopreserved for RNA isolation. Inflammatory components (IL-1beta, COX-2) were analyzed using gRT-PCR. Results: In soleus muscle of treated females the mitochondrial density was significantly 1.5-fold lower in comparison with control mice. Mitochondrial quality was markedly decreased in soleus muscle, because we found an increase of 2.2- and 1.4-fold of damaged mitochondria in treated female and male mice, and a 1.6-fold and 1.8-fold increase in gastrocnemius muscle compared with corresponding control mice. In addition, gene expression of inflammatory components (COX-2, IL-1beta.) were 2.8- and 4-fold increased in soleus muscle of treated versus control female mice.

Conclusion: Cold injury increases the number of damaged mitochondria and the expression of inflammatory components especially in soleus muscle of female mice. These changes may be responsible for an energetic imbalance, which accompanied by local proinflammatory conditions may cause the painful symptoms of fibromyalgia, predominantly found in females.

#### Rubrik: Zellbiologie

Titel:Cartilage tissue engineering using alginate-based scaffolds and mesenchymal stroma cells

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### Abstract:

To overcome the limited repair capability of cartilage, autologous chondrocyte or mesenchymal stroma cell (MSC) implantation attracts increasing interest. To provide sufficient cell adherence and initial mechanical stability chondrogenic cells should be combined with suitable biocompatible scaffolds.

The present study should indicate whether a recently developed highly porous alginatebased scaffold allows the attachment, survival and chondrogenesis of MSCs and articular chondrocytes.

The alginate foam scaffolds (pore size ~250  $\mu$ m, porosity ~90%) were prepared using a freeze-dry method. MSCs were seeded on the scaffolds and cultured for 1 and 2 weeks using either chondrogenic differentiation or maintenance media. For comparison, scaffolds were seeded with articular chondrocytes (passage 2-3) under similar conditions. Cell vitality was assessed by live death assay and confocal laser scanning mitcroscopy. Histology was performed using hematoxylin-eosin and alcian blue staining to detect sulfated glycosaminoglycan deposition. Additionally, cultures were immunolabeled for cartilage-specific type II collagen and non specific type I collagen.

Most of the MSCs survived on the scaffolds during the whole observation time forming rounded clusters within the scaffold pores. The growth of MSCs and primary articular chondrocytes did not show major differences. The MSCs, irrespective of whether cultured under non/chondrogenic conditions produced an extracellular matrix (ECM) containing sulfated glycosaminoglycans, types II and II collagen after 7 and 14 days.

The cell cluster formation induced by the scaffolds allowed intense cell-cell contacts and might stimulate chondrogenesis. Chondrocytes maintained cartilage-specific type II collagen synthesis on the scaffolds. Further analyses are required to assess whether the scaffolds have chondroconductive properties.

#### Rubrik: Zellbiologie

Titel:Effect of chronic intermittent hypoxia (CIH) on vascularisation in slow- and fast-twitch skeletal muscles in wild type and inducible nitric oxide synthase knockout (INOS-/-) mice.

Autoren: Dill L.(1),Bonaterra G.(1),Schwarzbach H.(1),Then H.(1),Schulz R.(2),Kraut S.(3),Weissmann N.(3),Kinscherf R.(1),Hildebrandt W.(1),

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#### Abstract:

Introduction: The obstructive sleep apnea syndrome (OSAS) causes arterial hypertension and insulin resistance. Whether skeletal muscle vascularisation contributes to decreased vascular conductance or glucose delivery is unclear in view possible neo-angiogenesis with OSAS. Methods: We therefore studied fiber type-specific capillarisation in the soleus, gastrocnemius and vastus muscles of wild type (WT) mice under normoxia (NOX, n=12) and after 6-weeks of chronic intermittent hypoxia (CIH, n=14) as a model of OSAS. Moreover, the role of inducible nitric oxide synthetase (iNOS) was evaluated by studying iNOS-/- mice under NOX (n=8) and after CIH (n=8). Results: CIH induced a significant angiogenesis that was limited to soleus muscle, significant for all fiber types (types I: 11%, IIa: 16%, IIx: 9%) and well reflected by a 4-fold up-regulation of VEGFa. As an indicator of nutritional capillarisation the fiber area to capillary contact ratio significantly decreased, with some contribution arising from atrophy of all fiber types in soleus muscle (not observed in gastrocnemius / vastus muscles). Strikingly, iNOS-/- mice under NOX revealed a soleusspecific angiogenesis and an up-regulation of VEGF gene expression compared to WT, but lacked any further angiogenic response upon CIH. Conclusions: These data exclude a muscular capillary rarefication contributing to OSAS-related insulin resistance. However, angiogenesis upon CIH is different between slow- and fast-twitch muscles, but not between fiber types within slow-twitch muscle. iNOS deficiency in NOX appears to strikingly mimic the angiogenic responses to CIH in WT mice, however it abolishes any further vessel growth upon exposure to CIH.

Rubrik: Zellbiologie

Titel:Mouse aortic adventitia-derived macrophages and their influence on vascular sprouting

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#### Abstract:

The paradigm of bone-marrow as the only origin of monocytes and macrophages has been reconsidered in the last years. Among different other cell types macrophages can additionally be generated from vessel-wall resident stem- and progenitor cells. We studied the impact of adventitial-derived macrophages on differentiation and sprouting capacity of vessel wall-resident stem- and progenitor cells.

In this context mouse aortic ring assay (ARA) was performed with and without clodronate liposome-mediated macrophage depletion. Cell sprouting capacity and density were assessed by morphometric measurements. Macrophages were detected by immunostaining for F 4/80. Furthermore, immunostainings for CD34, CD44, alpha SMA and VEGF as a known potent angiogenic factor were performed.

In contrast to freshly isolated aorta almost lacking macrophages, in untreated aortic rings macrophages were distinctly detectable in the adventitial and partially in the subintimal region after 11 days culture. After clodronate treatment macrophages were reproducibly depleted in the adventitia whereas subintimal localization was not always affected. Treated rings showed reduced density and branching of sprouting cells compared to controls. The number of CD44(+) cells among sprouting and partially cords forming cells was significantly reduced after macrophage depletion.

In conclusion our results suggest that macrophages generated from aortic adventitial progenitors can specifically be depleted by clodronate which impairs the sprouting capacity of aortic rings and also the differentiation capacity of vessel wall-resident CD44(+) multipotent stem cells.

Rubrik: Zellbiologie

Titel:Homesotasis of human periodontal ligament cells under stress conditions is influenced by IGF-2

Autoren: Götz W.(1), Memmert S.(2), Frede S.(3), Rath-Deschner B.(2),

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### Abstract:

The periodontal ligament (PDL) of the human tooth supporting apparatus is subject to biomechanical forces and other stressors like inflammation or hypoxia. We have shown that components of the Insulin-like Growth Factor (IGF) system are expressed in the PDL and regulated by biomechanics. Now we wanted to investigate the influence of IGF-2 on cellular functions and the IGF system of PDL cells in relation to different stress conditions in vitro. PDL cells from healthy premolars were cultured under normoxic and hypoxic conditions and stimulated with IGF-2. In order to mimic functional loading, dynamic tensile strain (3%) was applied using a strain device with membranes. In a further experiment, phosphoinositid-3kinase (PI3K) was blocked by Wortmannin. Proliferation was investigated by cell counting and BrdU assay, apoptosis using a detection kit. The effects of IGF-2 on the IGF system were analyzed with RT-PCR. Unstimulated PDL cells served as controls. PDL cell proliferation was increased after IGF-2 stimulation and could be blocked by Wortmannin incubation. Hypoxia aggravated IGF-2 induced proliferation. However, apoptosis rate was not regulated. While there was no significant influence on the gene expression of IGF-1. stimulation induced IGF-2 upregulation indicating an autocrine effect. The expression of IGFBP-1, -3 and -5 was differentially regulated by the factors investigated. Our data show that IGF-2 influences PDL cell proliferation via the PI3-Akt signal transduction pathway and that mechanical and hypoxic effects are partly mediated by IGF-2. These effects are modulated by IGFBPs in a promoting or inhibiting manner. Supported by DFG (KFO 208)

Rubrik: Zellbiologie

Titel:The role of ProSAP/Shank scaffold proteins in muscular tissue

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#### Abstract:

The ProSAP/Shank family of scaffold proteins link cell surface receptors to the actin cytoskeleton. The three family members Shank1, ProSAP1/Shank2 and ProSAP2/Shank3 are highly enriched in the postsynaptic density (PSD) of excitatory synapses. Mainly known as PSD scaffolding proteins that build large protein-protein interaction platforms, the ProSAP/Shanks are also widely expressed in other tissues. Analysis of protein levels exhibits the presence of ProSAP1/Shank2 in non-neuronal tissues like pancreas, lung, liver and heart. Intriguingly, high mRNA levels of the family member ProSAP2/Shank3 were also detected in heart and only moderate levels were observed in brain and spleen. The widespread expression of the ProSAP/Shanks in peripheral tissues indicates additional roles for these scaffold proteins apart from their scaffolding function in the PSD. In our study, we investigate the occurrence and localization of the ProSAP/Shank proteins in cardiac, smooth and skeletal muscles. Our data demonstrate that differing from the expression in neuronal tissue; predominantly the largest ProSAP/Shank isoforms are found in muscular tissue. Interestingly, our analyses show also high expression levels of the F-actin binding and ProSAP/Shank-anchoring protein cortactin in skeletal and cardiac muscular tissue indicating a similar interconnecting mechanism of cell surface receptors to the actin cytoskeleton via the ProSAP/Shanks. Further investigations will be performed using ProSAP2/Shank3 knockout mice to get more detailed information about the physiological functions of ProSAP/Shanks in muscular tissues.

Rubrik: Zellbiologie

Titel:A cholinergic cell with brush cell characteristics in the thymic medulla

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#### Abstract:

Specialized epithelial cells with a tuft of apical microvilli ("brush cells") sense the luminal content and initiate protective reflexes in response to detection of potentially harmful substances. They utilize the canonical taste transduction cascade to detect "bitter" substances such as bacterial quorum sensing molecules. In the respiratory tract and in the urethra, the majority of these cells is cholinergic and they are approached by cholinoceptive sensory nerve fibers. Utilizing two different mouse strains expressing eGFP under the control of the choline acetyltransferase promoter (ChAT-eGFP), we observed strong immunoreactivity in a subset of thymic medullary cells with brush cell shape. ChAT expression was confirmed by in-situ-hybridization. These cells showed expression of villin, a brush cell marker protein, in the tufts at a tip of cell processes. Ultrastructurally, such cells exhibit lateral microvilli. These cells do not express neuroendocrine (chromogranin A, PGP9.5) but thymic epithelial (CK8+, CK18+, CK5-, CK14-) markers. They are immunoreactive for components of the taste transduction cascade (Ga-gustducin, TRPM5, PLC<sub>β</sub>2). RT-PCR shows taste receptor expression (Tas2R1, Tas2R105, Tas2R108). Thymic "brush cells" are not approached by nerve fibers (identified by PGP9.5 and CGRP antibodies). Utilizing mice expressing eGFP driven by the promoter of the nicotinic acetylcholine receptor  $\alpha$ 3-subunit, a distinct cell population (PLC $\beta$ 2 and TRPM5 negative) was found scattered in the thymic medulla. The data show a previously unrecognized chemosensitive cell in the thymus that utilizes acetylcholine for paracrine signaling. It might be speculated that it serves to detect bacterial products to initiate local responses, possibly influencing thymocyte maturation.

#### Rubrik: Zellbiologie

Titel:Effect of cancer cachexia on myocardial vascularization and inflammation in a transgenic mouse model of pancreatic cancer

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### Abstract:

Introduction: Cancer cachexia is characterized by a progressive loss of muscle and fat, poor function capacity and prognosis. Its development is most rapid with pancreatic cancer. Little is known to what extent myocardial morphology and function are affected, especially in early stages of cachexia or even cancer itself, including precancerous lesions. Methods: We therefore studied myocardial capillarization (lectin staining) and angiogenic as well as pro-inflammatory gene expression (RT-PCR, IHC) in the left ventricle of the 3xtransgenic mouse model of the ductal pancreatic adenocarcinoma (LSLG12D/+;LSL-Trp53R172H/+;Pdx-1-Cre-Mouse). Tumor-bearing 3x-mutant (TB, n=7) with non-significant weight loss were compared with WT (n=10) and tumor-free 2x- (TF2x, n=21) or 3x-mutants (TF3x, n=10) or their subgroups of precancerous PanIN stages 1a-b (n=20) and 2-3 (n=11). Results: Compared to WT, TB showed lower myocardial capillary density (21%, p<0.01) along with a decrease in angiogenic signals (VEGF-a 0.67-fold, Notch1 0.31fold, Notch3 0.19-fold), which were already present in TF2x and TF3x, i.e. all PanIN stages. Moreover, TB had significantly increased pro-inflammatory components compared to WT (IHC: COX-2 133%, CD68 85%, TNFa 24%, IL-1β 19%) with corresponding changes in RT-PCR including IL6. Strikingly, the expression of the suppressor of cytokine signaling-3 (SOCS-3) gene was 46-fold increased, that is compatible with attenuated angiogenesis, inflammation and apoptosis. Conclusions: Cancer cachexia affects the myocardium even before massive loss of weight or skeletal muscle mass. The observed myocardial capillary rarefication and inflammation warrant functional analyses early within the rapid cachexia development experienced by pancreatic cancer patients.

Rubrik: Zellbiologie

Titel:Influence of all-trans-retinoic acid (ATRA) on human meibomian gland epithelial cells

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#### Abstract:

Purpose. Meibomian Gland Dysfunction (MGD) is the key process leading to Dry Eye Disease (DED). It is currently thought that MGD is caused primarily by terminal duct obstruction due to hyperkeratinization of the ductal epithelium and an increased viscosity of meibum. However, the molecular mechanisms that underlie this process are unclear. Dermatological studies have shown that ATRA has a strong dose dependent influence on both, sebocytes and epithelial cells regarding differentiation and proliferation. Since the meibomian gland consists of both, sebocyte-like cells (meibocytes) in the acini and epithelial cells lining the ductal system, this study aimed to investigate the effects of ATRA on meibocyte differentiation and proliferation.

Methods. Human meibomian gland epithelial cells (cell line) were cultured with or without ATRA at a concentration of  $2\mu$ M for 96 h. Afterwards, treated and non-treated cells were analyzed by means of proliferation assay, real-time PCR, Western Blot and immunohistochemistry.

Results. Both, mRNA and protein of various genes, such as cytokeratins 5 and 14, involucrin, fatty acid synthase and stearoyl-CoA desaturase were altered by the ATRA treatment. In addition, ATRA decreased cell proliferation and increase apoptosis of meibocytes. Conclusion. ATRA inhibits cell proliferation, promotes cell death and alters gene expression and with that has a strong effect on keratinization and lipid-synthesis of meibocytes. Therefore, ATRA must be considered a substance promoting MGD.

Rubrik: Zellbiologie

Titel:The importance of melanocortin receptors concerning meibomian gland dysfunction

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#### Abstract:

Purpose. Meibomian gland dysfunction (MGD) is considered as one of the primary causes leading to Dry Eye Syndrom. It has well been established that changes in meibum composition and an altered meibum delivery are crucial factors provoking and maintaining MGD. As meibocytes and sebocytes share anatomically and physiologically similarities and it has been shown that ligands of the melanocortine receptor (MCR) family influence differentiation of sebocytes and sebum production the aim of this study was to investigate expression of MCRs and their potential effects in meibocytes.

Methods. Immortalized meibocytes were cultured in one of two different types of media (A or B) resulting in undifferentiated cells. Afterwards meibocytes were treated by medium C initiating cell differentiation. Cells were then evaluated with regard to MCR expression using RT-PCR, immunocytochemistry, realtime RT-PCR and Westernblot. Sudan-III-staining was used to visalize lipid production. In addition, cells were stimulated with MCR agonists alpha-MSH and beta-MSH as well as with MCR antagonist HS-024 and expression of MC5R and central enzymes of the lipid synthesis were analyzed via realtime RT-PCR.

Results. mRNA and protein of MC1R and MC5R were detected in differentiated and undifferentiated meibocytes. Depending on the medium (A, B, C) there was much or only little difference of MC5R expression. HS-024 inhibited MC5R expression and expression of fatty-acid-synthase and stearoyl-CoA-desaturase which take part in the lipid synthesis. Conclusion. Our data show that MCRs are expressed on meibocytes and that stimulation/inhibition of MCRs, especially MC5R, alter their differentiation and lipid synthesis. Therefore, the role of MC5R and its ligands should be considered with regard to MGD and possible treatments.

Rubrik: Zellbiologie

Titel:The barrier function of the nuclear envelope is compromised in fibroblasts lacking expression of lamin A

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#### Abstract:

Lamins are structural components of the nuclear lamina and integral parts of the nucleoplasm. They act as a scaffold for many transcriptional and also nuclear envelopeassociated proteins. Mutations in A-type lamins cause a wide range of human genetic disorders (laminopathies). Some laminopathies, such as those responsible for premature aging (e.g. Hutchinson-Gilford progeria syndrome/HGPS or restrictive dermopathy/RD) are associated with severe disruption of nuclear envelope integrity. Lamins play central roles in the functionality of nuclear pore complexes (NPCs), and are thus important players in nucleocytoplasmic protein transport. In this context, we and others demonstrated that in HGPS/RD cells protein import into the nucleus is compromised. Moreover, we observed that HGPS-/RD-causing mutations have a negative impact on the nuclear import of lamin A itself. These findings prompted us to extend our investigations with respect to the impact of the lamina on global nuclear protein transport. In the experimental set-up mouse fibroblasts lacking lamin A expression (LMNA-/-) were used in conjunction with the ARGENT™ Regulated Heterodimerization Assay to measure nuclear protein import of selected cargo molecules quantitatively. We found time dependent differences in the nuclear import of the cargo molecule carrying the nuclear localization sequence of the SV40 large tumor antigen. We also observed a significant loss of barrier function of the nuclear envelope in LMNA-/cells, as judged by the penetration of expressed GFP-beta-galactosidase into the nucleus. In conclusion, our results demonstrate the importance of the nuclear lamina in protein transport across the nuclear envelope.

Rubrik: Zellbiologie

Titel: Does platelet concentrate have anti inflammatory effects in rheumatoid arthritis?

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#### Abstract:

Einleitung und Fragestellung.

Die rheumatoide Arthritis (RA) ist die häufigste entzündliche Erkrankung der Gelenke. Über die positiven Wirkungen von autologem plättchenreichem Plasma (PRP) bei der Behandlung der Knorpelschäden wurde in den letzten Jahren berichtet Die intraartikuläre Applikation von PRP wird als möglicher Therapieansatz bei der RA diskutiert. In dieser Studie sollen die pround antiinflamatorischen Effekte von freigesetzten Wachstumsfaktoren aus Thrombozyten, die sogenannten "platelet released growth factor" (PRGF), auf den Knorpelzellen untersucht werden.

### Methodik.

Die Knorpel-anabole Faktoren wurden in PRP, PRGF und Serum Mittels ELISA quantifiziert. Ein in vitro Rheuma Modell wurde durch Zugabe von Tumor necrosis factor–alfa (TNFalfa) zu humanen C28/12 Zellen erzeugt. Diese Zellen wurden mit unterschiedlicher Konzentration an PRGF behandelt. Mit der quantitativen real-time PCR wurde die Expression von pro- und antiinflamatorischen Zytokinen sowie chondrogenen Markern Ferner wurde die Freisetzung von verschiedenen Mediatoren von den Zellen nach Behandlung mit PRPG mittels ELISA untersucht.

#### Ergebnisse.

In PRP konnten signifikant erhöhte Level an TGF-beta, VEGF, PDGF-BB und BMP-4 im Vergleich zu Serum quantifiziert werden.

Eine Behandlung der vorstimulierten Zellen mit 1%PRGF führte zu einer signifikanten Down-Regulation von TNF-alfa, welche durch steigende Konzentration an PRGF erhöht wurde. Die freigesetzten Level an VEGF, IL6, TNF $\alpha$  und IL-10 wurde ebenfalls nach Behandlung der Zellen mit PRGF quantifiziert.

Schlussfolgerung.

Es scheint, dass die freigesetzten Wachstumsfaktoren aus Thrombozyten in geringer Konzentration auf den hyalinen Chondrozyten antiinfalmatorisch wirken. Die Durchführung weiterer Experimente ist notwendig, um die Mechanismen zu verstehen und den positiven Effekt von PRP als autologes Therapeutikum bei der Behandlung der Gelenkschäden zu sichern.

Rubrik: Zellbiologie

Titel:Effect of GDF-15 on morphology and gene expression in the liver using an experimental model of atherosclerosis in mice

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Abstract:

Introduction: We have most recently shown that Growth Differentiation Factor-15 (GDF-15) deficiency inhibits atherosclerosis progression in hypercholesterolemic ApoE-/- mice. To address the question whether GDF-15 has an impact on the progression of dysfunctions in organs known to be involved in the development of atherosclerosis, we consistently studied initially possible effects of GDF-15 in the liver.

Methods: 10 weeks old GDF-15+/+/ApoE+/+ (wild type; WT), GDF-15-/-/ApoE+/+, GDF-15+/+/ApoE-/-and GDF-15-/-/ApoE-/-mice received a cholesterol-enriched diet (CED) for 12 or 20 weeks. The animals were sacrificed and the liver was removed and shock-frozen. Computer-assisted immunohistomorphometrical quantification of CD68+macrophages was performed. RNA was isolated and the mRNA expression of apoptosis (Casp-3, BAX, BCL-2), inflammation (MIF, TNF-alpha, COX-2) and proliferation (PCNA) relevant genes were analysed by qRT-PCR.

Results: GDF-15-/-/ApoE-/-mice revealed after 20 weeks CED in the liver around the central vein a significant 20 % increase of CD68+ macrophages compared with GDF-15+/+/ApoE-/-mice. Furthermore, after 20 weeks of CED, we found in the livers of GDF-15-/-/ApoE-/-mice increases in the mRNA expressions of caspase-3 (6-fold; 6x), BCL-2 (2.9x), BAX (2x), MIF (4.7x), TNF-alpha (2x) and PCNA (7x) compared withGDF-15+/+/ApoE-/-mice.

Conclusion: GDF-15 deficiency inhibits atherosclerosis progression in the arterial wall and induces a local increase of CD68+ macrophages in the liver, in parallel with an increased expression of pro-inflammatory, pro-apoptotic and proliferation relevant genes. GDF-15 deficiency seems to induce an alteration of the steady state of the cellular components in the liver, which may have an impact on development and progression of systemic atherogenesis.

#### Rubrik: Zellbiologie

Titel:Increased activity of thermosensitive transient receptor potential channels in pterygium cells

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### Abstract:

Purpose. A pterygium conjunctivae is a benign fibrovascular overgrowth of the conjunctiva, which may spread to the cornea. The pathogenesis of pterygia is uncertain at present. Notably, neoplastic properties of overgrowing cells are determined by calcium-dependent cellular mechanisms. Intracellular calcium is substantially regulated by transient receptor potential channels (TRPs). Therefore, this study was undertaken to investigate the properties of temperature-sensitive TRPs in pterygium cells.

Methods. Conjunctiva cells from a patient with pterygium were isolated and cultured to establish an in vitro pterygium cell model, since these cells spontaneously immortalized. Expression of different markers for pterygium, limbal stem cells, cornea, conjunctiva as well as expression of TRPs were studied by RT-PCR, Western Blot and immunohistochemistry. Finally, we used functional assays such as florescence Ca2+ imaging (fura-2) and planar patch-clamp technique to measure intracellular Ca2+ and whole-cell currents of TRP- channels.

Results. Expression of pterygium markers revealed similar pattern in pterygium cell line and resected pterygium by RT-PCR and Western Blot analysis. These results were verified by immunohistochemistry in resected pterygium. Calcium measurements and analysis of whole-cell currents showed an increased Ca2+ influx via TRPV1 and another TRP subtype channel such as TRPM8 in pterygium cells compared to HCjE cells.

Conclusions. The expression of pterygium markers in our in vitro cell model confirmed typical pterygium characteristics. For the first time, the functional expression of the temperature-sensitive TRP channel subtypes TRPV1 and TRPM8 and their increased activity could be described in pterygium cells. Overall, these findings contribute to elucidate the pathogenesis mechanisms of pterygium.

Rubrik: Zellbiologie

Titel:Cardiomyocyte alterations contributing to disease progression in homozygous desmoglein 2-mutant mice, a model for arrhythmogenic right ventricular cardiomyopathy

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### Abstract:

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is caused by mutations of desmosomal proteins. It is characterized by cardiomyocyte death, myocardial fibrosis and dilation of the right but also the left ventricle. We recently described knock-in mice, in which the gene coding for the desmosomal cadherin desmoglein (DSG) 2 was substituted by a mutant DSG2 allele. The encoded polypeptide lacks part of the adhesive extracellular domains 1 and 2. Homozygous DSG2 mutant mice (DSG2mt/mt) develop principal features of ARVC and are therefore a suitable model to study ARVC pathophysiology.

To examine, if hypertrophy and sarcolemmal leakiness are pathological events occurring in ARVC the diameter of cardiomyocytes was measured and the penetration of IgG into cardiomyocytes was analyzed in DSG2mt/mt and wild-type mice between 2 and 54 weeks after birth.

We found that cardiomyocyte diameter was significantly increased in 8 to 54 week-old DSG2mt/mt mice. Cardiomyocyte hypertrophy became more pronounced with increasing age. In 2 and 4-week-old mice cardiomyocyte diameter did not differ between DSG2mt/mt and wild-type mice.

IgG molecules were detected in blood vessels, within the extracellular matrix of lesions and in necrotic cardiomyocytes of DSG2mt/mt mice by immunohistochemistry. Cytoplasmic IgG immunostaining was detected in single cardiomyocytes and in small groups of morphologically normal appearing cardiomyocytes in DSG2mt/mt-mice. In addition, single IgG-positive cardiomyocytes were identified in old wild-type mice (39-54 weeks). Our data demonstrate that cardiomyocytes of DSG2mt/mt mice develop pathological hypertrophy and sarcolemmal instability. We conclude that both phenomena indicate intrinsic mechanical disturbances which lead continuously to cardiomyocyte decompensation and death.

Rubrik: Zellbiologie

Titel: Flow culture model for corneal epithalial cells

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Abstract:

<u>Aim</u> Aim of this study is to establish a flow culture model of human corneal epithelial cells to mimic physiological tear flow through blinking of the eye lid.

<u>Methods</u> Using an IBIDI pump system human corneal epithelial cells (HCE cell line) were cultivated under two different flow conditions: continuous (15mbar for three days) and discontinuous to imitate blinking (50mbar forward flow for 1s, 50mbar reverse flow for 1s, break for 3s). Afterwards, morphology and expression profiles of cultivated cells were compared with HCE cells which were cultivated under stationary conditions. Cytoskeleton morphology was investigated by immunofluorescence and electron microscopy. The mRNA expression of different markers like different cytokeratins, MUC5AC, MMP7 and Ki-67 was evaluated by semi-quantitative PCR and Western blot analysis. Furthermore, apoptotic cells were detected by TUNEL assay.

<u>Results</u> HCE cells expressed B-actin and Ki-67 mRNA in an equivalent manner independent of the way of cultivation. Cells cultivated under flow conditions showed an increased MUC5AC, but decreased MMP7 mRNA expression level. Depending on cultivation conditions HCE cells displayed a distinct cytokeratin expression profile detected by Western blot. The morphological studies demonstrated an increased formation of stress fibers and contacts under flow conditions. A higher number of apoptotic cells was observed under a permanent medium flow compared to stationary cultivation conditions.

<u>Conclusion</u> Our findings underline the necessity of tear flow for the physiology of corneal epithelial cells which can be mimicked by the advanced technique of the IBIDI pump system.

Rubrik: Zellbiologie

Titel:The impact of interleukin-1 (IL-1) on cell death, nitric oxide (NO) production and proteoglycan degradation in meniscal and articular cartilage tissue: a comparative study

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#### Abstract:

Pre-inflammatory conditions are risk factors for the initiation of degenerative joint diseases. Compared to articular cartilage (AC) very little is known about meniscal tissue destruction, even though meniscal damage might lead to symptomatic osteoarthritis. We therefore investigated the impact of IL-1 (0-10ng/ml) on cell death [measuring nuclear blebbing and condensation of nuclei in histological sections], NO production and proteoglycan degradation [measuring NO (Griess) and glycosaminoglycan = GAG release (DMMB assay) in supernatants and gene expression of enzymes (quantitative RT-PCR and casein/gelatine zymograms]] in bovine meniscal and AC explants after 3 and 10 days of culture. We found a significant IL-1 dose-dependent increase in cell death in meniscal tissue which was higher compared to AC. NO and GAG release also increased, but the meniscal response dropped down during culture to non-significant levels whereas AC showed longer lasting releases. Unfortunately, the reference genes GAPDH/18sRNA showed increased CT values in the AC control groups which did result in relatively low mRNA levels for the other studied genes in the AC/IL-1 group; this needs further investigation. However, IL-1 increased MMP-3, -13 and ADAMTS-4 levels significantly in both tissues whereas aggrecan and collagen type II were reduced. The AC zymograms showed a longer lasting release of enzyme activity compared to menisci, most likely MMP-3 and MMP2, which might be related to the prolonged GAG release. We conclude that IL-1 affects the meniscal tissue viability more compared to AC accompanied by a shorter NO and GAG release response. Whether these findings are related needs further investigation.

Rubrik: Zellbiologie

Titel:PLUNC, a new surfactant protein of the tear film

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#### Abstract:

**Purpose:** PLUNC (Palate Lung Nasal Clone) is a very hydrophobic protein that belongs to the family of surfactant proteins. It has been shown that PLUNC regulates the liquid volume of the airway surface by inhibition of the epithelial sodium channel (ENaC). This indicates that PLUNC could play a role in regulating the fluid balance of the lung. Furthermore the PLUNC protein directly acts antimicrobial against Gram-negative organisms. Objective of this study was to determine the expression and production of PLUNC at the healthy human ocular surface and to investigate potential differences in the presence of dry eye disease. **Methods:** PLUNC expression was analyzed and quantified by means of real time-RT-PCR, Western-blot analysis, ELISA as well as immunohistochemistry. Activation and regulation of PLUNC transcription was analyzed in a human cornea epithelial cell line (HCE) and a human conjunctiva epithelial cell line (HCjE) after incubation with ocular pathogens. Furthermore, PLUNC concentrations were measured by ELISA in tears from patients with dry eye disease (DED) and compared to healthy volunteers.

**Results:** PLUNC is expressed under healthy conditions at the ocular surface and secreted into the tear film. In tears from DED patients the PLUNC concentration is significantly increased compared to controls. Treatment of HCE and HCjE with bacterial supernatants also revealed a significant increase in PLUNC expression.

**Conclusions:** Our results show that PLUNC is a component of the lacrimal apparatus that seems to play a role in immune defense at the ocular surface and during DED.

#### Rubrik: Zellbiologie

Titel:Effect of chronic intermittent hypoxia (CIH) in mice on left myocardial vascularisation, inflammation and mitochondrial integrity

Autoren: Büttner F.(1),Bonaterra G.(1),Schwarzbach H.(1),Frewer A.(1),Wilhelm B.(1),Schulz R.(2),Kraut S.(3),Weissmann N.(3),Kinscherf R.(1),Hildebrandt W.(1),

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### Abstract:

Introduction: The obstructive sleep apnea syndrome (OSAS) imposes repetitive hypoxiareoxygenation-stress associated with arteriosclerosis and related cardiovascular events. Moreover, OSAS causes ventricular dysfunction of largely unknown morphological and molecular base. Methods: Using a mouse model of OSAS (chronic intermittent hypoxia, CIH, n=11), we studied capillary density (lectin staining), angiogenic and pro-inflammatory gene expression (RT-PCR and IHC) as well as mitochondrial density and integrity (transmission electron microscopy) in the left ventricle compared to control mice (normoxia, NOX, n=10). CIH consisted of 6 weeks (5days/week) of 8h/d of switching pO2 between 6-7% and 21% every 60s. Results: We found a dramatically increased pro-inflammatory expression of COX-2 and TNF- $\alpha$  (RT-PCR: 20- and 180-fold; IHC: 50% and 20% increase of positive cells, respectively). Moreover, the suppressor-of-cytokine-signalling-3 (SOCS-3) was up-regulated 80-fold. Despite a 3- to 7-fold increase in expression of angiogenic genes (VEGFa, VEGF-R, Notch-1, Notch-3), capillary density was only marginally enhanced (5%, p=0.22) in CIH compared to NOX. Moreover, CIH showed a 17- and 69-fold up-regulation of the plasmamembrane-Ca2+-ATPase-4a/b) and -4b (PMCA4a/b and 4b). Whereas mitochondrial density and size were similar, the fraction of intact mitochondria was significantly decreased (24% vs 35%, p<0.05) with a &gt;50% loss of cristae observed in 32% vs 26% of mitochondria in CIH compared to NOX, respectively. Conlusion: CIH results in insufficient myocardial angiogenesis and in mitochondrial damage, which may be attributable to up-regulation of SOCS-3 and cytokines. Moreover, an up-regulation of PMCA-4 may attenuate cardiac hypertrophy and contractility, i.e. adaptation to CIH-related sympathetic activation and hypertension.

Rubrik: Zellbiologie

Titel: Adducins may be involved in endothelial barrier regulation

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Abstract:

Regulation of actin dynamics is critical for endothelial barrier function. Tight regulation of actin polymerization can be achieved by adducins. As actin-binding proteins adducins were reported to regulate epithelial junctional remodeling by controlling either organization of the spectrin lattice or the assembly of actin filaments in the areas of cell-cell contacts. Here we investigated the role of alpha- and gamma- adducin isoforms for endothelial barrier formation by using human dermal microvascular endothelial cells and microvascular murine cells. Immunofluorescence analysis revealed that forskolin and rolipram, agents well-known to stabilize the endothelial barrier by increasing cAMP, induced accumulation of adducin along cell junctions. Moreover, transendothelial electrical resistance measurements revealed that silencing of  $\alpha$ - adducin destabilizated endothelial barrier function. To further test whether the peripheral localization of adducins is functionally linked with integrity of endothelial adherens junctions, endothelial junctional remodeling was induced by a Ca2+-switch assay. Ca2+depletion disturbed both linear VE-cadherin and adducin localization along cell junctions, while Ca<sup>2+</sup>-repletion restored the distribution of VE-cadherin and the membrane staining for adducin. Taken together, our results demonstrate that adducins may be involved in endothelial barrier regulation.

Rubrik: Entwicklungsbiologie

Titel:Developmental dynamics of occipital and cervical somites

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#### Abstract:

Development of somites leading to somite compartments like sclerotome, dermomyotome and myotome has been intensely investigated. Most knowledge on somite development, including the commonly used somite maturation stages, is based on data from somites at thoracic and lumbar levels. Potential regional differences in somite maturation dynamics have been indicated by a number of studies, but have not yet been comprehensively studied. Here, we present a detailed overview on the developmental dynamics of somites at occipital and cervical levels. We show that in these regions, the onset of sclerotomal and myotomal compartment formation is later than at thoracolumbar lev

els, and occurs simultaneously in multiple somites, which is in contrast to the serial cranialto- caudal progression of somite maturation in the trunk. Our data suggest a variant spatiotemporal regulation of somite patterning in occipitocervical somites.

Rubrik: Entwicklungsbiologie

Titel:Spar3 mutant lenses exhibit developmental delay in mice and xenopus

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#### Abstract:

Spar3 is a novel and non-characterized protein of post synaptic density (PSD) with a length of 1776aa. It is widely expressed in brain and belongs to Spar family of proteins, which comprises on Spar1, Spar2 and Spar3.

We generated and characterized a SPAR3 knock-out mouse (KO). As one of the key phenotypic alterations we found that SPAR3 mutant mice display an early onset of cataract. In this respect closer investigations revealed the presence of a heavily disturbed lens fibers shape, structure, arrangement and packing pattern. Surprisingly, the standard expression of major lens structural proteins e.g., alpha and beta crystallins are not altered; therefore it seems that crystallines are not a major contributing factor for the development of early cataracts in these mice. On the molecular level an upregulation of major neuronal marker proteins in homozygous mutant lenses was observed. In addition, the silencing of Spar3 using morpholino oligonucleotides (MO) technology in Xenopus laevis also leads to a very similar, abnormal eye development.

The exact physiological functions of SPAR3 are not known at present, but it seems that the protein has a crucial role during early lens (and eye) development. Further investigations of our KO models will help to further clarify the functional aspects of SPAR3.

Rubrik: Entwicklungsbiologie

Titel:Asymmetrical morphogenesis und signaling during early molecular left-right patterning in the chick

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#### Abstract:

Leftward fluid flow due to cilia rotation is thought to be a common left-right (LR) symmetry breaking mechanism in many model vertebrates but not in the chick. Here, perinodal leftward cell movement between embryonic stages 4 and 5 is suggested to cause the asymmetry of shh and fgf8 expression in the node. Our analysis of the temporal progression of morphological and molecular asymmetry reveals the emergence of early asymmetrical nodal expression with increasing node asymmetry and progressive breaking of the shh domain symmetry. Crucially, the node is morphologically asymmetrical prior to both early nodal expression and leftwards cell movement. This suggests that shh may be a necessary condition for paraxial nodal expression but may not be an inducer of paraxial node asymmetry. Moreover, peripheral expression of the shh receptor patched2 in the lateral plate mesoderm (LPM) and of its ligand in the tissue beneath indicate functional decoupling of asymmetrical nodal expression in the LPM from the paraxial shh domain. We propose a leftright patterning model in the chick in which the emergence of node asymmetry is due to redundant mechanisms such as differential cellular proliferation and asymmetrical cellular migration towards the primitive streak; these, in turn, may be influenced by asymmetrical activity of the gastric proton-potassium ion pump ATP4. Interestingly, cilia are present and left-right dynein is expressed in the notochord at stage 6, which suggests that cilia and ciliarelated proteins in the chick and possibly all vertebrates play a functional role during later stages of LR patterning.
Rubrik: Entwicklungsbiologie

Titel:Severe cardiac alterations in homozygous murine desmoglein 2-mutant embryos

Autoren: Venger K.(1),Kant S.(1),Leube R.(1),Krusche C.(1),

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### Abstract:

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a disease of the desmosome. It is characterized by ventricular dilation, myocardial fibrosis and heart failure. ARVC patients are prone to arrhythmia which may lead to sudden cardiac death. We recently described knock-in mice, in which the gene coding for the desmosomal cadherin desmoglein 2 was substituted by a mutant desmoglein 2 allele. The encoded polypeptide lacks part of the adhesive extracellular domains 1 and 2. Homozygous animals (DSG2mt/mt) survive embryogenesis and develop an ARVC-like phenotype. A large percentage of DSG2mt/mt mice, however, die before birth. The object of the current study was to identify the reason of intrauterine death.

Embryos were dissected at different time points of pregnancy, genotyped and subjected to routine histological staining. We found a near Mendelian ratio of progeny around E12. Serial hematoxylin and eosin-stained sections of paraffin-embedded embryos, however, revealed cardiac alterations of different severity in a large percentage of the DSG2mt/mt embryos. These alterations included disrupted cardiac walls with hemopericardium, transmural lesions in ventricular and atrial myocardium and abnormally thin atrial walls. Some of the embryos were already pale and tissues started to decompose indicating that cardiac dysfunction led to embryonal death. Immunohistochemistry of DSG2mt/wt embryos demonstrated that desmoglein 2 is localized at cell-cell contact sites of E12 cardiomyocytes. We conclude that desmoglein 2 is important for embryonic cardiac development. The manifestation after the start of coordinated cardiac contractility suggests a crucial contribution to the biomechanics of early heart action.

Rubrik: Entwicklungsbiologie

Titel:Longitudinal change in knee cartilage thickness and subchondral bone area in adolescent athletes

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### Abstract:

Objective: It is unclear whether joint cartilage thickens during growth, and whether thickness changes differ between girls and boys. The objective was to compare a potential increase in knee cartilage and subchondral bone areas (SBA) between young male and female athletes at the end of adolescence.

Methods: The dominant legs of 16 young top volleyball players (Olympiastuetzpunkt Berlin; 8 female, 8 male; age 15-17 years) were studied. Baseline and two-year follow-up MR images were acquired, and the thickness and SBAs of the medial and lateral tibial and femoral cartilages were computed after segmentation. Differences between both sexes were computed using unpaired t-tests.

Results: The increase in total femorotibial cartilage thickness was +2.7% (95% confidence interval: +1.1; +4.1) and amounted to +2.2% in boys (-0.5; +4.8) vs. +3.3% (+1.6; +5.0) in girls. The difference in the rate of change was not significant (p=0.867). The increase in femorotibial SBAs was +1.0% (+0.4; +1.6), and amounted to +0.8% in boys (-0.2; +1.9) vs. +1.2% (+0.2; +2.1) in girls. Again, the difference was not significant (p=0.871). Conclusion: A substantial increase in femorotibial cartilage thickness (and SBAs) was observed in young athletes towards the end of adolescence, i.e. a period during which the epiphyseal line is closing. These differences did not differ significantly between boys and girls. This thickness increase must be taken into account when determining longitudinal cartilage change in young athletes after injury, in order to adequately differentiate pathological (post traumatic) change from that occurring physiologically.

Rubrik: Entwicklungsbiologie

Titel:Expression of Atoh8 in vascular wall resident stem cells of the developing and adult mouse aorta

Autoren: Koc Ü.(1), Theiss C.(1), Stoll O.(2), Wagner N.(2), Ergün S.(2), Brand-Saberi B.(1),

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### Abstract:

Angiogenesis defines the outgrowth of new vessels from preexisting blood vessels, whereas vasculogenesis describes the de novo vessel development from stem and progenitor cells. Recently, different progenitor and stem cell types have been identified within adult vascular intima and adventitia suggesting the vascular wall as an important source of postnatal vasculogenesis. Atoh8 is a member of the basic helix loop helix transcription factor family and is known to be indispensable for early embryonic development. Our study aims at characterization of Atoh8 expression pattern in the developing and the adult mouse aorta using aortic ring assay analyses.

We performed immunohistochemistry for Atoh8, CD34, and a-SMA on sections of developing and adult mouse aorta after ring assay. The stainings were analyzed using confocal laser scanning microscopy.

Our analyses revealed a specific Atoh8 expression pattern in the aortic intimal layer of early development stages while in later development stages; also some single cells of aortic media and adventitia were positive for Atoh8. Furthermore, sporadically a coexpression of Atoh8 with a-SMA and CD34 could be found. Interestingly, after ring assay both cells covering the aortic lumen and cells forming capillary-like structures within the collagen gel were strongly positive for Atoh8. Also some cells of aortic adventitia exhibited a strong Atoh8 expression after ring assay.

In summary, our data show a shift of Atoh8 expressing cells within the developing aortic wall layers and demonstrate a strong Atoh8 expression in the adult vascular wall resident stem cells along with their activation in ring assay.

Rubrik: Entwicklungsbiologie

Titel:Gap junctional communication through connexin channels is essential for primitive endoderm formation in embryonic stem cell derived embryoid bodies

Autoren: Wörsdörfer P.(1), Edenhofer F.(1), Willecke K.(2),

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### Abstract:

Connexins (Cx) are expressed during pregastrulation development, form gap junctions and contribute to the establishment of defined communication compartments in the early conceptus. A critical function of gap junctions during early development, however, could not be demonstrated yet. The connexin isoforms Cx43 and Cx45 are coexpressed during that developmental stage. Here we describe, the generation and characterization of Cx43/Cx45double deficient mouse embryonic stem cells (mESCs). They were differentiated into embryoid bodies (EBs), an in vitro model for pregastrulation and early gastrulation. While Cxdeficient mESCs did not show any obvious phenotype regarding cell proliferation or apoptosis, we observed that the expression of Cx43 and Cx45 is required for the establishment of primitive endoderm (PE) in EBs. Lentiviral overexpression of either Cx43 or Cx45 rescues the observed phenotype in Cx43/45-deficient mESCs, indicating a redundant function of these isoforms during the process. Defective PE formation leads to a block in subsequent differentiation events, in particular germ layer specification. Viral overexpression of the oculodentodigital dyplasia associated mutant Cx43 G138R in Cx43/Cx45-deficient ESCs indicates that functional gap junctional communication is required for the proper differentiation of ESCs in 3-dimensional cell aggregates.

Rubrik: Entwicklungsbiologie

Titel:Axonal pathfinding of the accessory nerve

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Abstract:

The accessory displays a unique axonal pathfinding. Its axons ascend for many segments along the longitudinal axis after exiting the cervical spinal cord and medulla oblongata before they bend ventrally into the periphery. Little is known about how this organization is achieved. We here investigated the function of different embryonic structures in guidance of motor axons of the accessory nerve. First, we observed that the axon root pattern of the accessory nerve is controlled by intrinsic properties of the neural tube. Then we found that the motor axon exit point is required for the correct pathfinding of the accessory axons. Neural crest cells contribute also to the axonal pathfinding of this nerve. Moreover, sensory ganglia induce the ventral projection of the accessory axon.

Rubrik: Reproduktionsbiologie

Titel:Adiponectin modulates the glucose uptake of the preimplantation embryo in a diabetic pregnancy

Autoren: Fischer S.(1), Schindler M.(1), Thieme R.(2), Fischer B.(1), Navarrete Santos A.(1),

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### Abstract:

Worldwide the incidence of diabetes mellitus is increasing dramatically. Type 1 diabetes mellitus has been found to negatively affect pregnancy by causing miscarriage and poorer prenatal outcomes in humans. Hormones of the adipose tissue, like adipokines play a crucial role in the aetiology of metabolic diseases. Adiponectin is a 26 kDa peptide hormone, which regulates lipid and carbohydrate metabolism via AMPK.

The rabbit reproductive model was used to investigate the effects of a maternal diabetes mellitus on the embryonic adiponectin regulation and glucose metabolism. Diabetes mellitus was experimentally induced by alloxan in female rabbits resulting in a complete loss of endogenous insulin (hypoinsulinaemia) followed by elevated blood glucose levels about 14 mmol/L (hyperglycaemia).

An increased adiponectin concentration (1.5-fold) was measured in the blastocystic fluid of embryos from diabetic rabbits. To analyse the effects of a maternal diabetes mellitus on the embryonic glucose metabolism glucose transporters (GLUT) 1, 3 and 4 were analysed via qRT-PCR. All three GLUTs showed an increased mRNA expression in blastocysts of a diabetic pregnancy. An in vitro supplementation with adiponectin (1µg/ml) to these blastocysts activated AMPK and Akt. Furthermore adiponectin caused an increase in GLUT1 mRNA expression and forced the translocation of GLUT4 and consequently an increase in glucose uptake.

Blastocysts from diabetic rabbits compensate the lack of maternal insulin via adiponectin in order to maintain the embryonic glucose uptake. In diabetes, this failsafe system may compensate for the loss of insulin and helps to maintain embryo development.

Supported by: DFG (NA 418/4-2); EU (Epihealth 278418)

Rubrik: Reproduktionsbiologie

Titel:Expression of tight junction proteins claudin-3, -7, and -10 in murine endometrium during early implantation

Autoren: Schumann S.(1), Grümmer R.(1),

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### Abstract:

The synchronized crosstalk between the blastocyst and the receptive endometrium is crucial for successful implantation of the mammalian embryo. Prior to implantation, the uterine epithelial cells, which are the first to interact with the implanting blastocyst, exhibit an apikobasal polarity which is maintained by tight junctions. Members of the claudin multigene familiy represent one of the major constituents of these cell contacts which are important for the selective permeability and thus for the function of the epithelium.

Here, we analysed the expression of different claudins in the uterine epithelium of nonpregnant compared to pregnant mice prior to implantation and in the primary decidual zone during the peri-implantation period at 4.5 days post copulationem (dpc). Since gene expression profiles revealed a strong expression of claudin-3, -7 and -10 in the endometrium of pregnant mice at 4.5 dpc, expression of the corresponding proteins was analyzed by immunohistochemistry.

The non-pregnant murine endometrium showed a strong expression of claudin-3 and -7 in the luminal epithelium, whereas expression of claudin-10 was not detectable. In pregnant mice, expression of claudin-3 and -7 was reduced in the endometrial epithelium adjacent to the blastocyst at 4.5 dpc but remained in the inter-implantation sites. Interestingly, expression of claudin-10 was clearly induced in the cells of the primary decidual zone at 4.5 dpc.

This specific spatial and temporal regulation of the different claudin genes in the endometrium during the early implantation phase points to a role of these proteins in regulating embryo implantation and trophoblast invasion.

Rubrik: Reproduktionsbiologie

Titel:Cell-cell adhesion in decidualized human endometrium

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### Abstract:

Endometrial stromal cells differentiate into decidualized stromal cells under the influence of progesterone in the late secretory phase of the human menstrual cycle and during early pregnancy. Aim of our investigation was to assess the occurrence of cell-cell junctions between decidualized cells and between trophoblast and decidualized cells during early pregnancy and in cell culture.

Decidual tissue was obtained after elective termination of normal healthy pregnancies (6-14 weeks of pregnancy) and was investigated on paraffin and cryostat sections.

Immortalized human endometrial stromal cells (T-HESC) were decidualized in vitro and cocultured with the human hybridoma cell line AC-1M88, which is a fusion of primary extravillous trophoblast cells with the choriocarcinoma cell line JEG-3.

Desmosomes, adherens and tight junctions were assessed by immunofluorescence against the desmosomal proteins desmoplakin, desmoglein 2 and plakophilin, the adherens protein E-cadherin and the tight junction protein ZO-1.

Decidual tissue or in vitro decidualized stromal cells showed no staining for any adhesion proteins. In contrast, the cytotrophoblast cells stained positive for all adhesion markers. Interestingly, this was also the case for single extravillous trophoblast cells that invaded the decidual cell layer, both, in vivo and in vitro.

Although, during the process of decidualization, the uterine stromal cells change from a fibroblastic to an enlarged, rounded epitheloid-like morphology, no typical adhesion markers could be detected between decidualized cells. The expression of adhesion markers in single extravillous trophoblast cells might be a prerequisite for the invasion of glandular and vascular structures.

Rubrik: Reproduktionsbiologie

Titel:Localisation of prostaglandin synthesizing enzymes (cylooxygenase 1 and 2, prostaglandin E synthase) in the ovary of the ostrich (struthio camelus)

Autoren: Rodler D.(1), Sinowatz F.(1),

Adressen:(1)Ludwig-Maximilians-Universität München|Lehrstuhl für Anatomie, Histologie und Embryologie, Department of Veterinary Sciences|München|Bayern; email:d.rodler@anat.vetmed.uni-muenchen.de

### Abstract:

Prostaglandins have been recognized as key molecules in female reproductive functions, like ovulation and implantation. Recent studies in mammalian ovaries suggest that prostaglandins produced from arachidonic acid by cyclooxygenases and specific terminal prostanoid synthase enzymes may be involved in these processes, but almost nothing is known in birds.

A total of 24 female ostriches were used in the present study. Tissue samples were fixed in Bouin's fluid for 12 h. Immunostaining for cyclooxygenase 1 (cox 1) cyclooxygenase 2 (cox 2), and prostaglandin E synthase (PTGES) was performed using the avidin-biotin-complex technique. Additional ultrastructural studies were performed to characterize the fine structure of the follicular wall. Whereas the expression of cox 1 was confined to the ovarian surface epithelium, endothelium and smooth muscle cells of blood vessels, a complex immunostaining pattern could be demonstrated for cox 2: Whereas the granulosa cell layer of primordial follicles ((90-100 µm in diameter) showed weak immunostaining, the pseudostratified columnar granulosa cell layer in late pre-vitellogenic follicles (150–400 µm in diameter) was distinctly immunopositive. The oocytes in vitellogenic follicles contained a variable amount of yolk depending on the stage of development. Their simple cuboidal granulosa cell layer showed only a weak immunostaining. During the follicular development, the growing oocytes were always negative for cox 2. The immunostaining pattern for PTGES was similar to that observed to cox 2. The attenuation of cox-2 and PTGES immunostaining in the follicular wall of vitellogenic follicles does not support the idea that prostaglandins are involved in ovulation in ostrich.

Rubrik: Reproduktionsbiologie

Titel:Impact of the endocrine disrupting chemical DEHP on female fertility and adipogenesis in mice

Autoren: Schmidt J.(1), Schädlich K.(2), Fiandanese N.(3), Pocar P.(3), Fischer B.(2),

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### Abstract:

The rising prevalence of obesity is of major health concern worldwide. Next to overnutrition and physical inactivity, epidemiological studies have shown a positive correlation between accumulation of EDCs and body mass and waist circumferences. The endocrine disruptor Di(2-ethyl-hexyl) phthalate (DEHP) and its metabolites are known

to interfere with energy metabolism, mainly by activation of PPARs. In addition, exposure to DEHP has been associated with adverse effects on gonadal development and subfertility in the male. However, effects of DEHP on female reproductive health and metabolism are still scarce.

We dietary exposed mature female C3H/N mice to environmental and pharmacological concentrations of DEHP [0, 0.05, 5 and 500 mg DEHP/kg bw/day] for a period of eight weeks. During this period the mice were mated, gave birth and nursed their offspring. After weaning dams were sacrificed, and samples of various tissues and blood were collected for analysis by qRT-PCR and ELISA.

Exposed mice gained significantly more body weight compared to controls and visceral fat tissue (control: 0.7% fat of bw; 5 mg DEHP: 3.2%) and had hypertrophic adipocytes. In the liver, key genes of the lipid metabolism and PPARs were deregulated. In-utero and lactational exposure led to adipogenic effects in the F1 offspring, too, with significantly elevated body weights and visceral fat tissue. F2 preimplantation embryos (exposed only via germ cells) had downregulated leptin and leptin receptor expression.

Dietary DEHP exposure at a critical ontogenetic window stimulates adipogenesis in female C3H/N mice and their offspring.

Supported by EU (FP7-REEF N 212885) and the Wilhelm Roux Programme of the Martin Luther University Faculty of Medicine

Rubrik: Reproduktionsbiologie

Titel:Peroxisomes are essential for regular spermatogenesis

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### Abstract:

Peroxisomes are cell organelles with important functions in the metabolism of lipids and reactive oxygen species. In germ cells, they have only recently been described by our groups. Their role for spermatogenesis has not been characterized in detail yet. In order to understand the function of peroxisomes for the development of male germ cells we have established a mouse model for conditional knockout of Pex13 in pre-meiotic germ cells. Pex13 is a peroxisomal membrane protein that is required for import of peroxisomes with loss of all metabolic functions. We have used a mouse strain with Pex13 flanked by loxP sites which is recognized by a cre recombinase. The floxed Pex13 mice were crossed with a transgenic mouse strain expressing the cre recombinase under control of the Stra8 promoter to generate mice with Pex13 knockout in male germ cells. Histological analysis of knockout mice revealed a severe distortion of cell differentiation during spermatogenesis with the generation of multinucleated giant cells instead of regular spermatozoa.

Rubrik: Reproduktionsbiologie

Titel:Influence of experimental diabetes mellitus type 1 on the expression of cholesterol metabolism genes in rabbit embryos and maternal tissues

Autoren: Pendzialek M.(1),Schindler M.(1),Gürke J.(1),Haucke E.(1),Plösch T.(2),Fischer B.(1),Navarrete Santos A.(1),

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### Abstract:

According to the developmental origins of health and disease (DOHaD) paradigm, a maternal diabetes mellitus type 1 is associated with an increased prevalence of neonatal macrosomia and metabolic diseases in offspring later in life. Recent studies indicate a link between dyslipidemia of diabetic mothers and an altered lipid metabolism in embryos and fetuses. Metabolic programming of postnatal growth and physiology can already be induced during preimplantation development. We have recently shown that rabbit blastocysts grown under diabetic conditions in vivo (expIDD) generate considerably more lipid droplets. Therefore alterations in cholesterol metabolism could be a possible target for metabolic programming. We have analyzed maternal cholesterol metabolism and its influence on preimplantation embryo development in rabbits under induced diabetes. Maternal cholesterol levels in serum and uterine fluid were increased and accompanied by changes in the lipoprotein composition of VLDL, LDL and HDL. Additionally, in hepatic and adipose tissue the mRNA expression of HMGCR, LDLR, VLDLR, SREBP-2, Insig-1 and CYP7A1 was altered. During preimplantation embryo development HMGCR, LDLR and SREBP-2 were expressed from day 3 p.c. onwards, whereas VLDLR and Insig-1 were first present at the blastocyst stage. CYP7A1 was not detectable until day 8 p.c. Blastocysts grown under in vivo diabetic conditions showed only an increased SREBP-2-to-Insig-1-ratio and an increase in LDLR mRNA expression. In vitro experiments revealed a glucose-, but not insulin-dependent HMGCR expression in blastocysts. We conclude that the altered maternal cholesterol metabolism due to diabetes mellitus type 1 has only a minor impact on the cholesterol metabolism of very early stages of embryogenesis.

Rubrik: Reproduktionsbiologie

Titel:Comparison of mouse and human sperm beat frequency in the early activation by HCO3

Autoren: Wandernoth P.(1), Mannowetz N.(2), Szczyrba J.(1), Wennemuth G.(1),

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### Abstract:

Bicarbonate is an essential factor to regulate motility in both murine and human sperm. It is present in the seminal plasma as well as in the female reproductive fluid and initiates the sperm-specific soluble adenylyl cyclase (sAC) pathway to enhance sperm beat frequency. By analyzing and comparing human and mouse sperm motility, we detect significant differences in the basal beat frequency as well as in the response to bicarbonate. Whereas the beat frequency of murine sperm rises from 3 Hz to a maximum of 7 Hz, the frequency of human sperm increases from 6 Hz to a maximum of 15 Hz upon application of bicarbonate. Also the recovery rates differ significantly from each other. It takes mouse sperm approximately 10 minutes to reach basal levels of beat frequency, whereas human sperm need over 80 minutes for recovery. Interestingly, the application of fresh seminal plasma to human sperm has no accelerating effect on beat frequency and the cells become bicarbonate-sensitive only after removing the seminal plasma. However, this inhibitory effect does not account for murine sperm which display increased levels of beat frequency by application of fresh seminal plasma. In summary, we show that components of the seminal plasma have a species-specific impact on sperm with regard to their bicarbonate-mediated acceleration of beat frequency. We therefore propose that during evolution, humans - compared to mice developed an additional selective mechanism during sperm maturation thus improving the fertilizing capacity of human spermatozoa.

Rubrik: Immunbiologie

Titel:Obese rats show altered NK-cell functions and increased lung metastasis

Autoren: Hanke J.(1),Knauf D.(1),Spielmann J.(1),Jahn J.(1),Jacobs R.(2),Ben-Eliyahu S.(3),Kielstein H.(1),

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### Abstract:

Introduction: Obesity was identified as a major risk factor for malignant diseases, but underlying mechanisms remain unclear. Natural killer (NK)-cells, a pivotal aspect of innate immunity, are capable of identifying and killing virally infected cells and tumor cells. Previous studies have shown altered NK-cell functions in obesity, and the c urrent study aimed at investigating the relationship between altered NK-cell functions and increased cancer risk in obesity.

Methods: To induce obesity, 64 male F344-rats received a high fat diet (34% fat) or a control diet (4% fat) for 6 - 10 weeks. Thereafter, animals received 106 cells of an adenocarcinoma syngeneic tumor (MADB106) or a vehicle by i.v. injection. 15 min after injection, 32 rats were killed, lungs removed and immunohistochemically stained. Numbers of NK-, MADB106-cells and NK-cell-tumor-cell-interactions were quantified. Three weeks after tumor-cell injection the second group of rats (n=32) was killed and lung metastases were counted.

Results: After short term MADB106-challenge, obese animals showed significantly decreased NK-cell-numbers (63 vs. 1064 per 40 mm2) and NK-cell-tumor-cell-interactions (0.3 vs. 1.3 per 40 mm2) in the lung as compared to their control littermates. Three weeks after injection, the lungs of the obese rats showed significantly more lung metastases than the control animals (49 vs. 22 per lung).

Conclusion: Induction of obesity in F344-rats leads to altered NK-cell functions against tumor cells and results in significantly enhanced lung metastasis as compared to lean animals. It can be hypothesized that the obesity-induced altered NK-cell functions play an important role in cancer growth and metastasis.

Rubrik: Immunbiologie

Titel:In NTera2-D1 cells, antibodies directed to the gram negative bacterium Neisseria gonorrhoeae (NG) cross-react with the heat shock protein Hsp60 and lead to impaired neurite outgrowth

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### Abstract:

In humans, maternal first trimester gonococcal infections lead to an increased risk for psychotic symptoms in later life (1). Since the underlying mechanisms have not yet been clarified, we investigated here interactions and effects of a commercial rabbit antiserum directed to Neisseria gonorrhoeae (alphaNG) in human NTera2-D1 cells. Fluorescent immunocytochemistry revealed alphaNG to label antigens within an intracellular organelle, which by subsequent Western blot analysis revealed a molecular weight between 56 and 72 kDa. In contrast, an antiserum directed to Neisseria meningitidis (alphaNM) reacts with an antigen of a higher molecular weight of between 72 and 95 kDa, revealing thereby the observed interactions to be antibody specific. By two-dimensional Gel-electrophoresis combined with partial Western transfer, a protein spot with strong alphaNG specific crossreactivity could be isolated, and identified by LC-Q-TOF analysis as mitochondrial heat shock protein Hsp60. This result could be confirmed by Western blot analysis for interaction of alphaNG with a commercial Hsp60 protein sample, with which alphaNM failed to interact. Finally, effects of alphaNG on neurite outgrowth in retinoic acid stimulated NTera2-D1 cells was analysed, demonstrating that 10µg/ml alphaNG leads to decreased neurite length, whereas 10µg/ml alphaNM has no such effect. These results demonstrate that alphaNG indeed interacts with human mitochondrial heat shock protein Hsp60, however how this interaction leads to diminished neurite outgrowth in NTera2-D1 cells, and whether this is of pathogenetic relevance for psychotic symptomatology remains to be clarified in the future. (1) Sørensen et al., 2009, Schizophrenia Bull. 35, 631-637

Rubrik: Immunbiologie

Titel:Imaging of epithelial repair processes in mouse trachea after laser induced injury

Autoren: Kretschmer S.(1), Linz N.(2), König P.(1),

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### Abstract:

The integrity of the airway epithelium is essential for a normal airway function. Small lesions in the airway epithelium occur frequently and have to be repaired to prevent prolonged disruption of epithelial integrity. To better understand the repair process of small lesions of the epithelium we used two-photon microscopy and an ex-vivo murine trachea model. The trachea was cut longitudinally and was imaged with the epithelium facing up. Epithelial lesions were induced by focussing Ti-sapphire femtosecond laser pulses to single epithelial cells for 1-5 seconds (average output power 80mW at 80 MHz). Staining with propidium iodide (PI) allowed identification of damaged cells. Specific damaging of cells in a distinct area of the epithelium was possible and repair processes were visualized for up to four hours. Depending on the irradiation time an area of 1-12 cells was damaged. Hyperfluorescence around the beam focus and/or loss of autofluorescence in adjacent cells was observed. Within this lesion nuclei were stained with PI. Damaged cells were expelled into the lumen. In small lesions with up to 3 cells expulsion was observed within 30 min but in larger lesions this process took up to several hours. Adjacent epithelial cells changed their shape and started to close the lesion by stretching. Damages of 5 or more cells did not close completely within the observation time. However, small lesions of 1-3 cells were closed within 2-3 hours. With this technique selective damaging of the epithelium and observation of consecutive repair processes are possible.

Rubrik: Immunbiologie

Titel:Two distinct CD3 antibodies reveal significant functional differences in t cell activation

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### Abstract:

T-cells interact via their T-cell receptor (TCR) with an antigen offered on antigen presenting cells (APCs). In the presence of co-stimulatory factors such as CD28-ligand this TCR-antigen interaction triggers T-cell activation. Because CD3 receptors are the signaling part of the TCR, immobilized anti-CD3 and soluble anti-CD28 monoclonal antibodies (mAb) are usually used to mimic this situation in vitro. We found that anti-CD3 mAb are able to trigger proliferation in human T lymphocytes even in the absence of a co-stimulus and applied soluble. Hereby monocytes seemed mostly required for this anti CD3 driven mitogenic effect on T-cells. Furthermore, we investigated the impact of two different soluble applied CD3 mAbs on freshly isolated human PBMC (peripheral blood mononuclear cells). PBMCs of all healthy donors tested to date responded to treatment with CD3/OKT3, but only 41% of them responded to CD3/UCHT-1 treatment. Hereby 60% of males and 24% of females were UCHT-1-responders. In case of leukemic as well as melanoma patients the amount of UCHT-1-responders increased up to 63% with significant more female responders (71%). Again OKT3 activated PBMCs of all donors. Cytokine assays, re-stimulation experiments and cell signaling analysis revealed some remarkable differences between these two antibodies. Our results allowed the hypothesis that binding of CD3 leads to cytokine release and proliferation by a mechanism distinct to the usual TCR signaling. The responsiveness towards CD3/UCHT-1 seems to reflect the priming status of the individual T-cells of a donor and could be considered as functional marker in the context of a personalized medicine.

### Rubrik: Immunbiologie

Titel:The significance of the immunohistochemistry in sebaceous gland carcinomas of the eyelid

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### Abstract:

INTRODUCTION: Ocular sebaceous carcinoma (OSC) is a rare entity of the eyelid that tends to occur in older adults 60 to 80 years of age, being more common in women. This uncommon cancer can have similar microscopical features with ocular basal cell carcinoma (OBCC), but the rates of distant metastases and tumor death in OSC have been reported to be higher. AIM: Because a strict differentiation of OSC from OBCC is required, our study identified the immunohistochemical findings that can be useful for differentiating those two entities. MATERIALS AND METHODS: The histopathological features of all cases with OSC and OBCC admitted in the Clinic of Ophtalmology, "Prof. Dr. N. Oblu" Emergency Clinical Hospital la?i in 10 years (01.01.2002 - 31.12. 2011) were retrospectively reviewed. In each case, an immunohistochemical detection was performed using Monoclonal Mouse Anti-Human Cytokeratin Clones AE1/AE3, Monoclonal Mouse Anti-Human Epithelial Membrane Antigen Clone E29, and Monoclonal Mouse Anti-Human Ki-67 Antigen, Clone Ki-67 (Dako, Denmark). Results: There were 2 female patients with OSC. 59 cases had OBCC, among them being 43 male patients. Regarding the immunohistochemical features, OSCs revealed strong positivity for EMA in all the areas with sebaceous differentiation, and moderate positivity for CK only in the peripheral areas of tumoral islands where basaloid features were identified. In contrast, in expression of EMA was uncommon in all the cases of OBCC, but CK was positive in all tumoral cells. The mean Ki-67labelling index was 20% in OSC and 10% in OBCC. CONCLUSIONS: Our study suggests that immunohistochemical staining for EMA can be a valuable marker of sebaceous differentiation.

Rubrik: Immunbiologie

Titel:The effect of carbon black nanoparticles on airway epithelial cells is dependent on their surface chemistry

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#### Abstract:

The airway epithelium is the first barrier that is contacted by airborne nanoparticles. To date it is not clear how surface modifications determine the reaction of the airway epithelium. Therefore, we tested the short term toxic effect of 14 nm Carbon black nanoparticles (CBNP) with unmodified, nitroanthracene-, benzo[a]pyrene- and polycyclic aromatic hydrocarbons (PAH)-modified surfaces on the tracheal epithelium ex vivo. Concentrations up to 30 µg/ml and incubation times of 6 to 24 hours were used. Video microscopy was utilized for functional analysis. Damage of tracheal epithelium was evaluated by scanning electron microscopy. Apoptosis and integrity of the cell membrane were detected by activated caspase-3 and ethidium homodimer-1 intake, respectively. The expression of pro-inflammatory cytokine and mucin mRNA was analysed by real time PCR. Unmodified and surface modified CBNP increased ciliary beat frequency. However, all reduced cilia-driven particle transport. Modified CBNP induced epithelial damage, where PAH was the most toxic as evaluated by scanning electron microscopy. PAH-surface caused cell apoptosis and cell membrane damage, but no mucus release. Cellular damage resulted in dead cells on the epithelial surface that interfered with particle transport. In contrast, unmodified CBNP did not induce obvious cell damage but induced mucus release resulting in particle-mucus-aggregates and reduced particle transport in the upper part of the trachea. Mucine mRNA expression was not increased indicating mucus release from submucosal glands. In addition to cellular damage PAH induced inflammatory cytokine mRNA levels. In conclusion, all CBNP influenced epithelial function, but the effect is dependent on their surface modification.

Rubrik: Immunbiologie

Titel:Intravital imaging of airways with optical coherence microscopy

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### Abstract:

Current imaging techniques lack the ability to analyze ciliated cell function through the intact airway wall. To circumvent this problem, we build an optical coherence microscope (OCM) which combines confocal microscopy and optical coherence tomography. For ex vivo OCM the trachea of the mouse was placed in a culture dish filled with Hepes-Ringer solution. For intravital experiments the trachea of an anesthetized and ventilated mouse was exposed. The microscope was triggered by the ventilator and imaging was done during inspiratory hold. Images were taken with a custom built OCM with a 40× objective (NA

0.8).

With OCM we could image the whole tracheal wall up to the airway lumen in the ex vivo and the ventilation triggered in vivo setup. Epithelial cells and ciliary beating could be observed though the tracheal wall. Time-lapse OCM enabled the analysis of cilliary beat frequency and the transport of added particles. In the connective tissue the most prominent signals were recorded from chondrocytes and from fibers which could be detected throughout the tracheal wall. In some z-stacks leukocytes were observed in the connective tissue. Lymph and blood vessels could be identified and erythrocytes were seen inside blood vessels. Multiple z-stacks over hours could be imaged without obvious damage of the tracheal tissue. OCM is a useful tool for imaging throughout the whole tracheal wall with cellular resolution and is a suitable method to analyze epithelial function in living mice.

Rubrik: Immunbiologie

Titel:Colocalization of TRKa and HLA-DR in a subpopulation of DCS is dependent on inflammation stage of human dental pulp

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### Abstract:

Dendritic cells (DCs) are specialized to maintain T cell tolerance in response to self components in the uninfected steady state and to initiate and regulate the response of T cells to pathogens in infection. Immature DCs are equipped with receptors such as Toll-like receptors, nucleotide-binding oligomerization domain proteins, cytokine and chemokine receptors in health and inflammation.

The biological effects of nerve growth factor (NGF) are mediated by the low-affinity p75 neurotrophin receptor and the tyrosine protein kinase receptor TrkA. In addition to the regulation of the neuronal cells, NGF is involved also in the survival, maturation, and differentiation of a variety of immune cells. In the dental pulp, however, the in vivo expression of TrkA on human DCs in health and in hyperaemia, acute and chronic dental pulp inflammation are unknown.

Using decalcified, frozen-sectioned, free-floating sections of healthy (n=6), hyperaemia (n=6), acute (n=4) and chronic (n=6) inflamed human molars, we detected by avidin-biotinperoxidase complex method TrkA in cells with long dendritic processes around blood vessels and beneath the odontoblast layer in hyperaemia and acute inflamed dental pulp. By confocal-double immunofluorescence analysis, a colocalization of TrkA and HLA-DR was confirmed in a subpopulation of HLA-DR-positive DCs.

We conclude that TrkA is involved in the regulation of a subpopulation of HLA-DR-positive DCs during the hyperaemia and acute serous inflammation stages to initiate and regulate the response of T cells against caries pathogens in the inflamed human dental pulp.

Rubrik: Immunbiologie

Titel:Insulin-like growth factor-1 is expressed in classical and nodular lymphocytepredominant Hodgkin's lymphoma tumour cells

Autoren: Eppler E.(1), Janas E.(1), Wenger M.(1), Weidmann L.(1), Tinguely-Kovarik M.(2), Moch H.(2), Fellbaum C.(3),

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### Abstract:

Hodgkin's lymphoma is among the most frequent nodal lymphomas in the Western world. Hodgkin's lymphoma is classified in two disease entities: nodular lymphocyte-predominant Hodgkin's lymphoma (NLPHL) and classical Hodgkin's lymphoma (cHL) which represent 95% of all Hodgkin's lymphomas. Hodgkin's lymphomas lesions are characterized by a minority of clonal neoplastic cells, namely in cHL Hodgkin and Reed-Sternberg (HRS) cells and their variants, and in NLPHL the lymphocyte-predominant (LP) cells, respectively, both within a microenvironment of reactive T and B cells, macrophages, plasma cells and granulocytes which are assumed to support proliferation and maintenance of the neoplastic cells through cytokines, chemokines and growth factors. Insulin-like growth factor I (IGF-I) is an important growth factor mainly produced in the liver with growth hormone as major stimulus for its synthesis and release into the circulation. IGF-I is involved in proliferation, differentiation, apoptosis and cell survival of numerous organs including immune tissues, and there exist indications for a role in tumour pathogenesis and sustainment. Although in recent years evidence has accumulated that Hodgkin lymphoma is characterized by a profound disturbance of cell differentiation and apoptosis mechanisms, a potential involvement of IGF-I in Hodgkin's lymphoma has not been systematically investigated to date. We have localized IGF-I by double-immunofluorescence in frequent neoplastic cells of all cHL and NLPHL cases investigated. Additionally, IGF-I immunoreactivity was detected in high endothelial venules and in different immune cell types within the surrounding tissue. We assume that IGF-I plays an anti-apoptotic role in tumour pathogenesis and in shaping the tumour microenvironment.

Rubrik: Neuroregeneration/Neurodegeneration

Titel: New insights towards protective effects of osteopontin (OPN) on degenerative changes of retinal neurons *in vitro* 

Autoren: Lehmann J.(1), Garreis F.(1), Neumann C.(1), Paulsen F.(1), Scholz M.(1)

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### Abstract:

Osteopontin (OPN) is an age-dependently increased agueous humor factor, associated with degenerative changes of the optic nerve and the retina in the well described animal model for neurodegenerative changes in the eye, the DBA2/J mouse. Furthermore, OPN contributes to wound healing, neovascularization, neuroprotection and remodeling of extracellular matrix in the eye. In our study, we analyzed in vitro the protective effect of OPN on murine neuronal precursor cells (RGC-5) of the mouse retina. Basal expression of OPN as well as certain integrin receptors and the cell-surface glycoprotein CD44 have been analyzed by RT-PCR. Immunofluorescence was performed to confirm expression of all investigated proteins on protein level. Regulation of OPN receptor expression after stress induction with H<sub>2</sub>O<sub>2</sub> (300 uM) was analyzed by Real time RT-PCR. The metabolic cell activity was analyzed by using the CellTiter 96 Aqueous MTS Assay, with and without blocking of Integrin and CD44-Receptors. We currently examine the morphological and physiological eye phenotype (Retina and *N. opticus*) of the OPN<sup>-/-</sup>//DBA2/J mouse, a new established inbred strain, at different ages compared to the OPN<sup>-/-</sup>, DBA2/J and the pigmented wild-type control C57/BI6. In further studies, we aim to investigate the (patho-)physiological loss-of-function effect in the OPN<sup>-/-</sup> mouse.

Rubrik: Neuroregeneration/Neurodegeneration

Titel:Whole body vibration (WBV) and manual locomotion therapy (MLT) fail to restore glutamaergic input to the ventral horn and clarke's column after spinal cord injury (SCI) in rats

Autoren: Scholl C.(1), Stein G.(2), Ankerne J.(1), Papamitsou-Sidoropolou T.(3), Eisel L.(1), Harrach R.(1), Schempf G.(1), Wirth F.(1), Ozsoy U.(4), Ozsoy O.(5), Masson I.(1), Wolfsdorff N.(1), Irintchev A.(6), Angelov D.(1),

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### Abstract:

Accumulating evidence asigns an important role of the glutamatergic input to spinal motoneurons (VGLUT1-positive terminals in lamina IX of the spinal cord) for recovery after SCI. These terminals, which belong to medium- to large-sized neurons in the dorsal root ganglia and convey mechano- and proprioceptive information, are crucial for restoration of limb locomotion. In the present study we looked for correlation between functional and electrophysiological measurements and morphological (density of VGLUT1-positive terminals) parameters after SCI and several therapeutic trials. We performed severe compression SCI at low-thoracic level in adult female Wistar rats and subjected them to do daily training with whole body vibration (WBV) starting 7, 14 or 28 days after injury (WBV7, WBV14, WBV28 respectively) and continuing over a 12-week post-injury period. Physical therapy (WBV and MLT) caused positive effects on body weight support and bladder function, but failed to improve motoneuron excitability (no H-reflex enhancement). These findings correlated with the measured intensity of CY3-fluorescence after immunostaining of terminals for anti-vesicular glutamate transporter 1 (VGLUT1; 1:500; Synaptic Systems). We found a remarkable loss of VGLUT1-positive terminals, as indicated by grey level measurements, in the ventral horn and Clarke's column of the lumbar spinal cord after SCI compared with intact animals and no effect of WBV or MLT. WBV and MLT do not restore the excitatory glutamatergic afferents, which are essential for locomotor recovery after SCI. This failure may explain the lack of pronounced treatment effects on specific locomotor parameters like stepping ability and BBB scores, and spinal reflex excitability.

Rubrik: Neuroregeneration/Neurodegeneration

Titel:Improved long-distance regeneration and functional recovery in heterozygous Spry2 knock-out mice following peripheral nerve injury

Autoren: Klimaschewski L.(1),Letizia M.(1),Hausott B.(1),Thongrong S.(1),Ronchi G.(2),Pritz C.(3),

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### Abstract:

Sprouty2 (Spry2) is a negative feedback inhibitor of ERK-signaling and highly expressed in sensory dorsal root ganglia (DRG). Here, we investigated the involvement of Spry2 in axon outgrowth and peripheral nerve regeneration in Spry2+/- mice as compared to wild-type (wt) littermates. Adult dorsal root ganglion (DRG) neurons obtained from heterozygous Spry2 mice exhibited enhanced axonal elongation, which was further stimulated by FGF-2 or by NGF treatment. The strongest increase in axon outgrowth (axon elongation and total axonal length) was observed in TrkA/CGRP-positive neurons. Primary sympathetic (SCG) neuron cultures confirmed the enhanced elongative axon outgrowth of sensory neurons. The intensity of the pERK signal in DRG neurons was significantly increased by both growth factors in Spry2+/- neurons as compared to NGF- or FGF-2-induced pERK activation in wt neurons. In response to a sciatic nerve lesion, faster sensomotor recovery was observed by Rotarod testing. Histological analysis demonstrated an elevated number of regenerated myelinated axons and enhanced myelin thickness in Spry2+/- mice one month after nerve crush. The present results corroborate the functional significance of the ERK pathway for axon elongation and support a role for Spry2 as a potential novel target for pharmacological inhibition to accellerate axon elongation in lesioned peripheral nerves.

Rubrik: Neuroregeneration/Neurodegeneration

Titel:Better recovery in spinal cord-injured blind SD/RCS rats is not associated with alterations in motoneuron excitability

Autoren: Alsolivany K.(1), Rink S.(1), Stein G.(2), Angelova S.(3), Manthou-Halvadzhi M.(4), Abdulla D.(1), Masson I.(1), Wolfsdorff N.(1), Irintchev A.(5), Angelov D.(1),

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### Abstract:

Previous work has shown that the plantar H-reflex is a useful tool to assess motoneuron excitability after spinal cord injury (SCI) in rodents correlating with severity of injury and locomotor outcome. Specifically, recovery of better locomotor abilities after incomplete SCI is associated with enhanced H- but not M-wave and attenuated frequency dependent depression (rate depression) of the H-reflex. In the present study we compared electrophysiological parameters after SCI compression injury in two groups of adult rats. The first group consisted of Sprague Dawley (SD) rats with normal vision, poor recovery after peripheral nerve injury and normal expression of trophic factors in the denervated muscles. The second group consisted of Royal College of Surgeons (RCS) rats that are blind, recover completely after peripheral nerve injury. Recent own work showed that the blind SD/RCS animals significantly improved body weight support and skilled limb movements by 6 - 12 weeks after SCI. Following severe compression of the spinal cord at midthoracic level (Th8) H-reflex was analyzed at 1, 3, 6, 9, and 12 weeks. Several variables were measured at baseline stimulation frequency (0.1 Hz): maximum of M- and H-wave amplitudes and M- and H-wave latencies. In addition, we analyzed the alterations of the M- and H-waves upon incrementally increasing the stimulation frequency from 0.1 to 5 Hz. In contrast to the Mwave, which showed no frequency-dependant depression, a marked rate depression was found for the H-wave. This depression was, however, similar in both experimental groups.

Rubrik: Neuroregeneration/Neurodegeneration

Titel:Whole-body vibration (WBV) improves revascularization after compression spinal cord injury (SCI) in rats

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### Abstract:

Based on earlier own results indicating positive effects of WBV after SCI, we looked for correlation between functional (analysis of locomotion), electrophysiological (H-reflex) and morphological (density of functioning capillaries) measurements after SCI and WBVtreatment. We performed severe compression SCI at low-thoracic level (T8) in adult female Wistar rats and subjected them to WBV twice a day (2 x WBV) over a 12-week post-injury period. Intact rats and rats with SCI but no WBV training (sham) served as controls. Recovery of locomotion was determined by BBB-locomotor rating, foot stepping angle (FSA), rump-height index (RHI), correct ladder steps (CLS) and H-reflex at 1, 3, 6, 9, and 12 weeks after SCI. Animals were sacrificed by an overdose of Isoflurane (Forene, Abbott, Germany) and their spinal cords fixed in 4% PFA for 24 h. Samples from the thoracic cord containing the lesion site were cut into 10 µm thick longitudinal frozen sections. The endogenous peroxidase of the erythrocytes filling the capillaries was visualized with 0.05% DAB. Serial equidistant sections were photographed using a Leica DMLB2 microscope equiped with a Zeiss AxioCAM MRc camera. Using the Zeiss AxioVision software (Version 4.7), all functioning capillaries (containg erythrocytes) were identified by their discrete grey value. A determination of their absolute and proportional area followed. Our morphological measurements indicated a significantly denser capillary network in the WBV treated rats. At the lesion site, the area occupied by capillaries in the "vibrated" rats was  $1.64 \pm 0.38$  % versus  $0.85 \pm 0.22$  % in the sham-treated animals.

Rubrik: Neuroregeneration/Neurodegeneration

Titel:Indigenously upregulated trophic factor expression promotes better recovery in spinal cord-injured blind SD/RCS rats

Autoren: Rink S.(1), Alsolivany K.(1), Stein G.(1), Manthou-Halvadzhi M.(2), Angelova S.(3), Abdulla D.(1), Masson I.(1), Wolfsdorff N.(1), Irintchev A.(4), Angelov D.(1),

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### Abstract:

In the present study we compared several functional parameters after spinal cord injury (SCI) in two grups of adult rats. The first group consisted of Sprague Dawley (SD) rats with normal vision, poor recovery after facial nerve injury and normal expression of trophic factors in the denervated muscles. The second group consisted of blind Royal College of Surgeons (RCS) rats that recover completely after peripheral nerve injury and show a massive (3 to 5 times) upregulation of mRNA for 3 growth factors (FGF2, IGF-2 and NGF) in the denervated muscles. All rats were subjected to severe compression of the spinal cord at midthoracic level (Th8). Recovery of locomotion was analyzed at 1, 3, 6, 9, and 12 weeks after SCI using video recordings of beam walking and inclined ladder climbing. Four functional parameters were used: the foot-stepping angle (FSA), the rump-height index (RHI) estimating paw placement and body weight support, respectively, the number of correct ladder steps (CLS) assessing skilled hindlimb movements and the locomotor rating score of Basso, Beattie and Bresnahan (BBB). Locomotor rating and numerical assessment of plantar stepping revealed no significant differences between both rat strains. However, compared with SD-rats, the blind SD/RCS animals significantly improved body weight support and skilled limb movements by 6 – 12 weeks after SCI. The present findings provide hints for functional benefits of upregulated expression of neurotrophic factors that has been induced by behavioral demand (blindness) and warrant further preclinical investigations to determine mechanisms underpinning this potential substitutive therapy for SCI.

Rubrik: Neuroregeneration/Neurodegeneration

Titel:Delayed whole body vibration (WBV) therapy restores GABA-ergic input to the lumbar ventral horn after low thoracic spinal cord injury (SCI) in rats

Autoren: Khan A.(1), Stein G.(2), Ankerne J.(1), Papamitsou-Sidoropolou T.(3), Eisel L.(1), Schempf G.(1), Wirth F.(1), Ozsoy O.(4), Ozsoy U.(5), Masson I.(1), Wolfsdorff N.(1), Wechsler M.(1), Irintchev A.(6), Angelov D.(1),

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### Abstract:

Changes in the amount of inhibitory GABA-ergic synapses affect spinal cord motoneurons excitability during recovery after SCI. Here we looked for correlations between functional and morphological (density of GABA-positive terminals) parameters after SCI and different therapeutic trials. Following compressive SCI at low-thoracic level, female rats were distributed into experimental groups and subjected to daily WBV-therapy starting at 1, 7, 14 or 28 days after injury (WBV1, WBV7, WBV14, WBV28 respectively) over a 12-week postinjury period. Intact rats and rats with SCI but no WBV-therapy (sham) served as controls. Recovery of locomotion was analyzed using video recordings of beam walking and inclined ladder climbing. H-reflex was analyzed at 1, 3, 6, 9, and 12 weeks after SCI. To analyze the inhibitory synaptic input to the lumber ventral horn motoneurons, we used immunofluorescent staining for the vesicular GABA transporter (VGAT; 1:1000; SySy), which has been employed in studies on changes in spinal motoneuron innervation after injuries and is expressed in both GABA and glycinergic synaptic terminals. Following quantification of the CY3-fluorescence intensity in transverse sections (grey scale range 101-200), we found a remarkable loss in the amount of VGAT-positive terminals in all injured rats compared to intact animals ( $34.84 \pm 0.86$  mpx). In the sham-treated rats we measured  $1.49 \pm 0.42$  mpx, in the WBV1  $3.49 \pm 1.79$  mpxl, in the WBV7  $0.92 \pm 0.32$  mpx and in the WBV14 ( $2.07 \pm 0.67$ mpx. Only the WBV28 group had a density similar to that in the intact rats  $(30.07 \pm 5.17)$ mpx).

Rubrik: Neuroregeneration/Neurodegeneration

Titel:Patient specific dopaminergic neurons from IPS cells as a human in vitro model of Parkinson's disease associated with mutations in LRRK2

Autoren: Raab S.(1), Klingenstein M.(1), Linta L.(1), Liebau S.(1),

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### Abstract:

Parkinson's disease (PD) is the second most common neurodegenerative disease in Germany. The main features of this disease result from the loss of dopaminergic neurons in the Substantia nigra in the midbrain region and the presence of Lewy bodies. This leads to movement related symptoms like shaking, slowness of movement, difficulty with walking and gait. Genetic mutations in leucine-rich repeat kinase 2 (LRRK2) are responsible for both inherited and sporadic PD. The LRRK2 protein contains several distinctive structural and functional motives like a GTPase and kinase domain. Until now there are no certain correlations found between LRRK2 mutations and the age of onset. Beyond this, the exact function of the LRRK2 protein remains still unknown. The most common variant is the G2019S LRRK2 mutation which is found in the kinase domain. As a possible mechanism the increased toxicity of the kinase activity of LRRK2 is widely discussed. Published data show a correlation between the mutation of the kinase domain and reduced axonal length and number of neurites. As a model for the in-vitro analysis of patient specific mutations we want to generate induced pluripotent stem cells (iPSCs) from hair keratinocytes and differentiate them to dopaminergic neurons. Further experiments will be focused on the maturation of these neurons and their synaptic contacts to examine the impact of the changed kinase activity as well as the neurodegenerative mechanism. An additional focus will be set on initiating signals of cell death to find potential interventions of neuronal degradation.

Rubrik: Neuroregeneration/Neurodegeneration

Titel:Investigating rare neurodegenerative diseases with patient specific induced pluripotent stem cells

Autoren: Klingenstein M.(1), Raab S.(1), Linta L.(1), Pfänder S.(1), Liebau S.(1),

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### Abstract:

Analyzing rare diseases of patients with developmental defects play an increasing role in modern science. Here I used a model with human induced pluripotent stem cells (iPSCs) which already plays a crucial role in the analysis of patients with neurodegenerative diseases. In this project, keratinocytes from plucked human hair are used for reprogramming. This method is a well established non invasive possibility to gain patient cell samples for reprogramming compared to the widely used method with human skin fibroblasts. For the reprogramming process the keratinocytes have to be transduced with a lentivirus which contains the four so called Yamanaka factors Oct4, Klf4, Sox2 and c-Myc (OKSM). There are different publications which describe many ways to increase and optimize the efficiency of reprogramming patient keratinocytes. In addition, there are a lot of protocols to differentiate the iPSCs into almost all cell types of the human organism. With regard to patients with neurodegenerative defects in the nervous system differentiation into specific neurons is of particular interest, because every disease affects different types of neurons. Therefore, a better understanding of neurodegenerative diseases the protocols for reprogramming and especially differentiation into different neuronal cell types have to be improved. I received hair samples of patients who suffer from different diseases from all over the world. The aim is to reprogram the keratinocytes and differentiate the human iPSCs to disease specific neurons. Further morphological and cell biological analysis of these neurons will be done by protein immunofluorescence, guantitative Real Time-PCR (gRT-PCR) and western blot analysis.

Rubrik: Neuroregeneration/Neurodegeneration

Titel:Zinc biology of neurons generated from HIPSC of controls and Phelan-McDermid syndrome patients

Autoren: Pfänder S.(1), Achberger K.(1), Liebau S.(2), Grabrucker A.(3), Böckers T.(1),

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### Abstract:

ProSAP/Shank molecules are important scaffolding proteins in the postsynaptic compartment of excitatory synapses. They build platforms linking components of the postsynaptic signaling apparatus to the actin-based cytoskeleton. Thus, they build a framework for the formation of the postsynaptic density (PSD). Interestingly, two of the three ProSAP/Shank family members are targeted to and regulated at the PSD via their sterile alpha motif which is essential for protein assembly by binding to zinc ions. A role for ProSAP/Shank proteins in neurodegenerative diseases was first proposed when a deletion in the q13 region of chromosome 22, where the ProSAP2 gene is located, was identified as the main genetic cause for Phelan-McDermid Syndrome (PMS). This syndrome is characterized by features of autism spectrum disorders along with hypotonia and mental retardation. Intriguingly, an association of autism spectrum disorders with zinc deficiency in children was already shown and imbalances in zinc homeostasis have been associated with multiple brain disorders. Thus, here, we investigate the effects of zinc supplementation and depletion on the differentiation and synaptogenesis of neurons differentiated from human induced pluripotent stem cells. Moreover, we will use PMS patient derived stem cells to evaluate, if zinc supplementation could be a possible treatment strategy for PMS. In a first set of experiments we therefore evaluated expression of zinc homeostasis proteins of neurons differentiated from patient derived and control stem cells. Thus, indeed, zinc seems to be an important factor in neuro- and synaptogenesis and future stem cell based experiments will hopefully provide further evidences.

Rubrik: Neuroregeneration/Neurodegeneration

Titel:LPS cells and developmental studies of patients carrying a mutation in a translation initiation factor

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### Abstract:

The eukaryotic initiation factor 2 (eIF2) is a protein complex which is part of the cellular translation machinery. eIF2 comprises three subunits (?, ?, ?), being the ? subunit the one which contains a GTP-binding domain and binding sites for the other two subunits to interact. eIF2 acts in the initial part of the protein synthesis, forming a complex with GTP and MettRNAMet. This ternary complex associates itself with the 40S subunit of the ribosome and, in a cap-dependent manner, scans the mRNA with the help of other factors. The recognition of the first AUG codon leads to the final assembly of the ribosome and the protein is translated. Due to eIF2 importance, relevant genetic defects in their genes might be lethal, however a eIF2 mutation was recently found to be responsible for intellectual disability in male patients. In those patients, signs of problems in nervous system development are accompanied by a broad spectrum of other symptoms such as obesity, microgenitalism and ataxia gait. This finding has motivated the present study in investigating why function disruption in a central player of protein translation has mainly impact in the nervous system development. Patientderived iPS cells will be generated, as a model to understand more about the role of eIF2 in synaptogenesis and neuronal function. Characterization of neuronal differentiation from the patient cells might generate evidence about the role of eIF2 in nervous system development, and in other regulatory pathways which are so far not described.

Rubrik: Neuroregeneration/Neurodegeneration

Titel:Impairments in the sensory system of the wobbler mouse, a model for amyotrophic lateral sclerosis

Autoren: Zumholte B.(1), Schmitt-John T.(2), Brand-Saberi B.(3), Theiss C.(1),

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### Abstract:

There is increasing evidence that proteins related to intracellular transport mechanisms are affected in several motor neuron diseases. Especially in Amyotrophic Lateral Sclerosis (ALS) the mechanisms of motor neuron degeneration are not yet well known, besides the description of functional deficits in the spinal cord and cortex . For the present investigations we use the Wobbler mouse as a model for the sporadic form of ALS. This mouse model is characterized by a progredient paralysis of motor neurons that goes along with head tremor and loss of body weight. All relevant investigations up to now mainly focused on motor neurons, whereas information concerning the sensory system is only sparse. It is considered that the sensory system is not affected due to missing symptoms. In spite of this view, the aim of this study is to analyse dorsal root ganglia neurons (DRG) during the progression of this disease regarding morphological alterations potentially reflecting an involvement of the sensory system. We especially focused on the distribution of cytoskeletal proteins within the DRG using confocal laser scanning microscopy as well as electron microscopy. Our first results indicate an abnormal distribution and aggregation of neurofilaments accompanied by enlarged vesicles in the perikarya. Affected neurons also show signs of degeneration, like for example fragmented nuclei. Our results present thus first hints for impairments of the sensory nervous system in the Wobbler mouse.

Rubrik: Neuroregeneration/Neurodegeneration

Titel:VEGF triggers the distribution of cofilin in the axonal growth cone

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### Abstract:

VEGF is basically known as a factor promoting endothelial and vascular outgrowth and plays a certain role in cancer proliferation. Recent studies revealed that VEGF additionally increases axonal outgrowth and acts on the neuronal growth cone by modifying the activity of the actin-cytoskeleton. Actin-based motility is regulated by a large number of binding proteins, which affect its organization and the turn-over rate of filamentous actin, following different signaling pathways. But up to now the effect of VEGF on those signaling pathways is not well understood. The aim of this study was to reveal the effect of VEGF on the distribution of cofilin in the axonal growth cone. Cofilin is an actin-binding protein which severs filamentous actin leading to either actin assembly or disassembly. For the experiments to be presented, we microinjected plasmids encoding GFP-tagged -cofilin and RFP-tagged-actin into chicken DRG cultivated for several days. The injected neurons were stimulated with VEGF and time-lapse-imaging was performed, using confocal laser scanning microscopy. Time lapse microscopy revealed that there are stimulating effects of VEGF on the activity of cofilin within the neuronal growth cone. It was observed that the distribution of cofilin changed rapidly after stimulation. The cofilin relocalisation appeared to be similar to the distributional changes of actin. Based on these observations, we propose a stimulating effect of VEGF on pathways leading to an accumulation of cofilin in the axonal growth cone.

Rubrik: Neuroregeneration/Neurodegeneration

Titel:Neurodegeneration in the motor cortex of the wobbler mouse, an ALS animal model

Autoren: Dahlke C.(1), Schmitt-John T.(2), Brand-Saberi B.(3), Theiss C.(1),

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### Abstract:

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder of upper and lower motor neurons, characterized by rapid progressive weakness, muscle atrophy, dysarthria, dysphagia and dyspnea. The Wobbler mouse equally develops a progressive degeneration of motor neurons in the spinal cord and motor cortex and shows striking similarities to human ALS. Whereas astrogliosis has already been shown in the spinal cord of the Wobbler mouse the aim of our study is to analyze the proliferation behaviour of astrocytes in mouse motor cortex at different stages of the Wobbler disease. In healthy neural tissue, astroglial cells play critical roles in biochemical support, regulation of blood flow, synapse function and remodeling, homeostasis and most importantly in repairing the brain and spinal cord after injuries by filling up the space to form a glial scar. Upregulation of glial fibrillary protein (GFAP) is known as a classical marker for reactive gliosis. By immunohistochemistry and electron microscopy techniques we show abnormal density of reactive astrocytes in the motor cortex region of the Wobbler mouse, while there was no conspicuous increase of astrocytes in motor cortex of contemporary control mice. Considering these results, inflammation might be an important contributing factor of motor neuron degeneration and progressive paralysis in the Wobbler mouse that has so far not received any attention.
Rubrik: Neuroregeneration/Neurodegeneration

Titel:Lysosomal aggregations in von economo neurons of patients with schizophrenia

Autoren: Krause M.(1), Brüne M.(2), Theiss C.(1),

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#### Abstract:

Von Economo Neurons (VEN), first described by von Economo in 1926, are an unique and distinguish type of neurons in layer Vb of the anterior cingulate cortex (ACC), fronto insular (FI) and the frontopolar cortex of higher primates. VEN have also been found, though less abundant, in the brain of macaques, cetaceans and elephants. All these species have a distinctive social life. It has therefore been suggested that VEN are relevant for higher-order cognitive processes. The spindle-shaped cell-body of VEN is strictly perpendicularly oriented to the pial surface and they often appear in small clusters of two to five VEN around small blood-capillaries, which might indicate a high metabolism. In human ontogeny VEN emerge in the 35th week of gestation and reach their adult number in year 4 after birth. Due to the late development, VEN might be vulnerable for neurodegenerative psychiatric disorders like fronto-temporal dementia and schizophrenia. Both disorders are characterized by the loss of social-cognitive skills like empathy and are known to show less VEN. Our recent electronmicroscopy investigations of total 61 patients (20 patients with schizophrenia, 19 patients with bipolar disorder and 22 psychiatric healthy people) show for the first time morphological alterations in VEN, that could be the linked to rarefaction. In contrast to healthy people VEN of patients with schizophrenia show altered organization of cell-organelles, whereby especially lysosomes are pathologically increased.

Rubrik: Neuroregeneration/Neurodegeneration

Titel:Neurodegeneration in the cerebellum of the wobbler mouse, an ALS animal model

Autoren: Saberi D.(1), Schmitt-John T.(2), Theiss C.(1),

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#### Abstract:

Amyotrophic lateral sclerosis is a common motor neuron disease which is well described to affect motoneurons in the spinal cord and neurons in the motor cortex. To investigate the progression of the sporadic or familiar pathology different mouse models are well established. We use the Wobbler mouse as an animal model for the sporadic ALS type. Whereas neurodegeneration is well described in the spinal cord and the motorcortex the aim of our study is to investigate the cerebellar organization during the pre-symptomatic, evolutionary and stabilized phase of defects in motion. Here, we focus on neurodegeneration of Purkinje cells (PC), possibly caused by aggregation of proteins and morphology changes of cytoskeletal proteins. For these morphological analyses we use immunohistochemistry as well as electron microscopic techniques. As the function of the cerebellum takes an important part for fine tuning of movements, disorganization of dendritic trees of PC is a strong hint for impaired function of the cerebellum in these animals. Our first results show bloated somata of PC that are sometimes degenerated. Additionally, an abnormal organization of the PC layer, with numerous dying cells is detectable. To conclude, this is the first investigation of neurodegeneration in the cerebellum of the Wobbler mouse, which clearly shows that other structures related to motor activity beside the spinal cord and motorcortex are also affected.

Rubrik: Neuroregeneration/Neurodegeneration

Titel:The hippocampal autophagic machinery is slowed down in the absence of the circadian clock protein Per1

Autoren: Fekadu J.(1), Rawashdeh O.(1), Rami A.(1),

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Abstract:

We recently reported on the innate hippocampal vulnerability to cerebral ischemia in mice lacking the circadian clock protein 1 and found PER1 to be involved in the expression of apoptotic/autophagic markers (1). To exclude the contribution of vascular or glial factors to the innate vulnerability of Per1-KO-mice, we compared the autophagic machinery in primary hippocampal cultures from WT- and Per1-KO-mice, using the lipophilic macrolide antibiotic, Rapamycin to induce autophagy. Development of autophagy in WT cells involved an increased LC3-II-to-LC3-I ratio and an overall increase in the level of LC3-II. In addition, immunostaining of LC3 in WT cells revealed the typical transformation of LC3 localization from a diffused staining to a dot- and ring-like pattern. In contrast, treatment of Per1deficient-hippocampal cells with Rapamycin was not able to induce remarkable alterations of autophagy hallmarks. These results confirm that the autophagic machinery is slowed down in Per1-deficient hippocampal neurons. Basal autophagy occurs continuously as housekeeping function, and can be acutely expanded in response to injury. Insufficient autophagy as seen here, may lead under stress conditions to an accumulation of dysfunctional organelles that would be compensated under physiological conditions via lysosomal clearance. We speculate about a critical role of Per-1 in cellular homeostasis and underline that the insufficient autophagy may contribute to the innate vulnerability of Per1-KO-mice to cerebral ischemia.

(1) Wiebking N, Maronde E, Rami A (2013) Increased neuronal injury in clock gene Per-1 deficient-mice after cerebral ischemia. Curr Neurovasc Res.10:112-25.

Rubrik: Neuroregeneration/Neurodegeneration

Titel:A screening of autophagic factors during neuronal stress induced by epileptic seizures

Autoren: Benz A.(1), Langhagen A.(1), Rami A.(1),

Adressen:(1)Johann Wolfgang von Goethe-Universität|Institut für Zelluläre und Molekulare Anatomie (Anatomie III),|Frankfurt|Germany; email:rami@em.uni-frankfurt.de

Abstract:

Hippocampal cell loss remains the most commonly observed lesion after status epilepticus (S.E.) and growing evidence supports the role of autophagic stress-induced death of neurons in several models of neurodegeneration. Our goal was to examine whether autophagic pathways are involved in mechanisms of neuronal degeneration after epileptic seizures. To do so, we used the kainate-model of status epilepticus in mice and examined the dynamics in the expression of autophagic markers in the hippocampus by immunoblotting and immunocytochemistry. We were able to detect significant alterations in the levels of central proteins of the autophagic machinery. The expression-levels of the autophagic pacemaker phospho-mTOR/mTOR showed dramatic alterations at 6 and 48 h after S.E.. Beclin-1, one of the most important autophagy-inducing proteins, autophagocystosis associated protein 3 (Atg3), and MAP1LC3 (LC3-II/LC3-I), indispensable component of the autophagic vacuole's membrane, showed significant upregulation as early as 6 h after kainate-administration. Likewise, levels of Bag-3, Hsp70 and LAMP1 were increased after stimulus as well. The levels of Atg5 and Atg14 were also found to be elevated after S.E., whereas Atg7 and Atg12 remained unaltered. In summary, our results demonstrate that S.E. results in significant alterations of autophagic dynamics. It remains to be determined whether altered autophagy is causative or secondary to the pathological processes of neuronal death.

Rubrik: Neuroregeneration/Neurodegeneration

Titel:Induction of protection pathways in retinal ganglion cells by monochromatic light

Autoren: Hammer C. M.(1), Happel A.(1), Cuerda Galindo E.(2), Paulsen F.(1),

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#### Abstract:

Background: Progressive and irreversible apoptotic loss of retinal ganglion cells (RGCs) is a pivotal hallmark of glaucoma and diabetic retinopathy. Monochromatic red light (670nm) has been demonstrated to convey antiapoptotic effects in retinal neurons. Activation of the MEK/ERK pathway and/or the PI3K/Akt pathway may constitute a possible protective mechanism for RGCs to counteract apoptosis. Here, we examined the effect of blue, red and infrared light on the expression and activation of ERK-1, ERK-2 and Akt in RGCs. Methods: First, an irradiation chamber with exchangeable bandpass interference filters was constructed and calibrated. Immortalized murine RGCs were exposed to monochromatic blue (405nm), red (600nm) and infrared (800nm) light (16 $\mu$ W, 6.5  $\mu$ W and 10 $\mu$ W, respectively) for 1min, 5min and 10min each. Controls were not exposed to monochromatic light of any sort. Protein extracts were harvested after 4h and 24h. Western blot analysis of phosphorylated and dephosphorylated ERK-1, ERK-2 and Akt was performed in duplicate. **Results:** In the 405nm cohort, ERK-1 expression was significantly reduced at all times 4h post-treatment, while ERK-2 expression remained unaltered. ERK-2 phosphorylation, however, was increased due to an exposure of 10min. No effects on expression or activation of ERK-1 and ERK-2 were found following exposure to red and infrared light. After 24h, a steady increase of Akt phosphorylation over time was detected ensuing 405nm irradiation, while Akt expression was only slightly increased. Exposure to 800nm resulted in a steady reduction of phosphorylated Akt over time, while Akt expression remained unaltered. After exposure to 600nm for 1min, a slight reduction of phosphorylated Akt was detected. This effect was enhanced after exposure for 5min, while after 10min, the phosphorylation status appeared set to normal again.

**Conclusions:** Blue light activates the Akt- and ERK-2-dependent survival pathways. It is therefore tempting to speculate that blue light may have the potential to alleviate or delay RGC apoptosis.

Rubrik: Neuroregeneration/Neurodegeneration

Titel:Neuroprotective effects of mycophenolate mofetil - temporal dynamics in glial proliferation, apoptosis and scar formation

Autoren: Ebrahimi, F.(1),Koch M.(1),Pieroh P.(1),Bechmann I.(1),Dehghani(2),

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#### Abstract:

The immunosuppressant mycophenolate mofetil (MMF) has proven to reduce the extent of cell death induced by secondary injury cascades by inhibition of microglial and astrocytic activation. In this study we determined the effective neuroprotective time frame in which MMF elicits beneficial effects by analysis of glial cell proliferation, migration and apoptosis. Using organotypic hippocampal slice cultures (OHSC), temporal dynamics of proliferation and apoptosis following N-methyl-D-aspartate (NMDA)-mediated excitotoxicity were studied by quantitative morphometry of ki67 or cleaved caspase-3 immunoreactive glial cells. In the scratch wound model of astrocyte monolayers reactive astrocytic scar-formation was examined. Lesioned OHSC showed an increase of microglial and astroglial proliferation indices between 12 and 36h and of apoptosis indices between 24 and 72h after injury. The treatment with MMF caused a significant decline of the proliferation rates without affecting apoptosis. A continuous treatment with MMF reduced the amount of neuronal cell death when it was initiated within the first 12h postinjury. Significant neuroprotection was identified in the crucial time frame between 12 and 36h postinjury. In the scratch wound model the gap closure was reached within 48h in controls and was potently inhibited by MMF. The present data show that the immunosuppression by MMF significantly diminishes the extent of neuronal cell death when administered within a critical time frame postiniury. Inosine-5monophosphate-dehydrogenase, the rate-limiting enzyme of purine synthesis, is a potent target to modulate the temporal dynamics of proliferation and migration in glial cells leading to a reduction of the degree of secondary neuronal damage and scar formation.

Rubrik: Peripheres und vegetatives Nervensystem

Titel:Allometry of left ventricular myocardial innervation

Autoren: Schipke J.(1), Mühlfeld C.(1), Mayhew T.(2),

Adressen:(1)Medizinische Hochschule Hannover|Institut für Funktionelle und Angewandte Anatomie|Hannover|Deutschland; email:schipke.julia@mh-hannover.de; (2)University of Nottingham|School of Biomedical Sciences|Nottingham|United Kingdom

#### Abstract:

Body weights (BW) of mammalian species ranges from a few grams (Etruscan shrew) to a few tonnes (elephant). Given the differing cardiac capacity to adapt to metabolic demands in the species, we hypothesized that the left ventricular supply by the nervous system deviates from the usual size allometric relationship.

To test this, we used design-based stereology and electron microscopy to estimate parameters characterizing the myocardial innervation of a variety of species covering BW between 2 g in the shrew and 900 kg in the cattle. We analyzed also the cardiac blood supply and the composition of cardiomyocytes. Relationships between estimated variables and BW were examined using linear regression analysis applied to log-transformed data. The total length of nerve fibres (axons) in the left ventricle increased from 0.017 km (0.020 km) in the shrew to 7237 km (13,938 km) in the horse. The innervation density was similar across the analyzed species, but the mean number of axons per nerve fibre profile increased with rising body mass. The total length of capillaries increased from 0.119 km (shrew) to 10,897 km (horse); the volume of cardiomyocytes was 0.017 cmł in the shrew and 1818 cmł in the horse.

Scaling of the data against body mass indicated a higher degree of complexity of the axon tree in larger animals whereas the density of nerve fibres is independent of BW. It seems that the structural components of the autonomic nervous system in the heart are related to body/heart mass rather than to metabolic rate.

Rubrik: Peripheres und vegetatives Nervensystem

Titel:Canonical WNT signaling is involved in proliferation of neuronal progenitor cells in the enteric nervous system

Autoren:Neckel P.(1),Zhang Y.(1),Seid K.(1),Mack A.(1),Obermayr F.(2), Hirt(1),Just L.(1),

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Abstract:

Due to its complexity and functional diversity the enteric nervous system (ENS) is often referred to as "second brain" or "brain in the gut". The last decade revealed a population of neuronal cells in the ENS that preserves a high proliferative capacity throughout development and well into adulthood. We and others have shown that these enteric progenitor cells can be isolated, expanded, and finally differentiated into neurons and glia. During development and into adulthood, stem and progenitor cell niches are tightly controlled by Wnt signaling. Here we assessed the involvement of the canonical Wnt cascade in proliferation and differentiation of the ENS progenitor cell pool. Therefore, we isolated enteric progenitors from murine gut and kept them under proliferation conditions to promote the formation of spheroids. Application of Wnt agonists and GSK3 inhibitors to the sphere culture resulted in an enhanced proliferation and neuronal differentiation of enteric progenitors by canonical Wnt activation. Our results are a key to understanding the mechanisms underlying enteric progenitor homeostasis and are the basis for novel approaches necessary for potential future cell replacement therapies for enteric neuropathies. Future experiments will have to address the impact of canonical Wnt activation on enteric progenitors in vivo.

Rubrik: Zentrales Nervensystem/Signaltransduktion und Verschaltung

Titel:Analysis of neurotrophin signalling in ProSAP/Shank mutant mice

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#### Abstract:

Autism spectrum disorders (ASD), affecting approximately 1 percent of the human population, are based on three core features, including impaired social interaction, delay or absence of language development and repetitive and stereotypical behaviour. On the molecular level, ASD is overall associated with imbalances in the expression of synaptic components and disturbances in synaptic signalling.

The ProSAP/Shank family consists of three different members - Shank1, ProSAP1/Shank2 and ProSAP2/Shank3 – and build up a giant scaffold at the excitatory postsynapse. This scaffold serves as the contact point for numerous synaptic components and as the basis for proper synaptic signalling. The first correlation between ProSAP/Shanks and human neurological disease was found in patients with Phelan-McDermid Syndrome (PMS), which are lacking one allele of ProSAP2/Shank3 and finally show an autistic phenotype. To explore the molecular consequences of disrupting the ProSAP/Shank scaffold, we analysed the altered synaptic composition and function of ProSAP/Shank mutant mice, representing models with autistic traits.

Analysis of neurotrophin signalling in mutant mice, investigating the NGF neurotrophin family and insulin-like growth factors 1 and 2, revealed interesting consequences of ProSAP/Shank deficiency, showing alterations of neurotrophin levels, especially for BDNF. Since ASD patients are also reported to manifest neurotrophin levels, diverging from healthy controls, these findings suggest a critical role of aberrant neurotrophin signalling in ASD development and support a further in-depth analysis. Whether altered neurotrophin signalling represents the cause or consequence of ASD formation, still remains to be clarified to complete our understandings of autism spectrum disorders.

Rubrik: Neuroimmunologie

Titel:Trefoil peptide 3 (TFF3) reduces the expression of pro-inflammatory mediators in cultivated microglia

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Abstract:

Objektive: Trefoil factors are cysteine-rich peptides secreted by mucosal surfaces. Three mammalian TFFs (TFF1-3) are described so far. Previous studies suggest an important role of TFF3 in regeneration and immunomodulation in different tissue. As inflammation in the CNS is associated with many neurodegenerative diseases, we further elucidated the occurrence and possible pathophysiological implications of TFF3 in the CNS. Methods: Using gPCR and immunocytochemistry we investigated whether primary astrocytes or microglia express TFF3 and explored a possible regulation of TFF3-mRNA synthesis. To induce inflammation we utilized LPS. To show the influence of TFF3 on inflamed microglia we measured the activation of microglia via quantification of mRNA synthesis of iNOS, Cox-2, II-1beta, II-6 and TNF-alpha. In addition we explored the influence of TFF3 on the quantitative II-6 and TNF-alpha protein synthesis in inflamed microglia via ELISA. Finally, we investigated the intracellular signaling mechanisms using Western blotting. Results: gPCR and immunocytochemistry data revealed expression of TFF3 in astrocytes, but expression in microglia was missing. Quantification of mRNA synthesis of iNOS, Cox-2, II-1beta, II-6 and TNF-alpha showed an inhibitory effect of TFF3 on inflamed/activated microglia. Additionally, ELISA data demonstrated inhibition of protein synthesis of II-6 and TNF-alpha, possible via interaction with MAP-kinase pathway. Conclusion: Our results indicates that (i) cultivated astrocytes produce TFF3, which is able to (ii) decrease pro-inflammatory mediators and cytokines of activated microglia cells. A major unanswered question is wether pharmacological inhibition of neuroinflammation, e.g. via TFF3, will be able to slow neurodegenerative diseases.

Rubrik: Neuroimmunologie

Titel:GDNF fails to inhibit LPS-mediated classical activation of murine microglia

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Abstract:

Glial cell line-derived factor (GDNF) has been described as one of the most potent neurotrophic factors for midbrain dopaminergic neurons in vitro and in vivo. Several studies have shown that GDNF applications in toxic and inflammatory animal models for Parkinson's disease resulted in neuroprotection accompanied by decreased neuroinflammatory responses. Although a regulatory potential of GDNF on microglia activation has been suggested, no study directly addressed this question. Here, we analysed the effects of GDNF on lipopolysaccharide (LPS)-induced classical activation of primary murine microglia. We clearly demonstrate that GDNF is not able to inhibit LPS-mediated upregulation and subsequent release of pro-inflammatory markers such as interleukin-6 (IL6) and tumor necrosis factor-alpha (TNF?). Moreover, we analysed the expression of GDNF receptors GFR?1, c-RET and PSA-NCAM in primary murine microglia and observed that these cells lack expression of c-RET and PSA-NCAM. Thus, classical signalling cascades involved in GDNF signalling such as Akt and Erk1/2 could not be activated in murine microglia. These data indicate that GDNF fails to inhibit LPS-mediated classical activation of primary murine microglia, which might be due to lack of c-RET and PSA-NCAM expression. Reduced neuroinflammatory responses observed after GDNF applications in mice in vivo might therefore rather be caused by direct neuroprotective properties of GNDF and subsequent reduction in microglia activation.

Rubrik: Neuroimmunologie

Titel:Role of formyl peptide receptors in innate immune response after bacterial meningitis

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#### Abstract:

Bacterial meningitis is despite progress in research and the development of new treatment strategies still a cause of severe neuronal sequelae right up to death. The brain is protected from penetrating pathogens by the blood-brain barrier (BBB) and by the innate immune system. The representatives of the innate immune system in the brain are glial cells, astrocytes and microglia cells. The invading pathogens are being recognized via pattern recognition receptors such as formyl peptide receptors that are expressed by glial immune cells of the central nervous system (CNS). The expression of the G-protein coupled chemotactic formyl peptide receptors is up-regulated after bacterial meningitis, but the consequence of receptor for progression of inflammation are far from clear. Therefore, we used formyl peptide receptors (mFPR1 and 2) deficient mice to investigate the role receptors in lethality and inflammation after pneumococcal meningitis. We compared the lethality rate and bacterial growth between both mice strains and analysed the inflammation in the cortex and hippocampus using immunohistochemistry and realtime RT-PCR. Our results showed no change of lethality after bacterial meningitis for mFPR1/2-deficient mice compared to wildtype mice. But the mFPR1/2-deficient mice showed significant increased glial cell activation, whereas the immune response including cytokine and antimicrobial peptides expression are decreased after bacterial meningitis.

Rubrik: Neuroimmunologie

Titel: Is there any evidence for T cell stemness in experimental autoimmune encephalomyelitis?

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#### Abstract:

The concept of TH17 stemness is attracting increasing attention in the field of tumor immunology. In addition it has been shown that TH17 cells are less susceptible to apoptosis than TH1 cells. The expression of stem cell like-properties and the promotion of long-term immunity by TH17 cells is also of outmost relevance for autoimmunity.

To test the hypothesis of TH17 stemness, that is a shift toward TH17, we studied two mouse models of multiple sclerosis (MS), the MOG:35-55- and the PLP:139-151-induced experimental autoimmune encephalomyelitis (EAE). Using ELISPOT assays the frequency of autoantigen specific IFN-gamma and IL-17 producing cells was measured in the spleen and longitudinally in the blood. The CNS pathology was studied utilizing semi-thin sections stained with methylene blue.

In both mouse models CNS antigen-specific TH17 cells occurred in high frequencies and the ratio of TH1 and TH17 cells was comparable in blood and spleen in the induction phase of the disease. There was no preferential shift towards a TH17 response over time. The CNS pathology was associated with massive mononuclear infiltration in mice during the acute phase of the disease, though in the later phase the inflammatory activity vaned.

Thus, the apoptosis driven selection of TH17 over TH1 cells might not be particularly relevant for the MOG:35-55- and the PLP:139-151-induced EAE. Our data strongly suggest that the chronic outcome of EAE is due to permanent neurological damage in the CNS rather than being a reflection of ongoing TH17-mediated autoimmunity.

Rubrik: Neuroimmunologie

Titel:Flow cytometric analysis of the distribution of B cell subpopulations in patients with remitting-relapsing multiple sclerosis

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#### Abstract:

In this study we aimed to evaluate the frequencies of B cell subpopulations expressing CD3, CD20, CD27, CD43 and CD138 in patients with remitting-relapsing multiple sclerosis (RRMS). We wanted to analyze if the treatment with an immunomodulatory drug had an effect on the distribution of B cell subpopulations.

Peripheral blood mononuclear cells (PBMC) of 10 RRMS patients (5 patients were treated with glaterameracetat (GA) (tRRMS), 2 were untreated (utRRMS), 2 treated with nataluzimab (NA)) and 2 healthy control (HC) subjects were polyclonally stimulated for 96 hours subsequently the frequencies of B cell subpopulations were analyzed by 8-color-flow cytometry.

The data show that the frequency of the CD27+CD43+ B cell subset (B1 B cells) was significantly lower in tRRMS (GA) patients (18.84  $\pm$  6.41% mean  $\pm$  standard deviation) and tRRMS (NA) (20.33  $\pm$  8.33%) compared to HC subjects (39.65  $\pm$  2.75%; p = 0.01). Interestingly, the frequency of CD27+CD43- (Bmem) cells of the CD3-CD20+ B cells fraction was significantly higher in tRRMS (NA) patients compared to utRRMS patients and HC subjects (42.25  $\pm$  3.6% vs. 15.95  $\pm$ 2.85%; p = 0.01 and 42.25  $\pm$  3.6% vs. 15,70 $\pm$ 1.3%, p = 0.01, respectively). Furthermore, the CD27high fraction (plasmablasts) of the CD3-CD20-cells was decreased in tRRMS (NA) compared to HC subjects (1.48  $\pm$  0.17% vs. 20.60  $\pm$  0.4%, p = 0.01).

Here we demonstrate that the ratio between B1 B cells and Bmem B cells in RRMS patients is altered. These findings imply the need for further investigations of the B cell subpopulations and their functional characterization in patients with MS.