ANATOMISCHE GESELLSCHAFT
1886

114th Annual Meeting / 33. Arbeitstagung
der Anatomischen Gesellschaft
25. bis 27. September 2019

am Institut für Anatomie und Zellbiologie
der Universität Würzburg

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To find your abstract or
an abstract of interest
please use the alphabetical list of
first authors of lectures and posters
starting on next page.

Inhaltsverzeichnis/Contents

Erstautor/First author	Nr. des Vortrages (V) / Posters (P)
García Ponce A.	V7
Gasterich N.	V41
Gasthaus J.	P150
Geyer S.	P41
Gläser A.	P33
Gögele C.	P163
Groeneweg F.	P133
Gross I.	P123
Guy J.	V17
Haenssgen K.	P83
Hafner G.	V19
Hagedorn P.	P87
Hanika S.	P64
Hawlitschka A.	V34
Hecking I.	P117
Heilen L.	P156
Hensel N.	V32
Heun F.	P80
Hilmer J.	P78
Hinganu M.	P72
Hollenbach J.	P62
Hollenhorst M. I.	V39
Hörmann R.	P48, P84
Hu J.	V46
Iliev A.	P103
Islinger M.	V5
Ismail R.	P142
Jahr H.	P1
Jansing J. C.	P66
Jedlicka P.	V16
Johann S.	P138
Joost S.	V11
Kankowski S.	P134
Karnati S.	V15
Kavak D.	P61
Keiler J.	P2
Keller S.	V51
Kemnitz J.	P57
Kleefeldt F.	P9, V54
Kleine A.	P151
Kleinsasser B.	P7
Kliewe F.	V1
Klose L.	P28
Klymiuk M. C.	V45
Kneusels J.	P120
Knickmeyer M.	V23
Knöfler H.	V3
Korf H.-W.	P115
Krueger M.	P101
Kubo H. K.	P88
Kugelmann D.	V2
Kumar P.	P17
Kunke M.	P6
Kwiatkowski J.	P53
Labitzky V.	P59

Erstautor/First author	**Nr. des Vortrages (V) / Posters (P)**
Lambertz J.	P129
Lange T.	V8
Larionov A.	P74
Lehmann J.	P30
Lenz F.	P157
Lenz M.	V27
Li W.	P20
Lienbacher K.	V30
Lindhorst A.	P89
Lobachev O.	P95
Lückstädt W.	P22
Lutz D.	P136
Lutze G.	P42
Mages B.	P132
Mahmoud W.	P67
Malik I. A.	P8
Mann T.	V12
Marschalek A.	P21
Maurer-Gesek B.	P85
Maxeiner S.	P161
Mehlhorn J.	P108
Miroschnikov N.	P105
Müller J.	P152
Mutig K.	V4
Namm A.	P44
Nandigama R.	P26
Neckel P.	V44
Ortug A.	P116
Ortug G.	P82
Oswald J.	P45
Ottone N.	P10, P98
Petkova A.	P114
Pfeiffer V.	P23
Pfitscher K.	P93
Plantera L.	P107
Pous L.	V40
Pruidze P.	P86
Raisch H.	P111
Rana S.	P147
Reissig L. F.	P46
Richard M.	P122
Rietsche M.	V20
Rink S.	P154
Rodewald A.	P104
Rodriguez L. Z.	P25
Ruß T.	P127
Scharr M.	P32
Scheiner O.	P106
Scheld M.	V42
Schicht M.	P34
Schmidt P.	V56
Schnatz A.	V38
Schröder A.	P90
Schropp V.	V50
Schulte H.	P65

Erstautor/First author	Nr. des Vortrages (V) / Posters (P)
Schulze-Tanzil G.	P164
Schumacher S.	P160
Schwaerzer G.	P11
Semikasev E.	P145
Serrano N.	P81
Sharma K.	P76
Shoykhet M.	V43
Sigmund A. M.	P4
Sivukhina E. V.	P58
Slabik A.-K.	P55
Slowik A.	P144
Starzonek S.	P68
Stegemann L. N.	P118
Stein J.	P139
Steiniger B. S.	V10
Stofferin H.	P71, P96, P97, V35
Storsberg S. D.	P113
Sturm M. A.	V37
Stutzkowsky L.	P52
Thomas L.-Z.	P54
Thomson M. N.	V53
Tohidnezhad M.	P3
Torga T.	P63
Trattnig C.	P121
Trinh S. N.-B.	P119
Tsikolia N.	P40
Ullrich N.	P91
Upcin B.	P36
Vasiliev Y.	P70
Voelz C.	V33
Voigt M.	P110
Wagner N.	P14
Wandres M.	P102
Wanuske M.-T.	P27
Washausen S.	P39, P50
Wedel T.	V36
Welss J.	P16
Weninger J.	P148
Wicklein D.	V47
Wieghofer P.	V9
Wiegreffe C.	V21
Winokurow N.	P128
Wolniczak E.	P125
Wörsdörfer P.	V22
Wozniak S.	P77
Wunsch F.	P141
Wurm J.	P130
Xu Y.	P15
Yilmaz D. E.	P19
Zendedel A.	P146
Zhan J.	P140
Zimmermann J.	P131

Poster 1:

Titel:
Direct printed absorbable porous metallic bone implants

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Abstract:
An ideal load-bearing bone substitute material should provide sufficient mechanical support, is porous to promote angiogenesis, and disappears after its job is done. We used rational design and innovative Direct Metal Printing (DMP) to additively manufacture (AM) novel absorbable implants based on magnesium (Mg), iron (Fe) and zinc (Zn). We further developed bioreactor systems to evaluate degradation characteristics of the novel medical devices under in vivo-like in vitro conditions. The aims of this study were to i) show technical feasibility and ii) improving evaluation of absorbable medical devices according to ISO 10993-like standards.

Direct printing CAD-designed topographically ordered absorbable implants, using atomized metal powders. In vitro degradation under static and dynamic conditions in custom-built reactors under physiological conditions, for up to 28 days. Mechanical (ISO 13314: 2011), physicochemical and biological (ISO 10993 mod.) characterization during degradation period was compared to identically designed standard of care (Ti-6Al-4V.

Printed highly porous metallic implants revealed final morphologies closely resembling predefined CAD design values and superior mechanical properties, with Yield strengths and Young's moduli similar to that of trabecular bone, even after 28 days of in vivo-like degradation. In direct and indirect cytocompatibility assays, Fe performed poorly, followed by Mg and Zn; the latter showed level 0 cytotoxicity (modified ISO). Dynamic incubation resulted in improved cell viability as compared to static culture (2-way ANOVA, post-hoc Turkey's multiple comparison.

Accurately direct printed porous absorbable metal implants hold potential for dedicated Orthopaedic applications, with Zn showing best biocompatibility.

Poster 2:

Titel:
Quantitative post mortem morphometry of thrombofibrotic lead encapsulations in arrhythmia patients with implantable cardiac electronic devices

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Abstract:
About every hundredth arrhythmia patient with an implantable cardiac electronic device (ICED) is affected by a lead revision due to e.g. lead failure or infection. However, functionless leads are explanted in only around half of cases since the procedure is often challenging and risky for some patients due to lead adhesions caused by thrombofibrotic encapsulations. To gain information for the optimization in terms of reduced lead adhesion and better extractability, we evaluated the topographic and histological patterns of lead ingrowths in arrhythmia patients with ICEDs quantitatively.

Lead-bearing hearts and veins from corpses with an ICED (n=35) were dissected. The types of lead encapsulations (sheaths) were identified macroscopically:

type I) sheath with adhesion to vein/endocardium,

type II) sheath without adhesion.

In case of multi-chamber-systems further differentiation was performed:

subtype A) presence of lead-to-lead binding,

subtype B) absence of lead-to-lead binding.

Segment lengths were measured and sheaths were mapped topographically using anatomical landmarks for 56 leads. For 16 leads, about 70 sheath loci were analyzed histologically and degree of fibrosis and calcification and thickness of the sheath were assessed.

On average, 40.1 ± 17.5 % of the intravascular lead length was covered by an adhesive thrombofibrotic sheath. Correlations between ingrowth characteristics, lead properties and dwell time are discussed.

Quantitative post mortem analyses of ICED leads help to understand characteristics and formation of thrombofibrotic lead encapsulations. Specific modifications of technical lead parameters and integration of a drug eluting system in the outer lead insulation might improve extractability due to a reduced overall ingrowth.

Poster 3:

Titel:
Analysis of effects of modified beta tricalcium scaffolds on bone regeneration using a critical size fracture, healing model in mice

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Abstract:
The goal of this study was to compare the influence and effect of various sort novel tricalcium phosphate scaffolds particularly with regard to the inflammation process in bone healing.

Critical size fracture (CSF) on femur of NF-ƙB-luc transgenic mice was performed. The used Tricalciumphosphat scaffolds were modified by addition of strontium (TCP+Sr) or coating of co-culture mesenchymal stem cells and endothelial cells (TCP+OIM/EC). In consequence, the longitudinal expression levels of NF-κB could be monitored using the imaging system for two months.

The highest peaks of luciferase activity in the early phase of inflammation were observed by TCP+OIM/EC group. Sr reduced inflammation in the early phase of healing (15th days), but it was increased in the late healing stage. Newly tissue formation (NTF) in TCP+Sr group was significantly higher than in the TCP group, whereas the percentage of osseous tissue in relation to the NTF was in TCP group much more than in ß-TCP+ Sr groups. In control group a connective tissue without bony formation were observed.

This study presents the first data regarding NF-κB promoter activity profiles during fracture healing.

The higher percentage of bony tissue in β-TCP group compared to the Sr group lends this group a beneficial use to treatment of CSF.

Sr group leads to the higher tissue formation with less bone percentage, neverless, these soft and bony tissue bridges filled the fracture gaps faster in compare to the TCP group. This effect may prove to be advantageous for fracture healing by patient with less bone stability as osteoporosis patient.

Poster 4:

Titel:
Heterophilic Dsg3-Dsg2 interactions as a compensatory mechanism in pemphigus

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Abstract:
In the bullous autoimmune disease pemphigus, autoantibodies directed against the desmosomal cadherins desmoglein (Dsg)1 and 3 cause loss of intercellular adhesion in the epidermis which was reported to be paralleled by Dsg2 upregulation. We showed that homophilic Dsg3 interactions are directly inhibited upon autoantibody binding and recently observed that Dsg3 also undergoes heterophilic interactions with Dsg2. Thus, we here investigated the impact of this heterophilic interaction in pemphigus.

Atomic force microscopy (AFM), ex-vivo pemphigus skin model, Western blot, Immunoprecipitation, Immunostaining, Keratinocyte dissociation assay

Dsg2, which is almost absent in healthy, adult, interfollicular epidermis, was upregulated in epidermis of pemphigus patients. Similarly, pemphigus autoantibodies induced upregulation of Dsg2 in a human ex-vivo skin model. Further, a newly generated stable murine Dsg3-deficient keratinocyte cell line showed severely disturbed intercellular adhesion in keratinocytes dissociation assay paralleled by upregulation of Dsg2 in the cytoskeletal-bound membrane fraction. Heterophilic interaction was confirmed by Dsg2-Dsg3 co-immunoprecipitation in human keratinocytes. Thus, we characterized the heterophilic Dsg2-Dsg3 interaction by cell-free AFM, which showed binding strength and a catch-bond behavior comparable to homophilic Dsg3 interactions. Interestingly, a pathogenic pemphigus aDsg3 antibody directly impaired the heterophilic Dsg2-Dsg3 interaction less than homophilic Dsg3 binding.

Taken together, the data show that Dsg2 undergoes heterophilic interactions with Dsg3, which may serve as a compensatory mechanism to ameliorate autoantibody-induced loss of keratinocyte adhesion in pemphigus and further reflects the biological significance of heterophilic Dsg3 interactions under pathological conditions.

Poster 5:

Titel:
Roles of PKC and Erk1/2 in epidermal blistering and desmosome regulation in pemphigus

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Abstract:
To assess the role of PKC and ERK1/2 signaling pathways in mediating blister formation and regulation of desmosome ultrastructure in human epidermis.

Ex vivo human skin and mucosa model, transmission electron microscopy, cryosectioning, H&E staining, immunostaining.

Human skin explants were exposed to PV-IgG together with inhibitors for PKC or ERK1/2 signaling. Inhibition of PKC was not effective to prevent suprabasal blister formation or ultrastructural alterations of desmosomes. In contrast, inhibition of ERK significantly ameliorated blister formation and decrease in the number of desmosomes whereas shortening and splitting of desmosomes and keratin filament insertion were not different from samples treated with PV-IgG alone. However, desmosomes between basal and suprabasal cells remained unaltered when ERK1/2 signaling was inhibited.

Inhibition of ERK1/2 but not PKC signaling appears to be effective to ameliorate blistering and alterations of desmosome ultrastructure triggered by PV-IgG in human skin.

Poster 6:

Titel:
MTORC1 phosphorylates megalin to induce proximal tubular endocytosis

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Abstract:
Endocytosis is a hallmark of the proximal tubule and is one of the most important kidney functions to prevent loss of plasma proteins and to maintain body homeostasis. Protein uptake is mediated by the scavenger receptors megalin and cubilin, followed by internalization of the ligand-receptor complex via the clathrin-mediated pathway. The mTORC1 complex is a principle regulator of proximal tubular function, including endocytosis. Tubular deletion of mTORC1 reduces endocytosis of lactoglobulin and changes phosphorlyation of megalin despite normal megalin expression and distribution within proximal tubules. However, the effect of mTORC1 on megalin function remains unknown.

Plasmids containing megalin-minireceptor 2 (MMR2) were used to introduce mutations in the respective mTORC1 phospho-site S4577. To mimic phosphorylation S4577D and to inhibit phosphorylation S4577A were introduced. EBNA and MDCK II cells were transiently transfected with generated constructs for endocytosis assays with Alexa555-labeled albumin, localization studies by immunohistochemistry with markers for the endocytic vesicles or cell surface biotinylation and to study the stability of C-terminus by blocking proteasomal activity using MG132.

Phosphoproteomic analysis of megalin´s C-terminus upon mTORC1 stimulation and inhibition demonstrated a mTORC1-dependent phosphorylation at S4577 confirming previous published results. Subsequent analysis of phospho-site mutants revealed no influence on phosphorylation of PPPSP motif of megalin C-terminus and no significant difference in megalin expression and its cellular distribution. However, endocytosis was significantly increased using S4577D mutant and reduced using S4577A mutant compared to megalin wildtype under stimulating and non-stimulating conditions. Compared to megalin wildtype construct, inhibition of proteasome activity led to an augmented expression of megalin C-terminus using S4577A mutant whereas the amount was decreased using the S4577D mutant.

mTORC1 complex is an important regulator of proximal tubular function and phosphorylates megalin to influence megalin function.

Poster 7:

Titel:
Expression and function of ATP-binding cassette transporter in the lacrimal system

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Abstract:
The ABC transporter family is an important group of membrane-bound transporter for the cellular homeostasis as well as for efflux of endogenous substances and xenobiotics. Especially, the protein encoded by the ABCB1 gene (multi-drug-resistance-protein [MDR] 1/P-glycoprotein-1 [P-Gp]) shows a high variability for pharmacological substances and a well documented clinical relevance. In detail, expression and physiological relevance of most ABC transporter in the lacrimal system are yet unknown.

Expression of ABC transporter was analyzed in human lacrimal tissues from body donors immortalized human corneal epithelial (HCE) and conjunctival epithelial (HCjE) cells by RT-PCR and for selected ABC transporter by immunohistochemistry. Different stressors such as physical challenges using shear stress, hypoxia, and exposition to UV light, as well as chemical stressors like proinflammatory cytokines and variable salt concentrations were used to determine the regulation of MDR1 in cell culture experiments by qPCR, western blot analysis and immunofluorescence. Additionally, the functional activity of MDR1 was determined by functional calcein AM assays.

RT-PCR results revealed aberrant expression of the different ABC transporter in the examined lacrimal tissues and cell lines. Immunohistochemistry verified the presence of MDR1 at the human ocular surface. Furthermore, MDR1 expression was regulated by the used medium and through various mechanical and chemical stressors, especially in HCjE cells.

We show that a variety of ABC transporter is expressed to a significant extent in the lacrimal system. Further studies have to explore the importance of ABC transporter with regard to different eye diseases as well as their interaction with common drugs applicate at the ocular surface.

Poster 8:

Titel:
Development of a new mouse model for intrahepatic cholangiocellular carcinoma: Accelerating functions of Pecam-1

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Abstract:
Due to the lack of suitbale models the etiology of intrahepatic cholangiocellular carcinoma (ICC) is poorly understood. Cancers may arise from sites of inflammation. We previously showed involvement of platelet endothelial cell adhesion molecule-1 (Pecam-1/CD31) in acute liver damage. Here we aimed to develop a mouse model of ICC and to investigate the role of Pecam-1.

We treated male wild-type (WT) and Pecam-1-knock-out (KO)-mice orally with thioacetamide (TAA) for 22 weeks.

Gross inspection and microscopy revealed liver cirrhosis and ICC in both WT- and Pecam-1-null-mice. Severity of cirrhosis and ICC (demonstrated with anti-Ck-19 immunohistology) was reduced in Pecam-1-null-mice (stage 4 cirrhosis in WT vs. stage 3 in KO-mice; reduced Ck-19 quantity and Ki-67-ICC-labeling in KO-mice). Tumor cells were predominantly located in portal areas, and had mostly undergone epithelio-to-mesenchymal transition. Dilations of numerous bile ducts were indicative of cholestasis. In serum of WT- and Pecam-1-null-mice, TAA induced an increase in the hepatic damage markers alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, and bilirubin. Thereby, Pecam-1-null-mice revealed signficantly lower levels. With qPCR of liver, induction of Pecam-1 mRNA expression was noted in WT-mice, in addition to the adhesion molecules Icam-1 and EpCAM, as well as the cytokines Tgf-β, Tnf-α and Il-6. Interestingly, the levels of all investigated pro-inflammatory cytokines and EpCAM were signficantly lower in Pecam-1-null-mice. Lipocalin-2, a marker for liver and kidney injury, as well as Ccl5, cMyc and Mmp2 were significantly lower in Pecam-1-null-mice after TAA.

We present the first highly reproduceable mouse model for ICC, and show protective effects of Pecam-1-deficiency.

Poster 9:

Titel:
Bone marrow-independent macrophage precursor cells contribute to angiogenesis via VEGF/VEGFR-2-mediated activation of resident vascular progenitor cells

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Abstract:
Pathological angiogenesis promotes tumor growth, metastasis and atherosclerotic plaque rupture. Macrophages are key players in this process. However, whether these macrophages differentiate from bone marrow-derived monocytes or from local vascular wall-resident stem and progenitor cells (VW-SCs), is an unresolved issue of angiogenetic processes.

We analyzed vascular sprouting and alterations of aortic cell populations in the mouse aortic ring assay (ARA) using immunohistochemical methods and genetic in vivo labeling approaches. The contribution of macrophages to angiogenic activation in ARA was elucidated by clodronate-mediated depletion.

ARA culture leads to the generation of large numbers of macrophages especially within the aortic adventitia. Approximately 60% of these macrophages differentiate from bone marrow-independent Ly6c+/Sca-1+ adventitial progenitor cells. Furthermore, these macrophages represent the main source of VEGF in ARA that in turn promotes the generation of additional macrophages thereby creating a pro-angiogenetic feedforward loop. Additionally, macrophage-derived VEGF activates CD34+ progenitor cells within the adventitial vasculogenic zone to differentiate into CD31+ endothelial cells. Consequently, depletion of macrophages and VEGFR-2 antagonism drastically reduce vascular sprouting activity in ARA as well as remodeling of the adventitial stem cell niche.

In summary, we show that in angiogenically activated vessels a substantial number of macrophages differentiates from bone-marrow independent VW-SCs. These macrophages in turn promote angiogenesis by VEGF/VEGFR-2-dependent activation of the adventitial vasculogenic zone in a paracrine manner. Therefore, modulation of VW-SC-derived macrophages may become a key target to interfere with pathological angiogenesis in cancer and atherosclerosis as well as with regenerative angiogenesis in ischemic cardiovascular disorders.

Poster 10:

Titel:
Extraction of DNA from plastinated tissues

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Abstract:
Plastination allows anatomical samples to be preserved in excellent condition for an indefinite period, free of formalin, in a format that allows biosafe manipulation by students, academics and researchers. As with other tissue preservation techniques, it is important to establish the level of conservation of deoxyribonucleic acid (DNA) for use in future applications. The object of the present work was to extract and evaluate DNA from plastinated tissues.

We used samples of liver from Canis lupus familiaris and skeletal muscle from Rattus norvegicus, Sprague-Dawley strain, extracted from specimens plastinated with silicon at ambient temperature (Ottone et al., 2015). The samples were deplastinated by incubation of the tissues in sodium methoxide 5% dissolved in methanol for 24 and 48 hours. The samples were divided into two equal parts and DNA was extracted by two different protocols. After extraction, the quantity of DNA was measured by fluorometry and its integrity by electrophoresis in agar gel 1%.

A good yield was obtained in DNA extraction, with whole DNA extracted from the deplastinated samples.

Plastinated tissues have proved to be stable and easily managed. They can also be used for study under light and electron microscopes. This technique of deplastination and DNA extraction allowed us to obtain whole DNA from samples plastinated with silicon at ambient temperature, without previous fixing. It may allow pieces to be preserved for retrospective studies in basic, clinical and forensic sciences, epidemiology, and in archived samples of normal and pathological anatomy.

Poster 11:

Titel:
Corin expression is decreased in nephrotic syndrome independent of ER-stress

Autoren:
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Abstract:
The ANP/GC-A/cGMP system plays a pivotal role in regulating renal physiological function and counteracting pathophysiological conditions through increased sodium and water excretion. Recently, we found that the expression of corin, a serine-type endopeptidase activating the artrial natriuretic peptide (ANP), is decreased in proteinuric kidney diseases such as nephrotic syndrome and glomerulonephritis. The lack of ANP cleavage causes ANP resistance and hypertension in humans and mice.

To understand the decreased corin/ANP-signaling in the nephrotic syndrome, podocytes-specific Nphs2 knock out mice as FSGS model were used to study the time-dependent expression of corin, ANP and cation-dependent ion channels, ER stress and the infiltration with immune cells using qPCR, western blots and immunohistochemistry. In addition, we analyzed the impact of various parameters as salt, cytokines and ER stress on the transcriptional regulation of corin in vitro.

In Nphs2∆pod mice, the level of corin mRNA was reduced at 5, 9 and 14 days after knockout induction. ANP mRNA was increased at time points analyzed. Since induction of ER stress was shown to cause corin mRNA decay, marker for ER stress were analyzed and found to be increased in Nphs2∆pod mice. Additionally, mpkCCD cells were treated with tunicamycin to induce ER stress. However, the induced ER-stress did not affect the corin mRNA stability and, thus, the decrease of corin expression is independent to ER-stress.

In the nephrotic syndome the expression of corin is decreased independently on ER stress. The early onset of corin reduction may be related to a cellular response to inflammation.

Poster 12:

Titel:
Nitric oxide changes the biomechanical behavior and the expression profile of Schlemm canal cells

Autoren:
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Abstract:
Primary open-angle glaucoma is a chronic progressive neuropathy. The main risk factor is an intraocular pressure (IOP) causing a loss of axons of the optic nerve. IOP alterations are due to an increased outflow resistance in the trabecular meshwork and Schlemm's canal (SC), caused by accumulation of extracellular matrix and by stiffening of outflow tissues cells. Nitric oxide (NO) is involved in the regulation of outflow facility and mediates cell relaxation. For that purpose, we analyzed the effect of exogeneous NO on of endothelial cells from SC.

Primary SC cell strains (n=3) were treated with 50 and 100 µM of a NO donor (Deta-NO). Cell viability was tested by Calcein-AM staining and influence on actin cytoskeleton by phalloidin labeling. Stiffness was analyzed by atomic force microscopy (AFM). By qPCR analysis, the expression of connective tissue growth factor (CTGF) and transforming growth factor-BETA2 (TGF-BETA2) were analyzed.

Immunocytochemical analysis showed marked changes in the actin cytoskeleton after Deta-NO treatment by inducing pronounced actin depolymerization. Probing subcortical and cortical stiffness, AFM measurements showed that Deta-NO decreased stiffness significantly. Gene expression analysis revealed significant downregulation for CTGF and TGF-BETA2.

We report strong evidence that exogeneous NO leads to marked changes in the biomechanical behavior of SC cells. In addition, we observed reduced expression of genes causing glaucomatous changes in outflow tissues. We suggest that the reduction of the outflow resistance after NO treatment is linked to the observed alteration of cellular stiffness and to the expression changes of SC cells.

Poster 13:

Titel:
TNF-β-induced proliferation of colon cancer cells is supressed by resveratrol

Autoren:
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Abstract:
TNF-β (Lymphotoxin) is a pro-inflammatory cytokine closely related to TNF-α. Although the impact of TNF-α in promoting tumorogenesis via activation of the NF-kappaB signalling pathway is well understood, little is known how TNF-β may stimulate tumorogenesis. Resveratrol is a natural polyphenol that possesses anti-inflammatory and anti-cancerogenic properties. Here we investigated how resveratrol may influence TNF-β-induced cancerogenesis and NF-kappaB activation.

We investigated colon cancer (CRC) cell line HCT116 in monolayer and three dimensional in vitro cell culture models for proliferation, invasion and colony formation.

TNF-β, similar to TNF-α treatment significantly stimulated CRC cell proliferation, invasion and colony formation and these effects were significantly suppressed by anti-TNF-β-receptor treatment. Resveratrol, similar to anti-TNF-β-receptor, blocked TNF-β-induced proliferation, invasion and colony formation and this was correlated with decreased NF-kappaB signalling. Further, resveratrol, similar to inhibitor of IkappaB-kinase (IKK) BMS-345541, blocked TNF-β-induced tumorogenesis, suppressed TNF-β-upregulated activation of IkappaBα, phosphorylation and nuclear translocation of p65-NF-kappaB and stimulation of NF-kappaB-linked genes which regulate proliferation, apoptosis and invasion. Finally, we have shown by ultrastructural immune-electronmicroscopic studies that TNF-β- and TNF-α-Receptors are expressed on the surface of CRC cells.

For the first time we could demonstrate that apart from TNF-α also TNF-β/TNF-β-receptor signaling is markedly involved in proliferation of CRC cells and resveratrol could significantly suppress this by targeting the NF-kappaB signaling pathway. This highlights the possible therapeutic effects of resveratrol as multi-targeted anti-cancer agent.

Poster 14:

Titel:
Megakaryocyte-endothelial cell interaction during platelet biogenesis in the bone marrow

Autoren:
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Abstract:
The final steps of platelet biogenesis require entrance of megakaryocyte (MK) protrusions into bone marrow sinusoids and release of MK fragments into the circulation. The cellular mechanisms involved in MK interaction with sinusoidal endothelial cells (SECs) and MK protrusion passage still remain unclear. Therefore, this study aims for the ultrastructural characterization of the interplay of MKs, SECs and CXCL12-abundant-reticular-cells (CAR-cells) within murine bone marrow sinusoids.

Ultrastructural analysis was performed using transmission-electron-microscopy and high resolution 3D serial-block-face-scanning-electron microscopy.

At sites of protrusion, we could not detect any preformed pores in the SECs, arguing against proplatelet extension inbetween single/individual endothelial cells. SECs were partially underlined by a basal lamina and covered by CAR-cells to a large extent. At MK/SEC contact sites, MK protrusions provoke local retraction of CAR-cells and disintegration of the basal lamina, followed by invagination and stretching of SECs and subsequently transcellular pore formation. 3D imaging demonstrates MK extensions to cross the marrow-blood-barrier by trans-endothelial passage. Before release of mature platelets, (pro)platelets are still interconnected via small cellular bridges and remain associated with SECs.

Our analysis supports a model in which MKs approach the sinus wall, preceded by local retraction of CAR-cell processes from the sinus wall, disintegration of the basal lamina followed by invagination and stretching of SECs at MK/SEC contact sides. MK protrusions penetrate the sinus wall by trans-endothelial passage into the sinus lumen with luminal formation of pro-platelets that remain interconnected and associated with SECs until they are finally released as platelets into the blood circulation.

Poster 15:

Titel:
Cell-specific heterogeneity of Cx43-positive gap junctions in the mammalian kidney

Autoren:
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Abstract:
Connexin 43 (Cx43) is a gap junction-forming protein which mediates the intercellular passage of molecules < 1000 Da. In the kidney, Cx43 was reported in glomerular vessels and mesangium. Own results prompted us to reevaluate the distribution of Cx43 and its phosphorylated form, phospho-S368-Cx43 (pCx43), within the different zones of the rat kidney and in cultured fibroblasts in more detail.

Confocal immunohistochemistry and EM localization was applied in rat kidney sections and cultured human fibroblasts. Cultured human fibroblasts served to study intercellular transfer of fluorescent lucifer yellow.

Cx43 was distributed in renal arterial and arteriolar endothelia, lymphatic vessel walls, peritubular capillaries, and glomerular compartments. In the interstitium, Cx43 was present as linear or punctate signals of the cell membrane of fibroblasts, in colocalization with pCx43. Cx43-positive medullary fibroblasts co-expressed cyclooxygenase-2. Ultrastructural analysis showed Cx43 signals at membrane contact zones between the cells. Cultured fibroblasts showed extensive, punctate Cx43 and pCx43 signals between adjacent cells. Signal was further distributed in non-junctional areas of plasma membrane, suggesting Cx43 hemichannel functions. Lucifer yellow staining spread rapidly (3 min) between fibroblasts suggesting intercellular exchange via gap junctions.

Cx43 in rat kidney shows prominent glomerular, vascular, and interstitial distribution, while epithelia, with exception of the podocytes, were negative. Focusing on interstitial fibroblasts, Cx43-mediated information transfer was demonstrated. A constitutive nature of Cx43 phosphorylation was established in these cells. Our data suggest a cell-specific heterogeneity of Cx43-positive gap junctions mediating information exchange among renal cell types.

Poster 16:

Titel:
Changes in tears and ocular structures involved in tear film formation in chemotherapy-induced polyneuropathy in mice

Autoren:
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Abstract:
Polyneuropathy (PN) patients frequently show typical symptoms of dry eye disease. However, the impact of neuronal degeneration processes to DED remains unclear. In this study, we used a chemotherapy-induced PN mouse model to investigate the effect on tear film and ocular structures involved in tear film formation.

C57BL/6 mice were treated once with 3mg/kg Oxaliplatin (Ox) (n=8) or Saline respectively. In 8 animals/group Schirmers test was performed at day 0 and day 10. Tear proteome changes were characterized in 3 animals/group by label-free quantitative mass spectrometry (MS)-based proteomics strategy. Analysis was made by utilizing MaxQuant computational platform and pathway analyses. Serial sections of the whole eyeball and lids (10µm) of 3 control mice were cut along the sagittal axis from nasal to temporal (228 sections/eye) and stained with Periodic Acid Schiff stain. The number of goblet cells (gc) in the conjunctival fornix of all serial sections was counted and compared with data of 3 treated mice obtained accordingly.

In normal mice the distribution of gc's differs markedly from that seen in humans. Most cells are found in the temporal quadrant. In PN mice, the number of gc's significantly decreased compared to control group ($P<0.01$). The tear volume was normal, but 7 proteins (ATP13A2, RHOX4B, LPO, LTF, 1600014C10RIK, KRT1 and BOD1L1) were found to be significantly ($P<0.05$) differentially expressed.

Our preliminary data show, that even a single Ox treatment alters the number of gc's as well as specific proteins in tears. It is currently unclear whether Ox affects the gc's or tear film directly or indirectly through intervention in neuronal processes.

Poster 17:

Titel:
Nicotine stimulation of apical chloride secretion in airway epithelium is dependent on the presence of the beta4 subunit in nicotinic acetylcholine receptors

Autoren:
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Abstract:
The epithelial ion and fluid secretion as a part of the mucociliary clearance is essential for the clearance of respiratory pathogens. In the murine tracheal epithelium, we have previously shown that nicotinic acetylcholine receptors (nAChR) mediate an apical chloride secretion driven by basolateral potassium secretion. However, the receptor subtype responsible for these ion transport changes remains unknown.

Transepithelial short circuit current (ISC) was measured with the help of an Ussing chamber in freshly isolated mouse tracheas in the presence of agonists and antagonists of different nAChR-subtypes.

Apical application of the general nAChR agonist nicotine transiently increased ISC with an EC50 of 23.14 µM. The nicotine-effect (100 µM) was inhibited by the general nAChR inhibitor mecamylamine (25 µM). 100 nM α-bungarotoxin (α7 nAChR inhibitor) had no effect. Epibatidine (α3β2, α4β2, α4β4, α3β4 nAChR agonist) transiently increased ISC with an EC50 of 50.69 nM. Moreover 100 µM A-85380 (α4β2, α3β4 agonist) increased ISC. 10 µM dihydro-β-erythroidine (α4β2, α3β2, α4β4, α3β4 antagonist) and 1 µM α-conotoxin MII (α3β2 antagonist) significantly reduced the nicotine-effect, while lower concentrations of both inhibitors had no effect. In β4-deficient mice nicotine (100 µM) and epibatidine (1 µM) had no effect, while in β2-deficient mice, the nicotine- and epibatidine-induced currents were similar to wild type mice.

Heteropentameric nAChR containing the β4 and α3 or α4 subunit are responsible for stimulation of the transepithelial ion transport which is followed by fluid secretion. Selectively targeting these nAChR-types in the trachea might represent a useful target for stimulation of the mucociliary clearance.

Poster 18:

Titel:
Peroxisomes in pancreatic β-cells: protectors against Diabetes type 2

Autoren:
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Abstract:
Pancreatic β-cell failure is one of the characteristics of type 2 diabetes and has been attributed to deleterious effects induced by chronically elevated glucose and fatty acids levels, which cause oxidative stress and the alteration of the intracellular energy metabolism.

Peroxisomes are organelles involved in the degradation of a variety of lipid derivatives and in the metabolism of reactive oxygen species. We investigated whether the dysfunction of peroxisomes leads to metabolic dysfunction and cell-death in β-cells by increasing oxidative stress and promoting the accumulation of intracellular lipids.

Peroxisomal dysfunction was induced in β-TC3 cells by siRNA-mediated knockdown of the peroxisomal biogenesis protein Pex13 and cells were challenged using the fatty acids palmitate and phytanic acid. qRT-PCR, Western blot, immunofluorescence analysis, catalase-assay, reactive oxygen species measurements, and lipid droplet accumulation analysis were carried out to investigate the obtained phenotype. The capability of the β-TC3 cells to store/secrete insulin was also assessed.

Peroxisomal dysfunction led to increased H2O2 production and caused mitochondrial alterations in β-TC3 cells. Furthermore, it affected the lipid storage capability of the β-TC3 cells alone as well as in the presence of toxic fatty acid concentrations. The cytotoxicity of fatty acids was exacerbated by the peroxisomal dysfunction and was accompanied by decreased insulin release.

Our results suggest the importance of intact peroxisomes for the function and protection of the β-cell during lipotoxicity and show that they are actively involved in the detoxification of excess fatty acids and in the regulation of lipid storage in β-cells.

Poster 19:

Titel:
Calcineurin inhibitors induce endoplasmic reticulum stress in kidney epithelial cells via PERK- and ATF6-dependent mechanisms

Autoren:
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Abstract:
Calcineurin inhibitors such as cyclosporine A (CsA) or tacrolimus (Tac) are instrumental for immunosuppression after organ transplantations but may cause substantial nephrotoxicity with induction of endoplasmic reticulum (ER) stress and unfolded protein response (UPR) in kidney epithelia. Calcineurin may alleviate ER stress via PERK- and ATF6-dependent UPR pathways. We have studied the roles of PERK and ATF6 in managing the ER stress upon calcineurin inhibition.

To this end, we generated PERK-deficient and ATF6-deficient human embryonic kidney (HEK293) cell lines using CRISPR/Cas9-mediated gene editing. Effects of CsA on expression and cellular distribution of two critical UPR transcription factors, CHOP and spliced XBP1, were evaluated.

Treatment of control HEK293 cells with CsA (10 µM for 6h) significantly increased CHOP and spliced XBP1 protein levels in cell lysates. Immunofluorescence analysis revealed CsA-induced nuclear translocation of these transcripton factors. In contrast, PERK-deficient or ATF6-deficient cells showed no significant CsA-induced increases of CHOP expression along with much weaker stimulation of spliced XBP1 compared to control cells. To extend these results, we evaluated effects of Tac in cultured murine distal convoluted tubule (DCT) cells. In this model, treatment with Tac (10 µM for 6h) increased CHOP expression by two-fold but did not affected spliced XBP1.

In sum, these results suggest that CsA causes ER stress and UPR in kidney epithelial cells, which depends on activation of PERK or ATF6 functions.

Poster 20:

Titel:
Metalloprotease meprin BETA cleavage alters the expression of fibrosis associated genes in the cell manner.

Autoren:
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Abstract:
Meprin BETA is a multi-domain metalloprotease which is involved in the regulation of various physiological and pathophysiological processes including cancer cell migration and fibrosis. With procollagen I it maturates the most important structural protein in the human body. In recent years meprin BETA was found upregulated in fibrotic conditions such as lung fibrosis or idiopathic pulmonary hypertension.

In this study we use biochemical (Western blot, qRT-PCR) cell culture (protein expression, IF) and animal models (knock-out or inducible overexpression mice) to evaluate the role of meprin BETA in different cell types with regard to extra cellular matrix deposition.

The cleavage of BETA1-integrin by meprin BETA leads to the down-regulation of matrix deposition related genes, like CTGF (Connective tissue growth factor) in fibroblasts, thus activating a negative feedback loop of matrix deposition. On the contrary, meprin BETA cleavage of BETA1-integrin in smooth muscle cells induces the transcription matrix deposition associated genes and thus, activates a positive feedback loop. When we overexpressed meprin BETA in the smooth muscle compartment of mice, we observed an increased matrix deposition in the tunica muscularis of the colon.

We found that the metalloproteinase meprin BETA induces cell specific expression patterns with regard to matrix deposition related genes. While a negative feedback loop is induced in fibroblasts, that protects cells from excess matrix production, a positive feedback loop is induced in smooth muscle cells that results in increased matrix deposition even under steady state conditions.

Poster 21:

Titel:
CEACAM1-deficieny significantly increased tumor metastasis in TRAMPC1 mice

Autoren:
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Abstract:
Since the cell adhesion molecule-1 (CEACAM1) plays a key role as tumor suppressor gene in prostate cancer (PCa) and vascularization, we used the experimental TRAMPC1 PCa mouse model and analyzed besides PCa development tumor cell (TC) metastasis depending on CEACAM1 expression.

We used the following mouse models: TRAMPC1, TRAMPC1/CEACAM1-knockout and TRAMPC1/CEACAM1-knockout/endoR (global CEACAM1-knockout with endothelial CEACAM1-rescue). Here, we were able to follow up tumor progression by even single TC detection using SV40 antibodies.

Besides significantly rapid PCa initiation and significantly increased PCa tumor weight, TC metastasis to periphery organs appeared significantly accelerated in the global absence of CEACAM1. Whereas TC metastasis to lung, liver and kidney were detected in TRAMPC1 mice as early as 15 weeks of age in mostly single mice, TRAMPC1/CEACAM1-knockout and TRAMPC1/CEACAM1-knockout/endoR mice showed a significantly earlier and increased TC outbreak. Hereby, single TC metastasis were detected as early as 9 weeks of age in liver and kidney of TRAMPC1/CEACAM1-knockout/endoR mice. Already at the age of 12 weeks TC metastasis were observed in lung, liver and kidney with even solid tumor formation. These solid tumors behave like PCa tissue forming glandular structures with expression patterns like normal prostate tissue for Cytokeratin, Prostate specific antigen (PSA), SV40 and with exception of CEACAM1-knockout a luminal expression of CEACAM1.

Therefore, we hypothesize that endothelial overexpression together with epithelial absence of CEACAM1 accelerates the hematogenic outbreak of PCa cells and subsequent formation of distant metastasis.

Poster 22:

Titel:
CD109 - Proteolytic Processing Alters Signaling Properties and Sorting to Extracellular Vesicles

Authors -->
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Abstract:
CD109 is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein belonging to the ALPHA2-macroglobulin family. The full-length form of CD109 is reported to be upregulated in many tumor cells and in extracellular vesicles (EVs) which is associated with a bad prognosis for patients. There have been reports of several signaling pathways including JAK-STAT and YAP-TAZ that relate to CD109. As we found global CD109 levels downregulated in a proteomics approach after stimulation of cells with meprin BETA, we further investigated the connection between both proteins with regard to cleavage, sorting to EVs and cell signaling.

Protein-protein interaction was investigated in cell culture experiments. Western blot analysis was used to determine meprin BETA cleavage of CD109 and downstream signaling effects. Furthermore, EVs from different cell lines were purified using ultracentrifugation or exosome purification kits. Characterization of EVs was performed using IF, TEM and Western blot analysis.

We found that CD109 is cleaved by meprin BETA at the cell surface and preliminary analysis revealed a reduced STAT3 phosphorylation after co-expression of CD109 and meprin BETA. We identified numerous glioblastoma cell lines with different expression levels of CD109 and checked again for alterations in signaling intensities after co-expression of meprin BETA. We purified EVs from these cells, found full-length CD109 present on them and analyzed its function by different means.

Here, we show that CD109 is cleaved by meprin BETA and that this affects downstream signaling. We could identify CD109 localization on EVs that may alters signaling properties on distant cells.

Poster 23:

Titel:
Endothelial presence of CEACAM1 promotes the lymphatic metastasis of prostate cancer in the murine TRAMPC1 model

Autoren:
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Abstract:
Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is involved in tumor development and vascularization including human prostate cancer (PCa) while its role in lymphatic metastasis of PCa remained elusive.

To this aim we performed immunostaining for CEACAM1 and some other tumor markers on tissue sections from human PCa of different Gleason Stages and corresponding lymph nodes (LN). Furthermore, following experimental mouse tumor models were used: TRAMPC1, TRAMPC1-CEACAM1-knockout and TRAMPC1/CEACAM1-knockout/endorescue that reflects the CEACAM1 pattern in human PCa. Subsequently, development and lymphatic metastasis of PCa were analyzed at different time points.

CEACAM1 expression was found in LN sinus of patients with an early stage of PCa, while the LN were diagnosed to be tumor free and did not show any positive tumor cell marker expression. These findings were further confirmed by experimental TRAMPC1 mouse PCa analysis, using SV40 as a specific marker for even single tumor cell metastasis. As observed in human PCa, newly formed blood vessels and lymphatics of prostate and LN tissue become CEACAM1-positive. Mouse PCa analysis furthermore revealed significantly accelerated PCa initiation together with significantly higher lymphatic metastasis rates into sentinel lymph nodes in the absence of epithelial CEACAM1 but presence of endothelial CEACAM1 expression.

Taken together, we demonstrate a dual role of CEACAM1 in PCa: epithelial CEACAM1 presence prevents PCa initiation while endothelial CEACAM1 expression accelerates the primary PCa development as well as its metastasis into the LN.

Poster 24:

Titel:
The actin binding protein EPLIN controls actin assembly during leukocyte transmigration

Autoren:
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Abstract:
Leukocyte transmigration through activated endothelium occurs either through endothelial cell (EC) junctions or the cell body. Firm adhesion of leukocytes via LFA-1 induces ICAM-1 clustering on EC which is accompanied by actin filament recruitment to this site, leading to the formation of docking structures. Actin dynamics is thought to mediate leukocyte transmigration and assist in VE-cadherin dynamics at the junction. In addition, actin participates in gap closure after transmigration with the help of Rho-GTPases. However, the impact of actin assembly by actin binding proteins remains to be better understood. Here we investigated the role of actin binding EPLIN-isoforms for the control of actin dynamics in the regulation of leukocyte transmigration

Fluorescence live cell imaging, immune labeling and biochemical approaches were used to study leukocyte transmigration through EC

We show that during neutrophil-mediated ICAM-1 clustering, actin (LifeAct-EGFP) is recruited together with EPLIN-□-EGFP and EPLIN-□-EGFP to docking structures in HUVEC cultures and in endothelium of the mouse cremaster muscle. Down regulation of both EPLIN isoforms in HUVEC cultures by siRNA disturbed the actin recruitment to docking structures leading to reduced human neutrophil transmigration under flow condition while in contrast leukocyte adhesion remained undisturbed. In contrast, overexpression of each fluorescence-tagged EPLIN isoform significantly reduced the crawling distance and the velocity of adherent leukocytes on the activated endothelium and increased transmigration of neutrophils likely by forced ICAM-1 clustering and increased actin dynamics

The data demonstrate a significant role of EPLIN isoforms in the regulation of neutrophil transmigration via actin remodelling

Poster 25

Titel:
Altered L-WNK1 expression in mouse models for FSGS and metabolic syndrome

Autoren:
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Abstract:
WNK1 is a kinase regulating renal salt reabsorption and blood pressure. High blood pressure is a symptom of focal segmental glomerulosclerosis (FSGS) and metabolic syndrome. In the kidney, the WNK1 gene produces a full-length kinase-active L-WNK1 and a truncated form KS-WNK1. The study aims to characterize the pattern of WNK1 isoforms and determine the role of the L-WNK1 isoform in FSGS and metabolic syndrome.

We used wildtype, db/db and Nphs2flox/podocinCre mice for FSGS to explore the expression of WNK1 isoforms at mRNA and protein level. In situ hybridisation for whole WNK1 mRNA and against mRNA transcribed from Exon 2 and immunohistochemistry were performed.

mRNA for both WNK1 isoforms showed high expression in the TAL, CNT, CCD and in renin cells. mRNA of exon 2 (L-WNK1) showed ubiquitous expression along the nephron, in glomerular, vascular and renin cells. Kidney sections from FSGS and db/db mice, showed compared to WT mice higher glomerular and tubular L-WNK1 mRNA expression and higher L-WNK1 mRNA expression in single proximal tubule cells, respectively. Immunohistochemical staining revealed strong WNK1 abundance in TAL, CNT and CCD. FSGS kidneys, WNK1 protein expression was augmented in podocytes and collecting duct cells. In db/db kidneys, WNK1 protein expression was increased in proximal tubule cells and strongly in the distal tubule.

KS-WNK1 is most abundantly expressed in the distal tubule, collecting duct and renin cells. L-WNK1 is ubiquitiously expressed and is increased on mRNA and protein level pathologically in FSGS in glomeruli and collecting ducts and in db/db in proximal tubule cells.

Poster 26

Titel:
Taxanes induce vascular permeability via TLR4/TRPC6 triggered Ca2+-influx

Autoren:
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Abstract:
Taxanes are among the most active chemotherapy agents used in the treatments of various cancer types. Previous studies showed that taxanes like paclitaxels (PTX) also have profound effects on endothelial cells (ECs) even at low nanomolar doses. However, the molecular mechanisms behind the vascular effects of taxanes remain unclear. Here we aimed to investigate the signaling mechanisms mediating PTX-induced rapid disruption of vascular integrity.

Electric Cell-Substrate Impedance Sensing (ECIS) was used to follow changes in endothelial barrier function. Immunofluorescence staining of EC-monolayers subjected to shear stress in channel slides was used to visualize stress tubulin polymerization, stress fiber activation and adherens junction integrity. We further used calcium imaging to monitor increase of cytosolic calcium-levels [Ca2+]I in response to PTX.

PTX exposure leads to a rapid breakdown of endothelial barrier function. Immunofluorescence staining showed the appearance of para-endothelial gaps within 30 min after PTX-exposure, characterized by EC rounding, laceration of adherens junction and the consolidation of actin stress fibers. Calcium imaging studies showed increased [Ca2+]i. Using Ca2+-chelators we confirmed the extracellular origin of the [Ca2+]i-influx. Subsequently, we were able to demonstrate that the PTX-triggered cascade leading to the disruption of the EC-monolayer can be prevented by pharmacological inhibition of TLR4 or TRPC6.

Our results show that PTX disrupts vascular integrity and increases para-endothelial permeability. The EC rounding effect of PTX that leads to breakdown of the endothelial barrier involves Ca2+ influx via TLR4/TRPC6 signaling.

Poster 27:

Titel:
Loss of desmoplakin leads to aberrant distribution but unaltered binding properties of desmosomal cadherins

Autoren:
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Abstract:
Desmosomes are intercellular structures conferring adhesion to tissues exposed to high degrees of mechanical stress such as the epidermis. The desmosomal cadherins, termed desmogleins (DSG) and desmocollins (DSC), are connected to the intermediate filament cytoskeleton by plakoglobin (PG), plakophilins (PKP) and desmoplakin (DP). We here investigated the role of DP in regulating cell-cell adhesion and mechanical properties of desmosomal cadherins.

We generated DP control and knockout (DP-KO) cell lines in human keratinocytes using the CRISPR/Cas9 system. Electron microscopy was used to visualize desmosomal ultrastructure. Cellular characteristics were determined by immunostainings, Western blot analysis, apoptosis, migration as well as TUNEL assay. Adhesive properties were analyzed using dispase-based dissociation assays and atomic force microscopy (AFM).

Knockout of DP led to complete absence of desmosomes. Intercellular adhesion was severely compromised, whereas proliferation, apoptosis and collective migration were largely unaffected. Primarily the protein levels of DSC3 were reduced, while most other cadherins were unaffected. As tested by AFM, the binding frequencies and binding forces of DSC3 or DSG3-coated cantilevers were not decreased. However, an increased amount of membrane tethers was detectable on the free cell surface, indicative of reduced cytoskeletal anchorage. In line with this observation, DSC3 as well as DSG2 were evenly distributed in the membrane in contrast to control conditions, in which they were mainly localized to desmosomes.

These data demonstrate that linkage of desmosomal cadherins to the intermediate filament network and their clustering seems to be the primary determinant of adhesion between keratinocytes.

Poster 28:

Titel:
Mitochondiral Parl has a Coat of Many Colours – Evidence für Tissue-Specific Stomatin-like 2 Complexes of the IMM

Autoren:
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Abstract:
Membrane-associated enzymes fulfil critical roles at the inner mitochondiral membrane (IMM), controling membrane shape, apoptosis and mitochondrial turnover; events linked to diseases like M. Parkinson or dominant optic atrophy.

Recent in vitro studies [1] have identified Stomatin-like protein 2 (StomL2) as a common IMM docking station for a multiprotein complex encompassing the rhomboid protease PARL and the iAAA protease YME1L1 as well as OMA1. This „SPY complex“ is involved in the cleavage of PINK, PGM5 and HTRA2/OMI via PARL and the regulation of the GTPase OPA1 via YME1L1 and the associated peptidase OMA1.

Here we demonstrate by immunohistochemical and biochemical screening of murine tissue different variations of the SPY complex occuring in vivo.

Squamous epithelia show a colocalization of StomL2 and YME1L1 apparently independent of Parl and OMA1, which in turn colocalize to STOM-L2 in ciliated epithelia without YME1L1. Genetic inactivation of individual components – shown here for Parl – deficient mice – may destabilize the remainder of the SPY complexes.

Taken together, the SPY complex appears to be a highly adaptable „workbench“ of enzymatic actions at the IMM whereby the mechanics of it’s regulation remain to be elucidated but will add even more complexity to the pathogenesis of neurodegenerative disease.

[1] Wai et al, EMBO Rep. 2016 17(12):1844-1856

Poster 29:

Titel:
Deletion of the neonatal Fc-receptor results in reduced function of the renal proximal tubule

Autoren:
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Abstract:
The neonatale Fc receptor (FcRn) transcytose albumin and immunoglobulin G (IgG) and therefor is essential for albumin and IgG homeostasis. FcRn is widespread in the body and expressed in renal podocytes and proximal tubule.

3- and 6-month-old FcRn knockout mice (FcRn-/-) were compared. Kidneys were examined morphometrically. Exogenous applied Alexa555-labeled albumin was used to determine proximal-tubule endocytosis capacity. Endogenous endocytosed proteins like albumin, IgG and ß2-microglobulin were detected immunohistochemically.

3-month-old FcRn-/- showed no significant difference in morphology (size of cortex: WT = 618±47 µm, KO = 632±82 µm), endocytosis capacity of Alexa555-labeled albumin or endogenous albumin, IgG and ß2-microglobulin. 6-month-old FcRn-/- displayed a significant reduction in cortex size (WT = 740±73 µm, KO = 558±139 µm, p=0,032), a reduced endocytosis of Alexa555-labeled albumin (urinary pole: -40%; proximal tubule: -25%) and endogenous albumin (proximal tubule: -25%), IgG and ß2-microglobulin.

The protein uptake was unchanged in 3-month-old FcRn-/-. We hypothesize that FcRn in the proximal tubule play a role in intracellular redistribution, but the uptake of albumin, IgG and ß2-microglobulin is independent of FcRn. The reduction in cortex size in 6-month-old FcRn-/- suggests a reduced metabolism and possibly a faster aging in FcRn-/-.

Poster 30:

Titel:
Molecular and functional details on the ACBD5-mediated contact zone between peroxisomes and the endoplasmic reticulum

Autoren:
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Abstract:
ACBD5 is the first tethering protein mediating membrane contacts between peroxisomes and the endoplasmic reticulum (ER) identified in mammals. Such contacts are supposedly required for metabolite exchange between both organelles but also for membrane expansion of peroxisomes which receive phospholipids synthesized at the ER. In this respect, this study aims at characterizing metabolic and proliferative potentials of peroxisomes deficient in ACBD5 as well as identifying proteins which are specifically enriched at ER contact zones.

To study the role of ACBD5 in peroxisome maintenance (1) we analyzed peroxisomes in ACBD5-deficient mouse embryonic fibroblast (MEFs) monitoring peroxisome abundance and morphology by immunofluorescence microscopy under different culture conditions. To allow the identification of proteins involved in functional cooperation between peroxisomes and the ER, (2) we aimed at isolating liver peroxisome fractions enriched in ER contact sites using density gradient centrifugation.

(1) While ACBD5-deficient MEFs exhibit comparable peroxisome numbers and morphology as control cells, induction of peroxisome proliferation applying docosahexaenoic acid appears to result in reduced peroxisome elongation in ACBD5 deficient cells suggesting that the organelle’s capacity for membrane expansion is hampered. (2) Isolation of a distinct peroxisome fraction, which is specifically enriched in ACBD5’s ER interaction partner VAPB, allows screening for further ER proteins copurifying with the tethering protein. Such an approach will allow identifying proteins fulfilling particular metabolic or transport functions at the organelle contact.

The ACBD5-mediated organelle contact site exhibits a specialized protein composition likely required for the transfer of metabolite between peroxisomes and the ER.

Poster 31:

Titel:
Cellular and subcellular alterations in cerebellum and liver of a mouse model for the peroxisomal disorder ACBD5-deficiency

Autoren:
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Abstract:
The tail-anchored membrane protein acyl-CoA binding protein 5 (ACBD5) was recently associated with a novel peroxisomal inherited disorder with a severe neurological phenotype. We discovered that ACBD5 interacts with VAPA/B to form a tethering complex mediating membrane contacts between the endoplasmic reticulum and peroxisomes. Thus, ACBD5-deficiency may be a first member of a novel class of disorders with a disturbed organelle interaction network, thus meriting a detailed analysis of its disease pathogenesis.

In order to delineate the pathomechanism of the ACBD5 deficiency we aimed at characterizing the phenotype of a correspondent mouse model (ACBD5 -/-) for the human ACBD5 deficiency applying immunofluorescence, electron microscopy as well as lipid and protein analytical methods.

While young animals show a normal development, adults develop a progressive motor dysfunction. In the liver, a highly significant reduction in ER-peroxisome contacts and an increase in peroxisome number were observed. These cytological changes were accompanied by elevated levels of very long-chain fatty acids in plasma and tissues suggesting a metabolic dysfunction of peroxisomes. In line with motor dysfunction, the cerebellum of ACBD5-/- mice showed several pathologic alterations in the cerebellum: numerous Purkinje cells exhibited axonal swellings implying degenerative processes, which explain the significant decline in total Purkinje cell numbers at the age of one year. In parallel, astro- and microglia were found to be activated suggesting the development of an inflammatory phenotype during disease pathogenesis.

ACBD5-deficient mice develop a cerebellar phenotype resembling other peroxisomal single enzyme deficiencies but lack pathological alterations in the liver.

Poster 32:

Titel:
R-Spondin1 – A further neurogenic regulator of murine and human enteric progenitor cells

Autoren:
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Abstract:
In the Wnt dependent stem cell compartment of the intestinal crypt, R-Spondin1 has a strong mitogenic activity on LGR5 expressing cells. Here we tested the hypothesis, that R-Spondin1 has a similar co-stimulating influence on progenitor cells derived from the enteric nervous system (ENS).

Therefore, we systematically evaluated the influence of R-Spondin1 stimulation during proliferation and differentiation of cultured enteric neural progenitor cells. Isolated ENS progenitors from Tunica muscularis of the small intestine of newborn and adult wild-type C57BL/6 mice as well as from Wnt1-Cre2 reporter mice were used. We also obtained intestinal tissue samples from patients and isolated ENS cells. Influence of R-Spondin1 was analyzed by proliferation assays, qRT-PCR, and immunocytochemistry.

Gene expression analysis of proliferating enterospheres verified gene expression of known Wnt target genes e.g. axin2 and lef1. Our cell culture experiments revealed that the total cell mass of proliferating enterospheres enlarged after R-Spondin1 treatment. Furthermore, total number of isolated neurons (HuC/D+) as well as the amount of BrdU co-labeled neurons increased in comparison to respective control groups after R-Spondin1 treatment.

Our results shed a light on the influence of R-Spondin1 on the proliferative capacity of enteric neural progenitors, thereby confirming our working hypothesis. Further experiments will verify the intrinsic Wnt regulation of the enteric progenitor niche.

Poster 33:

Titel:
A therapy with miglustat, 2-hydroxypropyl-ß-cyclodextrin and allopregnanolone restores splenic cholesterol homeostasis in Niemann-Pick Disease Type C1

Autoren:
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Abstract:
Niemann-Pick disease type C1 (NPC1) is an autosomal-recessive lipid-storage disorder with an estimated minimal incidence of 1/120,000 live births. Besides other neuronal and visceral symptoms, NPC1 patients develop spleen dysfunction, isolated spleno- or hepatosplenomegaly and infections. The mechanisms of splenomegaly and alterations of lipid metabolism-related genes in NPC1 disease are still poorly understood.

Here, we used an NPC1 mouse model to study a splenoprotective effect of a treatment with miglustat, 2-hydroxypropyl-ß-cyclodextrin and allopregnanolone and showed that this treatment has a positive effect on spleen morphology and lipid metabolism.

Disease progress can be halted and blocked at the molecular level. Mutant Npc1 (Npc1-/-) mice showed increased spleen weight and increased lipid accumulation that could be avoided by our treatment. Also, FACS analyses showed that the increased number of splenic myeloid cells in Npc1-/- mice was normalized by the treatment. Treated Npc1-/- mice showed decreased numbers of cytotoxic T cells and increased numbers of T helper cells.

In summary, the treatment promotes normal spleen morphology, stabilization of lipid homeostasis and blocking of inflammation, but alters the composition of T cell subtypes.

Poster 34:

Titel:
SFTA2, a novel surfactant protein of the ocular surface that might play a role in inflammation and dry eye disease

Autoren:
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Abstract:
Surfactant proteins (SP) are well known from human lung and have also been described in tears and tissues of the ocular surface. The objective of this study was to determine the possible expression of SFTA2 (SP-G) at the ocular surface and in tears and to analyze possible functional aspects.

Ocular tissues, human corneal epithelial cell lines (HCE) as well as tear fluid from volunteers were analyzed by means of RT- PCR, western blot as well as immunohisto- chemistry. Possible immunological aspects were investigated by stimulation of HCE cells using cortisol. The effect of recombinant SFTA2 on the surface tension of tear fluid was estimated using spinning drop tensiometer.

Lacrimal gland cells, corneal epithelium and endothelium, conjunctival epithelium as well as Meibomain gland cells express SFTA2 which also could be detected in tears of volunteers. In vitro stimulation of HCE cells with cortisol revealed a significant increase of the SFTA2 mRNA expression level. ELISA quantification showed a significantly in- creased up-regulation of SFTA2 protein expression in case of dry eye tears compared to healthy tear fluid. Recombinant SFTA2 is lowering the surface tension of tears.

The results indicate that SFTA2 is part of the tear film and the ocular surface. There it seems to be involved in immunological processes. Furthermore, SFTA2 is able to lower the surface tension of the tear film.

Poster 35:

Titel:
Pseudomonas aeruginosa quorum sensing molecules stimulate mucociliary clearance through brush cell mediated cholinergic signaling

Autoren:
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Abstract:
Patients with chronic lung diseases often suffer from infections caused by gram-negative Pseudomonas aeruginosa (PA). Recently, tracheal brush cells (BCs) emerged as cholinergic chemosensors expressing bitter taste signaling cascade components (e.g. TRPM5, transient receptor potential channel M5) that can evoke protective respiratory reflexes upon stimulation with PA 3-oxo-C12-HSL. Here, we investigated if PA quorum-sensing-associated metabolites (QSM) can be detected by BCs and induce a BC-mediated acetylcholine-release that has an effect on mucociliary clearance (MC).

Changes in [Ca2+]i levels in BCs in response to PA QSM and denatonium were studied on tracheal explants of TRPM5-GCaMP-mice. The impact of PA products on MC was estimated by the cilia-driven particle transport speed (PTS) on explanted mouse tracheas. PTS was calculated by tracking the dynabead transport on the mucosal surface. Signal transduction cascade has been investigated using immunohistochemistry, inhibitors and transgenic mice.

TRPM5 was found only in BCs. In BCs denatonium, PA products PQS (3,4-dihydroxy-2-heptylquinoline), 2-AA (2-aminoacetophenone) and DHQ (2,4-dihydroxyquinoline) increased [Ca2+]i. These substances also significantly raised PTS. Atropine and mecamylamine blocked the denatonium-induced PTS-increase. The stimulatory PQS-effect on PTS was abolished with the TRPM5 antagonist triphenylphosphine oxide and reduced in TRPM5-deficient mice. Interestingly, two other PA QSM of the same group, HHQ (4-hydroxy-2-heptylquinoline) and HQNO (2-n-heptyl-4-hydroxyquinoline N-oxide), decreased PTS.

Denatonium and PA QSM directly activate BCs and increase PTS. This stimulation of the MC is at least partially mediated through cholinergic signaling from BCs. Thus, BCs are important innate sensors that stimulate the MC for protection against lung infection.

Poster 36:

Titel:
The role of Tulp1-Tam receptors signaling in new vessel formation using a modified aortic ring assay

Autoren:
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Abstract:
TAM receptors are member of receptor-tyrosine kinases activated by endogenous ligands. Recently, Tulp1 was also shown to be a TAM receptors ligand but biological role of Tulp1 activation of TAM receptors in angiogenesis is not known. To understand the role of TAM receptors and Tulp1 in new vessel formation we modified aortic ring assay (ARA) and tested on it.

Adventitial layer from thoracic aorta of C57BL/6 mice was removed and performed aortic rings with- and without adventitia in 0.5-1 mm thickness. They were embedded in collagen gel, cultured with substances for 7 days and captured phase-contrast images. Sprouts were quantified using ImageJ. After 7 days, ARA was fixed and embedded in paraffin for the preparation of tissue sections that were used for immunohistochemical staining and subsequently quantitative measurements and statistical analysis. A part of aortic rings were stained without sectioning as whole mount and analyzed using confocal microscopy.

With this data, we show that the vascular adventitia is not only important as a niche for vascular cells, but also for non-vascular cells like macrophages that contribute to angiogenesis and has effect on intima-derived macrophages. TAM receptors were involved in VEGF-A-induced capillary formation in the presence and absence of adventitia. TAM receptor blockage inhibited VEGF-A- and Tulp1-induced capillary-like sprouting.

We established a modified ARA that allows interrogation role of adventitia in the angiogenesis. With this modification, we showed the contribution of intimal endothelial cells to vascular sprouting from that of adventitia-derived stem and progenitor cells. Adventitia layer supported establishment of robust and complex capillary networks.

Poster 37:

Titel:
Reelin in cancer cell migration and invasion

Autoren:
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Abstract:
Reelin is a extracellular matrix glycoprotein that regulates neuronal migration and positioning in the developing brain. Reelin is secreted in different brain regions by specific neurons, such as Cajal-Retzius cells in the cerebral cortex and hippocampus, by cerebellar granule neurons and neurons of the deep cerebellar nuclei, by neurons of the diencephalon and mesencephalon, thus sustaining functionality of the adult brain neurons. Moreover, brain cancer invasion and proliferation of several types of brain tumor cells were previously reported to be stimulated by Reelin. In contrast, Reelin seems to inhibit migration of other tumor cell lines as well as invasion of cancer cells. Here, we studied the effect of Reelin on migration and metastasis in different cell-based models.

We used cell-based in vitro assays to study the migratory and invasive behaviour of different tumor cell lines after exposure different concentrations of recombinant Reelin. Scrape loading assays were applied to assess the cross-talk of migration-activated tumor cells with non-active cells. Expression levels of Reelin-mediated cell communication signaling target proteins were examined by immunoblotting.

Cell viability, cell communication and activation, migration and invasion of cancer cell lines was stimulated by Reelin. Moreover, expression levels of cancer cell gap-junction-specific proteins were affected upon Reelin treatment.

The Reelin signaling pathway plays not only a role in neuronal migration and lamination, but also modulates tumor cell migration and progression. We show that Reelin affects signaling cascades required for cell motility and cell-cell communication, that are involved in both brain development and emergence of brain cancer metastases.

Poster 38:

Titel:
Origin of specimens at the Institute of Histology and Embryology in Innsbruck during the NS-period

Autoren:
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Abstract:
Bodies of Nazi victims did not only serve for the education of medical students but were also used for scientific research and publications by members of both, the Anatomical Institute and the Institute of Histology and Embryology at the ‘Alpenuniversität’ Innsbruck. The Institute of Histology and Embryology has been headed since 1939 until 1945 by Jürg Mathis (1900–2005).

In 2018 we discovered over 300 drawers in an adjoining room of the Institute which stored about 15.000 histological slides. After a thorough examination, 237 slides were identified that might contain human tissue sections from victims of the Third Reich. Some of the slides were labelled with abbreviations such as “Cl. 40”, “hing. Clara” or “Hinger. Cl.” suggesting that they were obtained from specimens sent to the Institute by the histologist Max Clara who was at that time head of the Institute of Anatomy in Leipzig but still closely associated with the Institute in Innsbruck.

In 2018 we discovered over 300 drawers in an adjoining room of the Institute which stored about 15.000 histological slides. After a thorough examination, 237 slides were identified that might contain human tissue sections from victims of the Third Reich. Some of the slides were labelled with abbreviations such as “Cl. 40”, “hing. Clara” or “Hinger. Cl.” suggesting that they were obtained from specimens sent to the Institute by the histologist Max Clara who was at that time head of the Institute of Anatomy in Leipzig but still closely associated with the Institute in Innsbruck.

In Innsbruck, NS-victims were not only used for (gross) anatomical, but also for histological teaching and research. The sources of bodies and/or organs of these independent institutes were different.

Poster 39:

Titel:
Responses of epibranchial placodes to disruptions of FGF-signaling in embryonic mice

Autoren:
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Abstract:
Epibranchial placodes (EPs) are major contributors to the development of cranial sensory ganglia. In zebrafish, induction and neurogenesis of EPs depend on fibroblast growth factor (FGF)-signaling. In FGFR1 hypomorphic mice, neurogenesis is exclusively reduced in EP1. Here we investigate how neurogenesis of EPs is affected by three different other ways of disrupting FGF-signaling in mice.

C57BL/6N embryos (E8.5/E9) were cultured for 24h in the presence of the FGFR inhibitors SU5402 and PD173074, respectively. Furthermore, blocking experiments with anti-FGFR3 antibodies were carried out. For negative controls, culture medium was applied with DMSO only. Serially sectioned embryos were qualitatively and quantitatively analysed using immunohistochemistry with antibodies against the neuronal differentiation markers neurogenin (Ngn) 1 and Ngn2.

Application of SU5402 or PD173074 reduces the numbers of Ngn2+ (EP1>>EP2>EP3) and Ngn1+ neuroblasts (EP1, EP2), whereas the amount of Ngn1+ neuroblasts remains constant in EP3. Furthermore, exposure to PD173074 but not SU5402 results in increased Ngn1/Ngn2 ratios in EP1 and EP3. Different from SU5402 or PD173074 treatments, incubation with anti-FGFR3 antibodies causes decreases of Ngn1+ and Ngn2+ neuroblasts in all EPs. As another point of difference, the reduction rates of Ngn1+ and Ngn2+ neuroblasts are equal in the three EPs.

Disrupting FGF-signaling with pharmacological inhibitors (SU5402, PD173074) or anti-FGFR3 antibodies differentially affects total neuroblast numbers and/or Ngn1/Ngn2 ratios in the different EPs of embryonic mice. Contrary to earlier views, FGFR3 appears to be active in EP1. Whether neurogenesis in the different EPs is regulated by different subsets of FGFRs and/or by FGF-independent pathways is discussed.

Poster 40:

Titel:
The cytoskeleton and the evolution of breaking left-right symmetry in vertebrates

Autoren:
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Abstract:
Breaking left-right symmetry in the vertebrate embryo is a subject of ongoing controversy. Central questions in this controversy are the time of symmetry breaking and the evolutionary conservation of its putative mechanisms. Leftward ciliary flow during early neurulation is thought to be required for this process in several model vertebrates. Alternative views for chick and other model organisms lacking ciliary flow suggest the involvement of an asymmetric ion current and the cytoskeletal chirality prior to the gastrulation and the functional knock down of the actin-binding protein formin in snails and frogs seems to support this alternative scenario, even challenging the well-established model of left-right symmetry breaking by ciliary flow in Xenopus laevis.

We disturbed formin function in cultured Xenopus and chicken embryos and used (1) left-sided mRNA expression of nodal and pitx2 and (2) scanning electron microscopy of the emerging morphological asymmetry including the gastrocoel roof plate (the left-right organizer in the frog) as a read-out.

Formin inhibition in early Xenopus embryos did not affect molecular left-right patterning whereas treatment during gastrulation and early neurulation lead to an abnormal gastrocoel roof plate formation and randomized expression of Pitx2 in the lateral plate mesoderm. In contrast, formin inhibition in equivalent stages of the chick resulted in a remarkable stability of asymmetric gene expression and in asymmetric node morphogenesis

Our data suggests the involvement of actin cytoskeleton chirality in the morphogenesis of the left-right organizer in Xenopus upstream of the putative ciliary flow mechanism and contradicts the evolutionary conservation of the left-right symmetry breaking mechanism

Poster 41:

Titel:
The venous system of E14.5 mouse embryos – reference data and examples for diagnosing malformations in embryos with gene deletions

Autoren:
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Abstract:
The study aims at providing reference data and metric characterisations of the venous system of mouse embryos to permit the identification of vascular malformations in individuals, which already have finished organogenesis. Diagnosing morphological abnormalities in embryos stemming from lines with disrupted gene function will proof the value of these data for researching inherited pathologies and for identifying mouse models for researching rare human diseases.

Using High Resolution Episcopic Microscopy (HREM), we created digital volume data with voxel sizes of 3x3x3µm3 from 200 genetically unaltered mouse embryos and 5 embryos with targeted gene deletions. All embryos were bred on the C57BL/6 background as part of the deciphering the mechanisms of developmental disorders (DMDD) program (dmdd.org.uk) and harvested at embryonic day E14.5.

Our study provides highly detailed three-dimensional (3D) descriptions and surface models of the venous channels of embryos of all developmental stages between S21 and S23 according to Geyer et al. in respect to the embryonic bodies, tissues and organs. It also provides statistics of the occurrence of norm variations and developmental differences. Examples for diagnosing vascular malformations in single gene knock out embryos on the basis of the new reference data are shown.

We present comprehensive reference data based on over 200 individuals, which permit diagnosis of vascular abnormalities in mouse embryos with gene deletions, provide examples for their usefulness and demonstrate their value for identifying mouse models for inherited diseases.

Poster 42:

Titel:
Non-canonical WNT-signaling controls differentiation of lymphatics and extension lymphangiogenesis via RAC and JNK signaling

Autoren:
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Abstract:
We previously showed that Wnt5a is an essential regulator of lymphatic development in the dermis of mice, however, the mechanisms of action remained unclear.

Here, whole-mount immunostaining shows that embryonic day (ED) 18.5 Wnt5a-null mice possess non-functional, cyst-like and often blood-filled lymphatics, in contrast to slender, interconnected lymphatic networks of Wnt5a+/- and wild-type (wt) mice.

We compared lymphatic endothelial cell (LEC) proliferation during ED 12.5, 14.5, 16.5 and 18.5 between Wnt5a-/-, Wnt5a+/- and wt-mice. We did not observe any differences. TEM studies revealed multiple defects of LECs in Wnt5a-null mice, such as malformed inter-endothelial junctions and ruffled cell membrane. Application of WNT5A protein to ex vivo cultures of dorsal thoracic dermis from ED 15.5 Wnt5a-null mice induced flow-independent development of slender, elongated lymphatic networks after 2 days, in contrast to controls showing an immature lymphatic plexus. Correspondingly, tube formation assays with human dermal LECs revealed increased tube length after WNT5A application. To study intracellular signaling of WNT5A we used LEC scratch assays. Inhibition of autocrine WNTs suppressed horizontal migration, whereas application of WNT5A to LECs promoted migration. Inhibition of the RHO-GTPase RAC, or the c-Jun N-terminal kinase JNK significantly reduced migration, whereas inhibitors of the protein kinase ROCK, another member of the WNT-PCP-signaling pathway, did not.

Our data show that WNT5A induces formation of elongated lymphatic networks through proliferation-independent WNT-signaling via RAC and JNK. Non-canonical WNT-signaling is a major mechanism of extension lymphangiogenesis, and also controls differentiation of lymphatics.

Sci Rep. 2019 Mar 18;9(1):4739.

Poster 43:

Titel:
PIK3CA mutations are specifically localized in lymphatic malformation-derived lymphatic endothelial cells: therapeutic implications

Autoren:
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Abstract:
Lymphatic malformations (LM) are characterized by the overgrowth of lymphatic vessels during pre- and postnatal development. The cysts are lined by lymphatic endothelial cells (LECs).

Recent studies performed on LM specimens in the USA have identified activating mutations in the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) gene in LM. However, whole tissue but not isolated cell types were studied.

We studied LM tissues resected at the University Hospitals Freiburg and Regensburg, Germany. We isolated LECs and fibroblasts separately, and sequenced the commonly affected exons of the PIK3CA gene. We confirm typical monoallelic mutations in 4 out of 6 LM-derived LEC lines, and describe two new mutations. LM-derived fibroblasts did not possess such mutations, showing cell-type specificity of the defect. High activity of the PIK3CA - AKT- mTOR pathway was demonstrated by hyperphosphorylation of AKT-Ser473 in all LM-derived LECs, as compared to normal LECs. Additionally, hyperphosphorylation of ERK was seen in all LM-derived LECs, except for the one with Glu109del. In vitro, the small molecule kinase inhibitors Buparlisib/BKM-120, Wortmannin, and Ly294002, (all inhibitors of PIK3CA), CAL-101 (inhibitor of PIK3CD), MK-2206 (AKT inhibitor), Sorafenib (multiple kinases inhibitor), and rapamycin (mTOR inhibitor) significantly blocked proliferation of LM-derived LECs in a concentration-dependent manner, but also blocked proliferation of normal LECs. However, MK-2206 appeared to be more specific for mutated LECs.

In sum, children that are, or will be, treated with kinase inhibitors must be monitored closely.

PLoS One. 2018 Jul 9;13(7):e0200343.

Poster 44:

Titel:
Pax Proteins and the Control of the Neural Tube Patterning in Human Embryos

Autoren:
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Abstract:
Recent evidence from experiments made on mouse chicken and rat embryos have been demonstrated that Pax genes play important roles in the early neural tube formation. The aim of this study was to detect the expression of Pax6 and Pax7 proteins in the developing neural tube of the human embryos.

The expression of Pax6 and Pax7 was examined in 29 human embryos by immunohistochemistry. The embryos were collected after legal abortions, fixed in 4% paraformaldehyde, embedded in paraffin and tissue blocks were serially cut in transversal direction. The embryos were classified according to Carnegie stages. For immunohistochemistry the slices were incubated with primary antibodies of Pax6 and Pax7 and with a universal secondary antibody.

The results demonstrated spatially and temporally restricted pattern of the expression of Pax6 and Pax7 in the developing neural tube of the human embryos. The expression of proteins had a tendency to increase in the later stages of the development. In the earlier stages of development these proteins were detected mostly in the dorsal part of the forming neural tube. In CS 16-20 the Pax6 and Pax7 were expressed in the different regions of the developing forebrain midbrain and hindbrain.

In human embryos Pax6 and Pax7 were identified as signaling molecules that are involved in the formation of the neural tube development. Results of this study confirming the role of Pax6 and Pax7 in the dorsal-ventral regionalization of the spinal cord and subdivision of the neural tube into vesicles.

Poster 45:

Titel:
The impact of IL-6 in Trisomy 21 mediated perturbation of fetal B-lymphopoiesis

Autoren:
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Abstract:
Trisomy 21 causes several haematological abnormalities before and after birth; and is associated with an increased incidence of myeloid and lymphoid leukaemia. Previous work on human fetal liver has shown that there is a lack of B-lymphoid progenitors in fetal life. Whole transcriptome analysis of haematopoietic stem and progenitor cells (HSPC) and mesenchymal stromal cells (MSCs) from human fetal bone marrow (BM) suggested that the inflammatory pathways involving IL6 and IFNα were perturbed in T21 fetal B-lymphopoiesis.

The aim of this study was to investigate the role of trisomy 21 and IL6 in human fetal bone marrow B-lymphoid development.

We performed novel co-culture assays using primary human fetal MSC and HSPC to investigate the role of IL6 in the production of B cells. We sorted 100 normal HSPC (Lin-CD34+CD38-) for co-culture on normal or T21 fetal BM MSC in parallel. At 7 day intervals we harvested cells for identification by flow cytometry. qPCR and ELISAs were conducted to investigate the independent production of IL6 in fetal BM MSCs.

Results of these assays suggest that although there may be a marginal increase in B-lymphoid output on normal MSC, addition of IL6 alone was not enough to rescue the B-lymphoid defect caused by T21 MSC.

The role of IL6 in blocking T21 B-lymphopoiesis requires further investigation. Although it is not enough to rescue B-lymphopoiesis on its own, it is possible that a combination of different environmental cues are responsible for the defect.

Poster 46:

Titel:
The Col4a2 mouse line a model for researching autosomal dominant porencephaly

Autoren:
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Abstract:
We aim at providing detailed phenotype descriptions of homozygous embryos of the Col4a2em1(IMPC)Wtsi line produced in the Deciphering the Mechanisms of Developmental Disorders (DMDD) (https://dmdd.org.uk/) program in order to define the role Col4a2 plays in organogenesis and to identify the spectrum of abnormalities associated with autosomal dominant porencephaly2 (OMIM # 614483).

Four homozygous Col4a2em1(IMPC)Wtsi mutants were harvested at embryonic day 14.5 and High Resolution Episcopic Microscopy (HREM) was used for producing digital volume data with a resolution of 3x3x3µm3. Using standard software tools the phenotype of the mutants was systematically analysed following a standardized protocol. Abnormalities were diagnosed by comparing each mutant against the morphology of 11 to 37 wild type embryos matching exactly in their developmental stage as defined by Geyer et al..

All DMDD Col4a2em1(IMPC)Wtsi mutants had survived organogenesis. Thus, phenotype analysis permitted the identification of the full spectrum of organs and tissues, the development of which depends on Col4a2 encoded proteins. Our careful phenotype analyses revealed characteristic defects in the brain, cranial nerves, visual system, lungs, endocrine glands, skeleton, subepithelial tissues and mild to severe cardiovascular malformations.

Our results recommend embryos of the DMDD Col4a2em1(IMPC)Wtsi line as useful models for identifying the full spectrum of defects of and for researching the mechanisms underlying autosomal dominant porencephaly2 (OMIM # 614483), a rare human disease.

Poster 47:

Titel:
Cervical squamous cell carcinoma: Is the cervical risk zone for cancer already predetermined in fetal life?

Autoren:
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Abstract:
The postnatal cervix is a complex organ with original squamous epithelium, metaplastic squamous epithelium and columnar mucosa. We evaluated the distinct origins of the different epithelia during prenatal development and discuss possible implications for cervical carcinogenesis.

We analyzed 37 female embryos and fetuses between 8 and 34 weeks and 2 newborns by histology and immunohistochemistry (SMA, CK 7, CK 14, CK 17, p63).

The characteristic shape of the cervix became visible first by week 25 with CK 14 and CK 17 positive cells in all cervical epithelia, while CK 7 was restricted to the endocervix. Adult-type epithelia were identified for the first time around week 30. The squamous epithelium had ascended from the vagina, still expressed CK14 but had lost CK17 expression. p63 / CK 17 positive cells were identified in metaplastic squamous epithelium of the TZ and a small zone of the adjacent diffusely CK 7 positive columnar epithelium. The more cephalad CK 7 positive columnar endocervical epithelium lacked CK 17 positive cells.

The epithelial organization of the cervix is already predetermined in prenatal life with p63 / CK17positive cells in the metaplastic squamous epithelium, but also in an adjacent zone of columnar epithelium. These CK 17 positive cells of urogenital sinus origin are capable of squamous metaplasia and subsequent squamous cancers development. We propose that the area at risk for cervical squamous cell cancer after HPV-infection needs to be redefined by the presence of p63 / CK17 positive cells. These observations may lead to a new definition of the TZ as zone of risk for cancer and adaption of treatment strategies.

Poster 48:

Titel:
Macroscopic Sex Determination in Human Embryo-fetal Specimens

Autoren:
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Abtract:
The sex-gender gap is recognized in clinical research, but in basic sciences often ignored. For the research in early human development, we aim to reduce this gap with a detailed and structured dissection protocol for sex determination in human embryo-fetal specimens.

We performed macroscopic dissections on over 170 embryo-fetal specimens from gestational week 6 to 12. All specimens were procured after written informed parental consent as part of legal abortions according to Austrian law (§97 StGB).

First, we inspected the specimens for macroscopic abnormalities and defined the embryological age by measurement of the crown-rump length, external and internal morphology. If needed, we adjusted the estimated gynecological age set by ultrasound.

Second, we meticulously separated maternal from embryo-fetal tissue, which is often sorely afflicted, under a magnifier.

Third, we identified in younger specimens the mesonephric (wolffian) or paramesonephric (müllerian) duct according to our illustrated and defined characteristics, partially using a microscope. In more developed specimens, we also incorporated early features of male genitalia.

We were able to identify the sex of the human specimens in 70%. In the remaining cases, identification was not possible as a result of the alterations of the aspiration procedure.

We provide an easy to follow, step by step sequence to identify macroscopic sex determining structures in human embryo-fetal specimens from gestational week 6 to 12. Our approach simplifies the integration and application of sex-specific questions in human embryology research.

Poster 49:

Titel:
Math6 regulates reprogramming, pluripotency and stem cell fate by counteracting TGF beta signaling

Autoren:
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Abstract:
The basic helix-loop-helix transcription factor Math6 (Atoh8) has been identified to be involved in multiple developmental processes in animal models. In humans, Atoh8 is described to play an important role in shear stress response, iron metabolism and tumorigenesis. In our previous study, we have reported the expression of Math6 for the first time in the inner cell mass of murine blastocysts, and the dynamics in its localization upon the onset of differentiation which raises several questions regarding its significance in the maintenance of pluripotent state and early differentiation. In order to study Math6 in detail, we have generated Math6 knockout mice and Math6 Flag-tag mice due to the lack of good commercial antibody.

We evaluated the role of Math6 in the generation of induced pluripotent stem cells by reprogramming wildtype and Math6 knockout derived fibroblasts using Oct4, Sox2, Klf4 and Myc. Following this, we also evaluated its status in the maintenance of pluripotency and early differentiation.

Here we report that the lack of Math6 compromises mesenchymal to epithelial transition, an initial step of cellular reprogramming resulting in lower reprogramming efficiency. Investigations performed on iPSCs and ESCs revealed that, the lack of Math6 primes pluripotent stem cells into mesendodermal fate. Moreover, we demonstrate that Math6 acts through SMAD-dependent TGF beta/ Activin signaling pathway.

Taken together, our data suggest Math6 as a novel regulator of TGF beta/Activin signaling.

Poster 50:

Titel:
Senescence: a new player in the development of neurogenic epibranchial placodes

Autor:
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Abstract:
Senescence denotes a form of cell-cycle arrest that is linked to tumour suppression and aging. Other less well understood roles are related to embryonic development, as illustrated by the expression of key components of the senescence pathway in two major signaling centres (roof plate, apical ectodermal ridge). Here we aim to clarify, whether senescence contributes to the development of neurogenic epibranchial placodes in mice.

Histological serial sections from C57BL/6N mouse embryos (E8 to E11.5) were immunoreacted with antibodies against the senescence inducer cyclin-dependent kinase inhibitor p21. Established other features of epibranchial placode development were demonstrated using anti-cleaved caspase-3 (apoptosis), anti-phospho-Histone H3 (proliferation), and anti-neurogenin (neurogenesis) antibodies.

Strictly localized p21 immunopositivity was found in the lateral thirds of the pharyngeal pouches and, thus, immediately adjacent to the three paired epibranchial placodes. These are exactly the positions where Begbie et al. (1999) have identified epibranchial signaling centres that, in the chicken, express bone morphogenetic protein 7 and induce epibranchial neurogenesis. Proceeding rostrocaudally, the onset (E8.5 to E9), climax (E9.5 to E10.5) and decline of epibranchial neurogenesis in mice is followed by the onset, climax and decline of endodermal p21 immunopositivity with a delay of about 12 to 24h. Additionally, spatiotemporally regulated patterns of senescent ectodermal cells occur within as well as adjacent to each epibranchial placode.

Our findings suggest that senescence contributes to the maintenance and/or to the termination of epibranchial signaling centres as well as to the morphogenesis of epibranchial placodes. Whether senescence and apoptosis cooperate during these tasks is discussed.

Poster 51:

Titel:
Neurogenesis and apoptosis in the posterior placodal area of embryonic mice

Autor:
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Abstract:
In the posterior placodal area (PPA) of mice, apoptosis contributes to the segregation of otic and epibranchial placodes (OP, EPs) by eliminating rudiments of lateral line placodes (LLPs). Here we investigate the spatial, temporal and functional relationships between placode morphogenesis, neurogenesis, and apoptosis in the PPA.

47 C57BL/6N mouse embryos (E8 to E11.5) were evaluated using reconstructions and immunohistochemistry with antibodies against neural stem cell markers (anti-Sex determining region Y-box (Sox) 2 and Sox3) or neuroblast differentiation markers (basic helix–loop–helix transcription factors neurogenin (Ngn) 1 and Ngn2).

Sox2 and Sox3 are widely expressed in the early PPA. Thereafter, segregation of high-grade thickened placodes either precedes (EP1, OP) or follows (EP2, EP3) gradual restriction of Sox expression to the mature EPs. Exceptions are Sox2- dorsal parts of EP1 and the Sox3- EP3. Ngn1/Ngn2 ratios in EP1 and EP2 constantly remain below 1, whereas they transiently exceed 5 in EP3. ”Doomed rudiments” of LLPs express Sox2 and/or Sox3. This explains why LLPs rescued by pharmacological inhibition of apoptosis retain their neurogenic potential (Washausen and Knabe 2018). Regression of EPs is driven by ventral (EP1, EP3) and dorsal apoptotic foci (EP1, EP2, EP3) that eliminate (mainly) Sox2+/Sox3+ placodal remnants.

Epibranchial neuroblasts are generated either from Sox2+/Sox3+ (EP1, EP2) or from Sox2+/Sox3- ectoderm (EP3). These facts may contribute to the observed differences regarding the production of Ngn1+ and/or Ngn2+ neuroblasts in different EPs. Whether epibranchial neurogenesis varies individually due to differences in inductive FGF-signaling (Washausen and Knabe, see accompanying abstract) is discussed.

Poster 52:

Titel:
Low intensity pulsed ultrasound (LIPUS) influence the cell migration and proliferation of osteoblasts

Autoren:
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Abstract:
Low intensity pulsed ultrasound (LIPUS) is a non-invasive and regenerative potential treatment option, which has clinical application in wound and fracture healing. The exact effect of LIPUS on cells is not investigated as far.

The formation of microvasculature is an essential stage for bone remodeling and fracture healing, therefore we investigated in this study the effect of LIPUS on VEGF release from osteoblasts.

Human osteoblast cell line SAOS-2 were used for this study. Cells were treated using LIPUS for five days. Cell proliferation and cell viability of osteoblasts after treatment with LIPUS was determined using CyQuant cell proliferation assay and cell titer blue cell viability assay respectively. The cell migration was tested by scratch assay. VEGF protein level was quantified using ELISA.

the cell viability was not influenced during the treatment periods, we observed slightly higher proliferation rate after 24 h if the cells were treated with LIPUS. LIPUS treatment accelerate also the cell migration and effect the actin cytoskeleton. Die release of VEGF was increased in LIPUS group compared to the control group.

Our date confirm the proliferative effect of LIPUS on cells in other studies. The accelerative act of LIPUS by cell migration and increase of VEGF protein release could impact positively the fracture healing.

Further studies are recommended to analyze the effect of LIPUS on other cellular processes

Poster 53:

Titel:
The effect of Math6 on the development of fast and slow twitch muscle fibers

Autoren:
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Abstract:
Skeletal muscles are composed of slow and fast twitch fibers.

Each skeletal muscle shows a genetically determined distribution of these fiber types. Previous results showed that Math6 knockout mice are reduced in size and have less body weight. Since these mice failed at endurance tests on the rotarod, we went on to examine the effect of Math6 on the development of slow and fast twitch muscle fibers on muscles of the hindlimb.

Our recent analysis of three muscles in Math6 knockout mice suggests that the highly conserved bHLH transcription factor Math6 may have an important regulatory role on the ratio and distribution of muscle fiber types.

Male Math6 knockout and wildtype mice were sacrificed at the age of six months. We then dissected the quadriceps femoris, the triceps surae and the tibialis anterior muscle. We generated cross sections which were subsequently subjected to ATP staining using the ATPase method. We scanned the sections and counted the cells.

We were able to show that both, the tibialis anterior and the triceps surae muscle in knockout mice have more slow twitch fibers than their corresponding wildtype (62 % vs. 48 % and 67 % vs. 48 %).

The effect was not seen in the quadriceps femoris muscle (67 % vs. 66 %).

We conclude that Math6 has an effect on the distribution of muscle fibers. In the shank, more slow twitch muscle fibers were present. Further analysis regarding the underlying regulatory and metabolic mechanisms is currently on the way.

Poster 54:

Titel:
Medio-lateral patterning of the early chick neural plate

Autoren:
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Abstract:
The arrangement of the diverse types of neurons along the dorso-ventral axis in the central nervous system depends on the molecular patterning of the neural tube and an important part of this pattern is a highly specific, conserved pattern of transcription factor mRNAs. This pattern is believed to require a ventral to dorsal gradient of the signaling molecule sonic hedgehog generated by the early notochord and, later, by the floor plate of the neural tube, which itself is induced by the notochord. As our previous work suggested that the induction of the floor plate occurs prior to notochord formation we ask whether dorso-ventral patterning of the neural tube is independent of hedgehog signaling. As the basis for the experimental analysis of this question the molecular patterning in the neural plate prior to neural tube formation needs to be established.

The spatio-temporal pattern of transcription factors involved in dorso-ventral differentiation was investigated by in-situ-hybridization in late gastrulation chicken embryos followed by histological analysis. In addition, preliminary sonic hedgehog inhibition experiments were carried out to test possible changes in some of these patterns.

In situ hybridization analysis reveals a medio-lateral patterning of nkx 6.1, nkx 6.2 and pax 7 in the neural plate which reflects their dorso-ventral patterning in the neural tube. First experimental results suggest that strength and distribution of medio-lateral patterning factors remain unchanged after hedgehog inhibition.

Our results reveal a specific molecular patterning of the early neural plate and will be discussed in the context of axial differentiation and the evolution of neural patterning.

Poster 55:

Titel:
Loss of Math6 promotes skeletal muscle differentiation

Autoren:
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Abstract:
Math6 (Atoh8), a transcription factor of the basic helix-loop-helix (bHLH) superfamily, influences a variety of developmental processes and is known to be expressed in a wide range of tissues throughout embryonic development. During skeletal muscle development, Math6 has been implicated to regulate the transition of myoblasts from the proliferative phase to the differentiation phase. Within this study, we aimed to gain more insight into the role of Math6 in skeletal muscle development.

To understand its role in mammalian muscle differentiation, we have compared and analyzed in vitro differentiation of primary myoblasts, induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) derived from wildtype (Wt) and Math6 knockout (KO) mice by using qRT-PCR and morphological data.

The in vitro differentiation of primary myoblasts showed an enhanced differentiation in the case of KO compared to Wt. The gene expression profile of myogenic markers corroborates the same. Surprisingly, the differentiation of Math6 knockout derived iPSCs and ESCs exhibited mesendodermal commitment in vitro. We observed upregulation of mesodermal markers such as Brachyury, Tbx6, Pax3. Furthermore, to check the status of Math6 in myogenic commitment within the mesodermal compartment, we subjected ESCs to cytokine guided myogenic differentiation. We observed an enhanced myogenic commitment and differentiation of KO-ESCs compared to Wt-ESCs.

Taken together, our data suggest that Math6 regulates the fate of pluripotent stem cells and governs the myogenic commitment and skeletal muscle differentiation.

Poster 56:

Titel:
Morphogenesis and axis specification occur in parallel during optic cup and optic fissure formation, differentially modulated by BMP and Wnt.

Autoren:
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Abstract:
During transformation from the optic vesicle into the optic cup, the optic fissure is formed in the ventral part of the optic cup. It is a transient gap, serving as entry route for tissue and embryonic blood vessels. It closes as development proceeds. If closure fails, coloboma, showing a wide range of morphological phenotypes, occur. In a previous analysis, we observed that optic cup morphogenesis is a highly dynamic process. Here, we aimed to elucidate to what extent the optic fissure is affected by these tissue dynamics to shed more light onto “morphogenetic coloboma”.

We addressed optic fissure morphogenesis by 4D in vivo time-lapse analyses using zebrafish (Danio rerio) as a model. We furthermore studied the role of BMP4-, and Wnt-signaling during optic cup morphogenesis and addressed dorsal-ventral axis specification by whole mount in situ hybridization.

We found that under normal conditions, at the temporal side, the tissue flow, driving optic cup morphogenesis, translates into a ventral flow, shaping the temporal fissure margin. Nasally however, a tissue flow from the optic stalk is largely shaping the fissure margin. Furthermore, we found that induced BMP expression and also inhibited Wnt- signaling both largely hamper optic fissure morphogenesis and in parallel affect the dorsal-ventral axis specification/ maintenance respectively.

Our analyses show, that induced BMP expression and Wnt inhibition both result in morphogenetic coloboma, also affecting the dorsal-ventral axis of the eye. Our data furthermore indicate that optic cup morphogenesis is crucial for a proper positioning of pre-specified dorsal-ventral optic cup domains.

Poster 57:

Titel:
Local MRI-based Measures of Thigh Adipose and Muscle Tissue are Highly Responsive to Bidirectional Change in Body Weight Using Fully Automated Segmentation Methods based on Deep Learning with Convolutional Neural Networks

Autoren:
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Abstract:
Thigh intermuscular fat (IMF), subcutaneous fat (SCF) and muscle cross sectional areas (CSAs) can be measured quantitatively using MR imaging. Their responsiveness to bidirectional weight change (loss and gain) has, however, not been studied. Here we explore the effect sizes using a conventional image analysis approach vs. automated segmentation by deep learning using a convolutional neural network (CNN).

Of 4,796 Osteoarthritis Initiative (OAI) participants we studied those who displayed a >10% weight loss/gain between baseline and 2-year (Y2) follow-up, and maintained some of that change (≥5%) at 4-year follow-up. Transverse spin echo MRIs were used to analyze thigh tissue composition. Longitudinal change in IMF, SCF and muscle CSAs were determined at baseline and Y2 follow-up, using a published segmentation method (reference) and a novel, fully automated approach using CNNs.

IMF and SCF both showed a statistically significant decrease with weight loss (n=52) and a significant increase with weight gain (n=51) in the range of 10-30%. The standardized response means (SRMs) were similar for the fully automated vs. the reference segmentation method, for weight gain and loss, and for men and women (SRM range 0.82 to 1.38). Subjects with weight loss also encountered significant reductions in extensor and flexor muscle ACSAs.

MRI-based measures of thigh adipose and muscle tissue are highly responsive to bidirectional weight change using automated measurement technology based on CNNs. These findings highlight the responsiveness of MRI measures of obesity, for instance during specific diet and/or exercise interventions, or under microgravity conditions.

Poster 58:

Titel:
Hypothalamic corticosteroid-binding globulin is malleable to functional status in rats

Autoren:
Elena V. Sivukhina (Institute of Anatomy II, University Hospital Jena, Friedrich-Schiller-University, Jena), Gustav F. Jirikowski (Institute of Anatomy II, University Hospital Jena, Friedrich-Schiller-University, Jena); elena.sivukhina@med.uni-jena.de

Abstract:
Corticosteroid-binding globulin (CBG) is among the factors regulating the availability of glucocorticoids (GCs) to target tissues. Intrinsic expression of CBG was demonstrated in various cell populations within the brain. The same gene encodes both hypothalamic and liver CBG in mouse. CBG was observed in brain areas associated with stress response. There is only partial overlap between the CBG- and GC-immunoreactivities (IR). In human hypothalamus CBG is co-localized with vasopressin (VP) and oxytocin (OT). Chronic osmotic stimulation is known to affect both parvocellular and magnocellular hypothalamic functions.

We subjected adult male Wistar rats to chronic osmotic stress by drinking 2%NaCl solution instead of water for 7 days. Double or triple immunofluorescence stainings were carried out on free-floating vibratome brain sections and examined with a confocal laser scanning microscope. CBG levels in the cerebrospinal fluid were determined by immunoassay.

In intact rats slight CBG-IR was found in OT and VP neurons of SON and magnocellular PVN. Chronic osmotic stress led to up-regulation of CBG expression there. Only partial coexistence of CBG with CRH was not significantly changed in osmotically challenged rats. Number of axons containing both CBG and VP or OT in the internal zone of the median eminence was significantly increased by drinking 2%NaCl solution. Immunoassays of cerebrospinal fluid revealed prominent increases of CBG amounts after chronic osmotic stimulation.

Our study demonstrated a first morphological evidence for up-regulation of brain CBG expression at time of increased hypothalamic activity which may be of significance for endocrine stress response.

Poster: 59:

Titel:
Comparison of two subclones of the human small cell lung cancer cell line H69AR: Crucial importance of sialyl-Lewis A for E-selectin binding, endothelial adhesion and spontaneous metastasis formation

Autor:
Vera Labitzky (Institut for Anatomy and Experimental Morphology, University Medical-Center Hamburg-Eppendorf, Hamburg), Hanna Maar (Institut for Anatomy and Experimental Morphology, University Medical-Center Hamburg-Eppendorf, Hamburg), Daniel Wicklein (Institute of Anatomy and Experimental Morphology, University Medical-Center Hamburg-Eppendorf, Hamburg), Michal R. Schweiger (Center for Molecular Medicine Cologne, University of Cologne, Colonge), Udo Schumacher (Institute of Anatomy and Experimental Morphology, University Medical-Center Hamburg-Eppendorf, Hamburg), Tobias Lange (Institute of Anatomy and Experimental Morphology, , University Medical-Center Hamburg-Eppendorf, Hamburg); v.labitzky@uke.de

Abstract:
One major challenge in cancer research is that one and the same cancer cell line can show divergent biological phenotypes when cultured in different laboratories. We identified two subclones of the Adriamycin-resistant small cell lung cancer (SCLC) cell line H69AR (H69AR-1 and H69AR-3), which both show a full match in the short tandem repeat (STR) profiling but differ clearly in their spontaneous metastatic potential in xenograft experiments. The aim of this study was to use these closely related cell line clones to identify the molecular drivers of metastasis formation.

Short tandem repeat profiling, spontaneous metastasis xenograft model, Alu-PCR, flow cytometry, laminar flow adhesion assay and transcriptome analysis.

Subcutaneous H69AR-1 xenografts developed a 4-fold higher number of lung metastases than H69AR-3 xenografts. In vitro characterization showed a higher binding capacity of H69AR-1 cells for human E-selectin. E-selectin is expressed on activated endothelial cells and promotes the adhesion of circulating tumor cells to the vessel wall by binding to glycan structures on the tumor cells like sialyl-Lewis A. Accordingly, H69AR-1 cells were found to express sialyl-Lewis A while H69AR-3 cells did not. Transcriptome data of the xenograft primary tumors showed up-regulation of several glycosyltransferases involved in the synthesis of selectin ligands in H69AR-1 as compared to H69AR-3 tumors.

These observations stress the importance of E-selectin binding for adhesion to the vessel wall during the metastatic process. Further analyses are necessary to investigate the mechanisms underlying the regulation of glycosyltransferases in the described H69AR subclones.

Poster 60:

Titel:
The effect of high-fat diet on the morphological properties of the forelimb musculature in hypertrophic myostatin null mice

Autor:
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Abstract:
Obesity is a worldwide nutritional disorder affecting body performance including skeletal muscle. Inhibition of myostatin not only increases the muscle mass but also it reduces body fat accumulation. We examined the effect of high-fat diet on the phenotypic properties of forelimb muscles from myostatin null mice.

Male wild-type and myostatin null mice were fed on either normal diet or high-fat diet (45 % fat) for ten weeks. Five forelimb muscles were processed for fiber type composition using immunohistochemistry and morphometric analysis.

We show that high-fat diet reduces the cross-sectional area of the fast (IIB and IIX) fibers in M. triceps brachii Caput longum and M. triceps brachii Caput laterale of both genotypes. In contrast, increases of fast fibers area were observed in both M. extensor carpi ulnaris of wild-type and M. flexor carpi ulnaris of myostatin null. Meanwhile, a high-fat diet increases the area of the fast IIA fibers in wild-type, myostatin null displays a muscle-dependent fiber type alterations. Despite, a high-fat diet causes a reduction in the area of the peripheral IIB fibers in both genotypes, only myostatin null shows an increase in the area of the central IIB fibers. We provide evidence that a high-fat diet induces a muscle-dependent fast to slow myofiber shift in the absence of myostatin.

Taken together, the data suggest that the morphological alterations of muscle fibers under combined high-fat diet and myostatin deletion reflect a functional adaptation of the muscle to utilize the high energy intake.

Poster 61:

Titel:
Comparison of the effects of maternal and postnatal application of protein malnutrition, yogurt diet and intra-uterine growth restriction on testes morphology and spermatological parameters in adult rats

Autoren:
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Abstract:
In the period of adulthood until the adulthood stage of male rats born from mother rats fed with different dietary groups in the embryonal period; To examine embiryonal development.

LPD 4%;CPD 24%, MPD 12% and Yugurt groups fed with different diets were followed by the same method in rats.The female rats whose uterinearteries were ligated at day 17 of gestationbilaterally Intrauterine growth restriction(IUGR) began to be fed with CPD 24%.

The epididymis was finely minced with anatomical scissors in 10 ml of physiologic saline, placed in a rocker for 10 min, and allowed to sit at room temperature for 2 min.

The fluid obtained from the cauda epididymis with a pipette was diluted to 2 ml with Tris buffer solution.

To determine the percentage of morphologically abnormal spermatozoa in the cauda epididymis, the slides stained with eosin-nigrosin were prepared.

Right testes from the rats were placed in 10% Bouin solution for 24 h for fixation and further pathologic examination.

Statistics; Mann Whitney U test and Kruskal Wallis tests were used.

The average weight of the experimental groups was different in testis location. Epididymis and vesicula seminalis locations; The mean weight of the experimental groups was different.The results of previous researchers support our findings.

The spermium development in the locations is examined; The development of testis, epididymis, vesicula seminalis, prostate and motility were found to be different according to the groups.Density did not differ between groups.

Poster 62:

Titel:
Voluntary activity alleviates sugar-induced mechanical and structural changes of the lung

Autoren:
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Abstract:
Diabetes and respiratory diseases are frequently comorbid conditions. However, the mechanistic links between hyperglycemia and lung dysfunction are not entirely understood. This study examined the effects of a high sucrose intake on lung mechanics and alveolar septal composition and tested voluntary activity as an intervention strategy.

Male C57BL/6N mice were fed a control-diet (CD; 7% sucrose) or a high-sucrose-diet (HSD; 35% sucrose) and were housed with or without running wheels (experimental groups: CD, CD-active, HSD, HSD-active; n = 7-10). After 30 weeks, lung mechanics were assessed. Left lungs were instillation-fixed and analyzed according to design-based stereological standards, right lungs were frozen for protein expression analyses.

HSD resulted in hyperglycemia as well as higher static compliance and lower elastance H compared to CD. Lung and septal volumes were increased and the septal ratio of elastic-to-collagen fibers was decreased. Moreover, the septal elastic fibers appeared more loosely arranged, what was accompanied by an increase in elastin protein expression.

Voluntary activity prevented hyperglycemia in HSD-fed mice. In the HSD-active group, the parenchymal airspace volume, but not the septal volume, was increased. The septal extracellular matrix (ECM) composition together with the protein expression of ECM components was similar to control levels in active HSD-fed mice.

High sucrose intake for 30 weeks caused elastic fiber remodeling and reduced pulmonary elasticity. Voluntary activity alleviated HSD-induced ECM alterations, possibly by prevention of hyperglycemia.

Poster 63:

Titel:
Expression of CILP-2 in the articular cartilage of patients with knee osteoarthritis: a pilot morphological study

Autoren:
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Abstract:
Osteoarthritis (OA) is a common joint disorder involving degeneration of the articular cartilage, but also affecting surrounding tissues. OA disables often weight-bearing joints including the knee. One of the important determinants of cartilage function is the structure and composition of extracellular matrix (ECM). CILP (cartilage intermediate layer protein) is one of the relatively recently described ECM components, originally found to be localized in the intermediate layer of the articular cartilage. However, accumulating data suggest that CILP in OA patients may have wider distribution in the cartilage and in the whole joint with possible involvement in the disease process. Therefore, the aim of this study was to detect CILP-2 expression in the articular cartilage of knee OA patients.

Material was obtained from 12 patients undergoing total knee arthroplasty. Cartilage samples were analysed using light microscopy, transmission electron microscopy and immunocytochemistry. CILP-2 antibodies were purchased from Abcam (UK).

Cartilage samples obtained from OA patients contained few singly located chondrocytes, which characteristically had short branching processes and contained many vacuoles. In perinuclear areas large bundles of filaments were seen. Nuclei were enlarged with partially disrupted chromatin. The number of distorted mitochondria was reduced. CILP-2 expression was detected in the cytoplasm of chondrocytes, in the pericellular matrix and in the interterritorial matrix.

CILP-2 expression seen in the articular carilage of OA patients may reflect the pathogenic steps of the disease process and thus CILP-2 may be a possible biomarker for the detection of early cartilage damage.

Poster 64:

Titel:
Shaping the tumor microenvironment: Regulatory role of Integrin β4 and E-/P-selectins for tumor-infiltrating leukocytes

Autoren:
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Abstract:
The crosstalk between tumor cells and components of their microenvironment plays a critical role for tumor initiation and progression. We focused on the role of the cell adhesion molecules Integrin β4 (ITGB4) and E- and P-selectin and their role in tumor cell invasion and metastasis. Former studies revealed that a knockdown of ITGB4 delays primary tumor growth in xenograft experiments. Interestingly, tumor growth was even more inhibited when the ITGB4-KD was combined with host E- and P-selectin deficiency. Our aim was to identify the molecular mechanism behind this synergism.

ITGB4-KD in PC3, PaCa5061 and SKOV3 cells, xenograft models (pfp-/-/rag2-/-), flow cytometry-based profiling of leukocytes, chemokine ELISA, Western Blot, 3D chemotaxis assays, in vivo tumor initiation assays, immunohistochemistry

Analyses of initial tumor nodules demonstrated an enhanced infiltration by mCD45+ leukocytes in PC3 ITGB4-KD tumors grown in control mice. Characterisation of these leukocytes revealed an increased amount of CD11b+/GR1+ positive cells (myeloid-derived suppressor cells, MDSCs). In contrast, ITGB4-KD tumors grown in E-/P-selectin deficient mice did not show elevated numbers of MDSCs. ITGB4-KD cells exhibited increased STAT1 expression and chemokine release.

Our results suggest that ITGB4 downregulation is compensated by activating pathways that support early tumor formation processes. ITGB4-KD seems to modulate tumor infiltration by immune cells such as MDSCs through enhanced chemokine secretion. MDSCs appear to be critical for growth of ITGB4-KD tumors and to depend on endothelial selectins for infiltration of the tumor.

Poster 65:

Titel:
Age-related structural and functional changes in the mouse lung

Autoren:
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Abstract:
Lung function declines with advancing age. To better understand the structure-function relationships leading to this decline, we investigated structural alterations in the lung and their impact on micromechanics and lung function in the aging mouse.

Lung function analysis was performed in 3, 6, 12, 18 and 24 months old C57bl/6 mice (n=7-8/age), followed by lung fixation and stereological sample preparation. Lung parenchymal volume, alveolar volume and number, septal volume, surface area and thickness as well as surfactant producing alveolar epithelial type II (ATII) cell volume and number were quantified by stereology.

Parenchymal volume and alveolar volume increased in old (18, 24 months) compared with middle-aged (6, 12 months) and young (3 months) mice. While the alveolar number decreased from young (7.5x10^6) to middle-aged (6x10^6) and increased again in old (9x10^6) mice, the septal surface area per alveoli was highest in middle-aged mice. The ATII cell number increased from middle-aged (8.8x10^6) to old (11.8x10^6) mice, along with the alveolar number and resulted in a constant ratio of ATII cells per alveoli in all age groups (1.4 ATII cells per alveolus). Lung compliance and inspiratory capacity increased, whereas tissue elastance and resistance decreased with age, showing greatest changes between young and middle-aged mice.

In conclusion, alveolar size and septal surface area per alveoli declined significantly with age concomitant with late alveolarization. These changes may partly explain the functional alterations during aging. Interestingly, despite age-related lung remodeling, the number of ATII cells per alveoli shows a tightly controlled relation in all age groups.

Poster 66:

Titel:
Functional and structural changes in acute lung injury are extenuated by the knock-out of miR-21

Autoren:
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Abstract:
Acute lung injury (ALI) is characterized by enhanced permeability of the air-blood barrier, pulmonary edema and hypoxemia. MicroRNA-21 (miR-21) was shown to contribute to pulmonary remodelling and the pathology of ALI. Here we hypothesize that structural and functional changes in ALI are reduced in miR-21 knock-out (KO) mice.

ALI was induced in male miR-21-KO and C57BL/6N (wildtype) mice by intranasal administration of 75 ug lipopolysaccharide (LPS; n=10 per group) in saline or saline alone (control mice; n=7 per group). After 24 h, lung function (compliance and inspiratory capacity) was measured and the mice were sacrificed. Stereological parameters such as parenchymal, septal and matrix volume as well as septal thickness were analysed. Inflammatory cytokines were measured by ELISA and gene expression of matrix remodelling enzymes by qPCR.

The main differences between the two strains within control animals were a decreased compliance and inspiratory capacity in miR-21-KO compared with wildtype mice, caused by an increased septal thickness in miR-21-KO mice. LPS exposure induced severe inflammation in both strains, accompanied with an elevated parenchymal and septal volume - an indicator of intra-septal edema. However, only wildtype but not miR-21-KO mice showed increased tissue resistance along with increased septal thickness and higher matrix volume upon LPS treatment.

Our results showed that both strains equally developed signs of ALI with pulmonary inflammation and intra-septal edema. C57BL/6N mice, however, showed more functional and structural changes in ALI than miR-21-KO mice, suggesting that structural and functional changes in ALI were extenuated by the knock-out of miR-21.

Poster 67:

Titel:
Remodelling of Murine Airway Epithelium after Stroke

Autoren:
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Abstract:
A serious complication following stroke is pneumonia, which affects the clinical outcome and is the leading cause of death in stroke patients. We hypothesised that disturbances in the mucociliary clearance, the primary defence mechanism of the airways, prepare the ground for the development of pneumonia and that changes are driven by beta-adrenergic signalling.

Middle cerebral artery occlusion (MCAO) was induced in wild-type and beta2-adrenergic receptor knockout mice (adrb2-/-). The tracheal epithelium from MCAO mice, sham and untreated controls was assessed by immunohistochemistry for the density of ciliated cells, proliferation and differentiation.

Stroke induced a distinct loss of ciliated cells detectable after 12 h, reaching a maximum after 1 day and gradually returning to control levels after 49 and 72 days. After 1 day, proliferation was increased in sham and MCAO mice and after 3 days in MCAO mice. Values returned to control levels after 72 days. The number of activated basal cells (keratin-14-positive) increased within the first 3 days in sham and MCAO mice and returned to control levels after 72 days. Preliminary data of a reduced loss of ciliated cells in the epithelium of adrb2-/- mice indicate that epithelial remodelling may be driven by beta2-adrenergic signalling.

Stroke induces a rapid and reversible remodelling of the respiratory epithelium, which may result in impaired mucociliary clearance. Communication between brain and trachea may be at least partially mediated by beta-adrenergic signalling.

Poster 68:

Titel:
Analysis of pro-adhesive ligands on different tumor cell subsets for endothelial adhesion in vitro

Autoren:
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Abstract:
The escape of circulating tumor cells (CTCs) from the blood vessels is one critical step of metastasis formation. This process requires the interaction of endothelial cells with carbohydrate ligands on CTCs. We aimed to achieve a more precise characterization of these ligands.

We analyzed the adhesion behavior of three subsets of human cancer cell lines to human endothelial cells under physiological flow conditions in a laminar flow adhesion assay. The cell subsets expressing either both canonical E-selectin ligands sialyl-Lewis A and X (sLeA/X), sLeX only, or none of them, were compared after different treatments. Tumor cell suspensions were perfused with a flow rate imitating the blood stream through flow chambers that were previously seeded with a confluent monolayer of human endothelial cells.

Dynamic adhesion to human endothelial cells did not exclusively require the presence of the aforementioned canonical ligands. All cells were able to form firm adhesions, so other (non-canonical) ligands must exist. E-selectin blocking experiments showed a selectin-dependent adhesion to human endothelium only for the subsets expressing canonical ligands. Cancer cell treatment revealed sialic acid-containing ligands for nearly all tested cell lines, but also differences in the glycan structures carrying the ligands on the tumor cell surface. However, the cells commonly showed glycoprotein-independent adhesion, so glycolipid ligands must be considered as well.

With the endothelial cell-coated flow chambers we generated a more physiologic adhesion assay compared to our previous studies on recombinant selectins. Nevertheless, the endothelium-CTC-interaction remains a complex process that requires future studies for a better understanding.

Poster 69:

Titel:
The invisible system - Pathophysiological alterations of the lymphatic system

Autoren:
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Abstract:
The anatomy of the lymphatic system is critically important for the treatment of lymph edema. The green fluorescence dye Indocyanine Green (ICG) is used for diagnostic classification of lymphatic disorders and for preoperative planning of lymphatic surgical procedures.

For ICG injection, 25 mg of indocyanine green sterile lyophilized powder (Diagnostic Green GmbH) is mixed with 10 mL of distilled water. 1 mL of ICG solution with a concentration of 2.5 mg ICG is used for injection. For each point of injection, about 50-100 µL is injected to the intradermal layer at a prolonged rate. ICG fluorescence is detected by IC Flow Fluorescence Detector.

ICG lymphography is a safe, minimally invasive, and simple examination that enables a real-time assessment of lymphatic vessel function and reflects the severity of lymphedema. Characteristic ICG lymphography patterns are consistent with the clinical conditions and can be categorized into several patterns. These patterns correlate with clinical severity. With this, the lymphedema pathophysiological stage can be evaluated.

With the correct interpretation of the ICG patterns, it is possible to not only diagnose lymphedema in general, but also to classify it into stages. Furthermore, ICG lymphography is a vital tool for intraoperative use, such as planning the incision for a supermicrosurgical lymphovenous anastomosis (LVA) and confirming the patency of an LVA.

Poster 70:

Titel:
Anatomical and radiological features of the structural organization of the mandibular mental region

Autoren:
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Abstract:
to study the intraosseous structural organization of the mandibular mental region

The following study was made on the 50 human mandibles. Immediately before the experiment, the samples were divided into two groups of 25 samples for wet and dry preparations.

Preparation of wet preparations for the study was carried out as follows by its own method. To the dry preparations, a sequential injection of a liquid gelatin solution with the addition of black ink was carried out on both sides through the mental foramen.

Using a dental tomograph (isotropic voxel size 0.1 in high resolution mode), a detailed study of cone-beam computer tomograms obtained from 561 patients aged 6-75 was carried out.

Based on our studies, we propose an original classification of the intraosseous structures of the anterior part of the mandible, consisting of a system of upper canals (7 subtypes) and lower canals (2 subtypes) of the mental spine region, as well as a transversal intraosseous canal of the mandible.

The prevalence of the upper canal of the mental spine was absolute in all studied drugs, the transversal intraosseous canal of the mental region was found in 78% of cases, and the system of the lower canals of the mental spine in 82%. Due to the contrast, the separation of the department from the main spongy substance of the mandible is determined.

As a result of studying X-ray anatomical and anatomic-topographical, constitutional-based, structural features of the mandible, intraosseous structures of the anterior mandible were clarified and classified, which solve not only terminological disputes, but also are important clinical guidelines for endodontic treatment, dental implantation and local anesthesia .

Poster 71:

Titel:
A Novel Technique for Ultrasound Guided Placement of an Electrical Stimulating Wire to the Superior Laryngeal Nerve

Autoren:
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Abstract:
Electrical nerve stimulation of the superior laryngeal nerve (SLN) is a common method to aid patients with aphonia second to superior laryngeal nerve impairment.

Our goal was to demonstrate the feasibility of an ultrasound-guided placement of needles and stimulation hooked wires next to the internal branch of the superior laryngeal nerve (ibSLN).

We initially performed several ultrasound exams of the SLN on volunteers and subsequently scanned one human embalmed cadaver to develop our sonographic approach. In the next step, we depicted the SLN in twelve fresh cadavers, placed a hooked wire, and injected a special solution to stain the ibSLN. Proving, after dissection, successful visualization and correct placement of the stimulation hooked wires next to the ibSLN.

We were able to identify the ibSLN bilaterally in all volunteers and in all human cadavers. We found the nerve immediately posterior to the thyrohyoid muscle as a round structure with fascicular pattern - characteristic for a nerve in an ultrasound image. The superior laryngeal artery was visualized consistently in the volunteers, but its relationship to the SLN was inconsistent. On anatomical dissection we were able to identify the inserted needle tips, the ibSLNs stained by the solution and the hooked wires adjacent to the nerve.

We were able to reliable identify the ibSLN and place the wires adjacent to it. Admittedly, identification of the nerve together with the placement of needles and wires has a steep learning curve.

Poster 72:

Titel:
Clinical anatomy of the nasal SMAS

Autoren:
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Abstract:
The existence of a superficial musculoaponeurotic system of the face (SMAS) in the nasal region as a continuous musculo-conjunctive and adipose layer has been reported to unite similar structures of nearby regions. In the present study, we aimed to highlight the arrangement of this structure in different topographies of the nasal region: nasal dorsum, ala nasi, radix, and nasolabial fold.

We performed an anatomical, histological, and radiological study on 24 formalin-fixed hemifacials, from 12 donors at the “Ion Iancu” Institute of Anatomy, U.M.F. “Grigore T. Popa” Iaşi.

The SMAS in the nasal region was defined by a superficial fascia and a subcutaneous adipose layer. The existence of the SMAS in the nasal region was demonstrated through dissection, quantitative and qualitative histological techniques, as well as MRI. The SMAS is different in the two sub-regions. At the radix and dorsum nasi, the adipose layer is thin. It is thicker at the alla nasi where the deep skin of the face does not adhere to the subjacent layers.

The results of our study definethe nasal SMAS as an entity of major importance in cervicofacial surgery, whether it is for facial rejuvenation, resolution of malformations, or tumourremoval.

Poster 73:

Titel:
Exceptional anatomical features at 4 skulls of the Meckel-Family

Autoren:
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Abstract:
We analyzed exceptional anatomical features at 4 skulls of the Meckel-Family: #1 Philipp Friedrich Theodor Meckel (1755-1803), #2 Heinrich Theodor Meckel (1785-1829), #3 Philipp Friedrich Meckel (1819-1847), #4 Bernhardt Albrecht Meckel (1823-1851).

All skulls were X-rayed and photographed. For a comparison concerning the conspicuous basal-temporal gyri in #1, two body donors brains from the Erlangen dissection course in winter term 2018/19 were used.

The skulls #2, 3 and 4 shared a low and flat posterior cranial fossa. Only #3 and 4 possessed a Sutura frontalis persistens. Surprisingly, #1 presented flattened basal-temporal gyri on both sides. Body donor brain of a 90-year-old male showed a similar feature, while this peculiarity was not observed at the respective one of a 101-year-old female. Top of the petrosum was grown together with the clivus in #2. The seldom expression of a Foramen Vesalii was observed in #3. Finally, clivus rose more steep than usually in #4.

All 4 members of the Meckel-Family, representing grandfather (#1), son (#2) from the second marriage of #1 and two grandchilds (#3 and 4) belonging to another son of the second marriage of #1, named Albrecht August Meckel (1798-1829), show a lot of similarities. This is reflected by the low posterior cranial fossa (#2-4) and the Sutura metopica (#3 and 4). However, the meaning of the flattened basal temporal gyri in #1 is difficult to interpret. Perhaps, it is related to an inflammation of the brain like in meningitis or it reflects age changes.

Poster 74:

Titel:
Innervation of clavicular part of deltoid muscle by branch derived from lateral pectoral nerve

Autoren:
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Abstract:
The axillary nerve has been considered the only nerve supplying the deltoid muscle. Only in 1997, Solomon et al. (The Anatomical Record: 249, 506-9) published a case report showing the innervation of the clavicular part of the deltoid muscle by a branch from the lateral pectoral nerve. As this variation has multiple clinical implications, we investigated the frequency of this variation.

29 bodies (26 male, 32 female; age range 68 to 101), embalmed according to the Jores protocol, were dissected for the anterior and posterior innervation of the deltoid muscle on both sides (58 cases) and photographic documentation was done. The neuromuscular anterior bundles were further investigated through histology and a trichrome staining procedure.

The clavicular part of the deltoid muscle was innervated by a branch from the lateral clavicular nerve in 87%, whereas it was innervated by a branch from the axillary nerve in 13% of cases. There were three topographical variations, including a deltoid branch that perforated the subclavian muscle, a deltoid branch where the lateral clavicular nerve crossed the pectoralis minor muscle proximally, or where it crossed the pectoralis minor distally. Inter-individual variation was also documented, with bodies having the clavicular part innervated by the axillary nerve on one side, and by the lateral pectoral nerve on the other side.

Our results are clinically relevant in the case of damage to the axillary nerve, as the function of the deltoid muscle can only reliably be tested on its acromial and spinal part, and in the majority of patients the function of the clavicular part may still be there, often attributed to compensatory function by the biceps brachii muscle.

Poster 75:

Titel:
M. digastricus – an unknown, but known muscle – morphofunctional and clinical relevance

Autoren:
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Abstract:
Although the digastric muscle (M. digastricus) with its two bellies is involved in numerous functions in the stomatognathic system and also important in plastic surgery, it is neglected in teaching and diagnostics. Based on a literature review, we wanted to present the importance of this muscle in the proper light and give a morphofunctional overview.

The literature search was carried out via EMBASE, MEDLINE, BIOSIS Previews, Zoological Records, LIVIVO and manually in university libraries. From a total of 3605 initially found sources, the full text of 668 sources was analyzed after assessing the thematic relevance. Finally 213 primary sources and 44 secondary sources of literature were evaluated.

The digastric muscle fulfills all criteria for striated (skeletal) muscles and has a complex innervation due to its embryological development (gill arches!). Both non-syndromal and syndromal anomalies are described, which however are not associated with relevant functional limitations. Both muscle bellies have distinct functions - the anterior belly (Venter anterior) is a an abductor of the jaw (mouth-opener), whereas the posterior belly (Venter posterior) initiates the act of swallowing and is important for phonation. Numerous factors influence the muscle causing hyper- and hypotrophy, such as jaw relation, TMD, facial morphology, occlusal changes and others. The digastric muscle is also a donor for local facial and pedicled flaps in surgery for defect coverage.

Due to its numerous important functions, more attention should be given to the M. digastricus - also in textbooks and manuals.

Poster 76

Titel:
Medial Meniscus Extrusion Deteriorates During Knee Osteoarthritis Progression

Autoren:
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Abstract:
To determine whether baseline meniscus position or size predict, or longitudinal change in quantitative MRI measures of the medial meniscus over time concurs, with structural and symptomatic progression of knee osteoarthritis (OA).

96 participants with radiographic knee OA (KLG1-3) of the Foundation of the NIH Biomarker qualification study, a sub-study of the Osteoarthritis Initiative, were investigated. Of those, 49 displayed radiographic and symptomatic progression over 2-4 years, whereas 47 did not. Manual segmentation of the tibial plateau cartilage and the medial meniscus were performed in the central 5 MRI slices of coronal reconstructions of a DESS MRI sequence, acquired at baseline and 2 year follow-up.

Baseline measures of tibial plateau coverage, medial meniscus extrusion, and medial meniscus size (width and height) differed only slightly between progressors vs. non-progressors; however, these differences did not reach statistical significance ($p>0.17$). Over 2 years, OA progressors displayed greater worsening in meniscus extrusion in the central 5 slices (+0.46 mm [95% CI 0.24, 0.69 mm]) compared with non-progressors (+0.10 mm [95% CI -0.11, 0.32 mm]; $p=0.02$, unpaired t-test). Whereas worsening of meniscus extrusion was also significantly different for other quantitative measures of meniscus position, longitudinal differences of tibia coverage and meniscus size did not reach statistical significance.

Patients with structural and symptomatic progression of OA show considerable worsening of meniscus extrusion that significantly exceeds that in non-progressor controls. However, baseline measures in tibial coverage, meniscus extrusion, or meniscus size, did not predict progression vs. non-progression.

Poster 77:

Titel:
Comparison between incomplete colonoscopies in children and adults due to differences in the anatomical characteristics of the colon

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Abstract:
Comparison of the differences in colonoscopy between children and adults based on the anatomical conditions of the large intestine.

We performed a retrospective evaluation of colonoscopies in 403 children aged 3-18 (192 girls, 211 boys) and 500 adults (252 women and 248 men) aged 40-65 years. The anatomical conditions describing the incompleteness of the colonoscopies were assessed and categorized as incomplete (ICAR) or modified incomplete anatomical conditions (MICAR).

In 59 (14.6%) children (27 girls, 32 boys) the examination was incomplete. Of these, we found in 31 (7.69%) (13 girls and 18 boys) no cause for the failure of colonoscopy. In 15 examinations the colonoscopy had to be stopped in the area of the right colonic flexure, in 13 at the left colonic flexure and in 3 cases at the transverse colon. The ratios were calculated for patients weighing less than 30 kg (the results were statistically significant; p=0.0006, chi-squared test): ICAR was between 0.0309 and 0.1889, MICAR between 0.0 and 0.1889. In adults, 30 colonoscopies had to be interrupted incompletely; most of them at the sigmoid colon (F: n=8, 38%; M: n=4, 44%) or below (F: n=4, 19%; M: n=2, 22%). The findings were correlated with the patients' BMI (p=0.03) (the lower the BMI, the higher the IC risk), sex (p=0.03) and previous surgical treatments (p=0.007) (Chi-square test). ICAR and MICAR were in the range of 0 - 0.17.

Body measurement is a statistically significant factor for the evaluation of incomplete colonoscopy risks in adults and children. The lower the ICAR and MICAR values, the lower risk of colonoscopy failure.

Poster 78:

Titel:
Radiological anatomical study on the difficulty of ulnar nerve treatment in distal humerus osteosynthesis

Autoren:
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Abstract:
The ulnar nerve describes an atypical course at the extensor side of the elbow joint. Osteosynthetic material at the distal medial humerus settles the nerve at risk for mechanical impairment that possibly results in postoperative neurological deficits. Interindividual differences in physiological nerve movement as well as the unknown influence of plating material give reason for closer evaluation of the ulnar nerve's dynamic passage at the elbow.

Under sonographic guidance three to five metal pins were injected transcutaneously into the ulnar nerves of four cadaveric arms close to the elbow joint. Before and after the implantation of an anatomical plate at the medial side of the distal humerus, radiographs were taken perpendicular to the X-Ray's beam to localize the pins at different angles of elbow movement (0 to 135 degrees of flexion). Stretching was defined as the increase of distance between two pins and was measured. Furthermore the nerve's approximation to the center of rotation was evaluated pre- and postoperatively.

Elbow flexion stretches all nerves increasingly and extensively in one case. Approximation to the center of rotation occurred in all test series within a range of ten millimeters. Plate implantation induced several aberrancies concerning the nerve's initial movement.

The ulnar nerve features a complicated system of movement including three-directional coping mechanisms for its particular course at the extension side of the humerus. Osteosynthetic plating might overstress this individual buffer.

Poster 79:

Titel:
The arterial ramification pattern of the horse's brain with regard to topographical sectors of the neopallium

Autoren:
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Abstract:
The complicated gyration pattern of the equine brain has hampered the detailed description of the neopallium per se, and that of the adjacent structures, like arterial blood vessels. Hence, a simplifying system of 21 neocortical sectors was applied (1) to check for a basically uniform pattern of arterial ramification and (2) to analyze whether separated or overlapping arterial supply areas existed in the horse's brain.

The heads of 6 dead horses were fixed by perfusion with 10% formalin and the cerebral hemispheres were macroscopically dissected ex situ. The ramifications of the main cerebral arteries (A. cerebri media [MCA], A. corporis callosi [CA], A. cerebri caudalis [CCA] and A. ethmoidalis externa [EEA]) were displayed in graphical sketches. For an interindividual comparison, sketches of the hemispheres were superimposed in one graphical representation to visualize identical and differing arterial branches.

(A) Certain main branches of first or second order, arising from MCA and CA, occurred with some slight variation of their extensions and courses in all examined specimens. In some sectors (compared interindividually), first-order branches were topographically replaced by second-order branches. (B) CCA and EEA displayed no regular ramification pattern. (C) Certain sectors of the neopallium were reached by arterial branches, given off from different main cerebral arteries (multiple supply); in others, a single supply was macroscopically encountered.

The 21 sectors served well for a topographical assignment of the arterial branches. The documentation of ramifications beyond the second order did not yield useful data for an effective, interindividually comparable arterial mapping.

Poster 80:

Titel:
A stereotactic approach for a topographical mapping of the neopallium in the horse

Autoren:
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Abstract:
Species specific anatomical/histological data of the equine neopallium are still rare at a time when the horse is gaining importance in veterinary neurological clinics or in scientific trials. Hence, a stereotactic procedure was elaborated to allow further neurological research in horse.

The frozen heads of 8 dead warmblood horses were sagittally cut (paramedian plane) and placed in the stereotactic apparatus using the Foramen supraorbitale, the Crista facialis (Hanover Line), and the median plane as anatomical landmarks. The drilling at selected external points of interest (drill points) in reproducible drill angles yielded distinct marks (target points) at the neocortical surface. The neopallium's laterodorsal (convex) surface was subdivided into 15 sectors related to primary sulci and derived auxiliary lines. The 3d coordinates of extracranial drill points and neocortical target points were used to determine topographical relations between extracranial landmarks and neocortical sectors.

Dorsally, the neocortex was only 10-20 mm below the skin, however, drillings in the lateral region of the M. temporalis took a way of 83 mm (maximum) to the brain. Rostrally, maneuvers towards the hemisphere's frontal pole varied due to the age-dependent extension of the Sinus frontalis. Sector I (directly rostral to the Fissura sylvia) lay 39±5 mm caudal to the Foramen supraorbitale, 42±5 mm dorsal to the Hanover Line, 50±5 mm lateral to the median plane.

The reproducible assignment of certain target points to distinct sites (Presence Probability) indicated that the stereotactic device facilitated reproducible maneuvers to distinct neocortical sectors in the horse (preliminary results).

Poster 81:

Titel:
Morphological and CT-based functional investigations of the glenohumeral joint in human body donors

Autoren:
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Abstract:
Osteoarthritic glenohumeral disorders may cause pain and an impaired range of motion, which ultimately leads to decreased quality of life. For the outcome of surgical treatment by total shoulder arthroplasty, optimal placement of the implant components is crucial and preoperative treatment planning is increasingly important. In this study on human cadaver specimens from the body donation program, we examined the gleno-humeral joint by morphological inspection and measurements on CT data.

Shoulder specimens from 20 male and 24 female body donors (mean age 79.5, range 44-98 years) were isolated and graded using a modified Outerbridge classification applied to the shoulder by experienced shoulder surgeons. Additionally, CT data sets were acquired prior to dissection for comparison with the morphological results. As a first step, CT-osteoabsorptiometry (CT-OAM), which allows the determination of the mineralization distribution of the subchondral bone plate as a marker for the long-term loading history of joints including the shoulder was applied. Results were correlated to the surgical classifications by two young scientists and were further discussed by experienced anatomists and shoulder surgeons. In selected pathologies, 3D-CT reconstructions of the glenohumeral joint were performed according to the method of Friedman as we described in a previous study (Mathews et al., 2017).

Preliminary data point to a pronounced correlation between the extent of osteoarthritic deformations or other defects as evaluated by the surgical grading's and the CT-based morpho-functional analyses.

This study is one of the first to combine anatomical dissection with surgical grading's and CT-based functional analyses of the glenohumeral joint for the investigation of osteoarthritic disorders in an elderly population. Findings may serve as a basis for future studies on radiological preoperative treatment planning of shoulder surgery in patients with osteoarthritis.

Poster 82:

Titel:
Non Metric Cranial Trait Variants of Modern Anatolian Population

Autoren:
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Abstract:
Analysis of non metrical cranial variants have been a central role for diagnosis in the identification of human population through osteologic analysis and genetic affinities. In making comparisons between populations in cases of bilateral traits, the sexes can be associated to evaluate what degree of the extent is. The aim of the current study was to examine the non metric cranial variants to see differences among sexes and inter side correlations in Anatolian dry skulls.

Eleven non metrical cranial variants were investigated in 84 crania. Only 50 crania of modern Anatolian population could be studied with the exclusions because of fractures of these regions or not being able to be identified. Both cranial and side incidences of the variants are analyzed to their sex differences and inter side correlations respectively. Results were anayzed by Mc Nemar Test and for analysis of statistics, the programme of IBM SPSS Statistics 22.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) was used.

Some variants in female crania seen more frequently than in the opposite sex. Most variants showed high correlations between right and left sides such as supra orbital notch, infra orbital foramen and zygomaticofacial foramen. At %10 level variant of supra orbital foramen in male shows difference between right and left sides.

On the whole there were not sex or side differences in significant level in Anatolian population.

Poster 83:

Titel:
The left phrenic nerve-Contribution to the vegetative innervation of the esophagogastric region

Autoren:
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Abstract:
The right and left phrenic plexus are networks of vegetative nerves originating from the celiac plexus, surrounding the inferior phrenic arteries and reaching the diaphragm and peritoneum. Both plexuses may communicate with phrenicoabdominal branches of the phrenic nerves. The cardiac end of the stomach receives branches mainly from the left phrenic plexus. The extent to which the branches of the left phrenic nerve contribute to this innervation is not well known.

The aim of this study was a systematic investigation of the phrenicoabdominal branches of the left phrenic nerve in humans and their contribution to the innervation of the esophagogastric region.

The nerves of 30 conserved human specimen (body donation program, Institute of Anatomy Bern) were dissected in situ. Nerve samples were examined by light- and electron microscopy.

In 46.6% of the specimen, a delicate left phrenic-nerve branch joined phrenic-plexus branches to the esophagogastric region. In 20%, the branch was more distinct showing variable courses and variable connections with the phrenic- and/or gastric plexuses and the anterior vagal trunk. In 10%, a distinct left-phrenic nerve branch directly connected to the celiac ganglion, which in turn, released phrenic-plexus branches. Missing left phrenic-nerve branches were compensated by phrenic-plexus branches. Comparative investigation of the left phrenic branches and left phrenic-plexus branches revealed similar morphological features representative for vegetative nerves.

The left phrenic nerve contributes to the vegetative innervation of the esophagogastric region displaying a high variability in course and connectivity and is thereby considered as a part of the phrenic plexus.

Poster 84:

Titel:
How does the vertebral artery pierce the dura mater? An anatomical study.

Autoren:
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Abstract:
The vertebral artery is often associated with different cerebral or cranial problems in the clinical field. The exact passage from the artery through the dura mater was still not described accurately.

Individuals donated their bodies to the Division of Clinical and Functional Anatomy of the Medical University of Innsbruck after written informed consent was obtained. Thirty-six heads were dissected to document the macroscopic situation. Additionally the area where the artery pierces the dura was observed by histological slides and electromicroscopy.

In the macroscopic observations there was no symmetry observed. In detail 72 different topographic situations around the vertebral artery were detected after piercing the dura from extradural to intradural space. In every case neural structures around the artery were found. Nearby the artery piercing the dura mater ganglion cells were seen in the ultrastructure, additionally to the structured connective tissue.

For the first time a detailed investigation was conducted how the vertebral artery is connected to the dura by piercing it. The results of this investigation support the hypothesis, that the neural topography around the artery has relevance for clinical problems.

Poster 85:

Titel:
Pregnance and birth-related modification of the bony pelvic skeleton– anatomical basics

Autoren:
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Abstract:
We aimed at examining the incidence and causality of anatomic features, which modern anthropology in general and the ERC funded VAMOS project in particular uses as indicator for pregnancies and childbirth in prehistoric skeletons. The focus will rest on dorsal pitting at the pubic bone, preauricular sulcus, additive pubic tubercle and piriform tubercle.

The pelves of 32 body donors (50% male, 50% female) were examined. 12 were fixed in a neutral buffered carbol/formaldehyde (4%/1%) solution and 20 were unfixed. All pelves were imaged with computed tomography (CT), before they were stratigraphically dissected and finally macerated. All important anatomic features and relations were carefully characterised and measured.

Based on quantitative and qualitative analysis of three-dimensional (3D) computer models created from CT data and macerated specimens, we provide descriptions and measurements of the osseous features and comparisons of their occurrence in males and females. The insertions and the topology of muscles and fasciae at and near these features are analysed.

Our results permit evaluating the value of dorsal pitting at the pubic bone, preauricular sulcus, pubic tubercle and piriform tubercle as indicators for pregnancies and child births and therefore contributes to evaluating the role women had in prehistoric societies, as researched in the VAMOS project. Furthermore, our data permit the formulation of a hypothesis, which explains the predominant occurrence of the examined features in females.

Poster 86:

Titel:
Functional aspects of axillary arches

Autoren:
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Abstract:
Our presentation aims at analysing the potential effect of arm movements on the neurovascular bundle passing through the axilla in individuals with axillary arches.

We examined a total of 93 body donors for the presence of axillary arches and measured the dimensions of the neurovascular bundle and the dimension of the area of the axillar outlet between the axillary arches and latissimus dorsi muscle. All donors were perfused and fixed for a minimum of 6 months in neutral buffered formaldehyde/carbol (1%/4%) solution. In all body donors a series of standardized arm movements were conducted and the position of the nerves and vessels in respect to the axillary arches was recorded.

From 5 cases we identified 3 different variations of axillary arches, for which we provide detailed morphologic descriptions and measurements of the ratios of the dimensions of the axillary outlet and neuromuscular bundle. Furthermore, we provide statistics on the minimal distances between each single arm nerve and the axillary arch after moving the arm into the defined arm positions respectively describe at which angle the nerves touched the arches.

Our results will permit estimating potential clinical symptoms patients with different types of axillary arch might face and define the arm positions at which they are most likely to occur.

Poster 87:

Titel:
A novel accessory muscle in the wrist and hypothenar musculature

Autoren:
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Abstract:
We describe a previously unknown accessory muscle, located on the right forearm. There are few reports on similar variants, however, the muscle we describe has a different origin and is extraordinarily large.

The body donor was a 65-year-old caucasian male. We used a formaldehyde-based intravasal perfusion fixation. We photo-documented each preparation step under standardized conditions and camera settings.

The novel muscle runs from the radial aspect of the distal forearm to the hypothenar muscles. It passes under the radial artery and M. flexor carpi radialis, thereby directly contacting the Carpal tunnel, Guyons Loge, and the ulnar vessel/nerve road. The nearly cylindrical tendon passes between the bellies of Mm. flexor et abductor digiti minimi, ending at the base of the proximal phalanx digitus minor. Its origin is flat and dichotomous inserting on the tendon of the M. brachioradialis and the very distal palmar part of radial bone. The muscle´s function was presumably a weak abduction of digitus minimus, a flexion-ulnarduction of the wrist, and an ulnar-rotation of the fifth finger. Additionally, we found a reverse M. palmaris on the left forearm of the body donor.

The accessory muscle described here differs from similar previously reported normal variants by size and its complex origin. The clinical relevance of similar variant muscles of the wrist, especially concerning neurovascular symptoms caused by a compression of the Loge de Guyon, is well documented. Also in surgical interventions, such anatomical variants should be considered to avoid confusion and resulting complications.

Poster 88:

Titel:
Is there a system beyond randomized distribution of elastic fibers in the shoulder joint capsule?

Autoren:
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Abstract:
Elastic fibers within joint capsules are supposed to protect folds from entrapments. There is, however, no functional description of their distribution available. Elastic properties are of particular interest for the shoulder joint due to its lax structure, which supports more extensive movements or shrinkage than in other joints. In the present study, we worked on a systematic histological analysis of the underlying elastic texture.

We used four areas of the shoulder joints from 6 donated bodies donors. We graded the alterations by arthrosis according to the Outerbridge-classification. The following parameters were investigated through standardized elastica staining using visual analog scales: fiber diameter, quantity, and distribution patterns of elastic fibers.

We identified an elastic boundary layer between the subsynovial and fibrous layers that could not be revealed in the scientific literature. We found significant differences in the fibers in diameter, quantity, and distribution pattern compared to the synovial and fibrous membrane. The elastic fibers were primarily curled fibers instead of parallel fibers. Following previous studies, the total amount of elastic fibers decreases with the incremental stage of arthrosis classification.

An elastic boundary layer exists between the loose synovial connective tissue and the fibrous membrane of the shoulder joint capsule. The concentration of restoring forces close to the traction solid collagenous fibers implicates a physiological role, that might contribute to capsule shrinking, degeneration, or healing processes. There was no specific alteration in one of our morphological parameters that correlate with arthrosis stages. The omnidirectional orientation of the fibers suggests a discrete biomechanic function.

Poster 89:

Titel:
The Role of Crown-like Structures in Adipocyte Degradation

Autoren:
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Abstract:
Obesity increases the risk of numerous diseases, including type 2 diabetes, coronary heart disease and hypertension. A chronic low-grade inflammation within adipose tissue seems to be the link between obesity and some of its associated diseases. One hallmark of this adipose tissue inflammation is the accumulation of adipose tissue macrophages around dead or dying adipocytes, forming so-called crown-like structures. Crown-like structures are more abundant in obesity, but a few are also found in lean animals, suggesting a physiological role in adipose tissue homeostasis as well. The number of crown-like structures therefore indicates the progression of the adipose tissue inflammation but the structures as such appear to reflect the physiological degradation process of adipocytes.

To further investigate the formation of crown-like structures and their impact on the activation state of adipose tissue macrophages, we established a model to induce crown-like structures in adipose tissue explants of lean mice by laser injury, coupled to a live imaging setup for real time detection.

Using this model, we were able to set a precise timeline for crown-like structure formation after adipocyte death, while also analyzing the expression of different pro- and anti-inflammatory markers in adipose tissue macrophages.

Our results indicate that adipocyte death leads to a locally confined activation of the immune system also in lean mice. Hence, disbalance of adipocyte degradation in obesity could contribute to adipose tissue inflammation and type 2 diabetes.

Poster 90:

Titel:
Influence of NaCl on human periodontal ligament fibroblasts during simulated orthodontic tooth movement

Autoren:
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Abstract:
Increased salt intake (NaCl) is associated with chronic diseases such as hypertension or osteopenia. Its effects on orthodontic tooth movement (OTM), however, have not yet been studied, although OTM as a pseudo-inflammatory process is significantly associated with general metabolism and the immune system with parodontal ligament fibroblasts (PDLF) playing a mediating key role. We therefore investigated the influence of NaCl on the expression pattern and activity of PDLF in a model of simulated orthodontic tooth movement.

PDLF were preincubated for 24h with 0mM or 40mM NaCl. At each concentration, PDLF were exposed to a compressive force of 2g/cm2 for another 48h or incubated without pressure (control). After 72h, we analysed the expression of genes and proteins involved in OTM by means of RT-qPCR, ELISA and immunoblot. Coculture experiments were performed to study PDLF-mediated osteoclastogenesis.

40mM NaCl increased prostaglandin-E2 secretion while reducing expression of IL-6 under pressure. NaCl also affected the expression of alkaline phosphatase and remodelling of the extracellular matrix (EM). 40mM NaCl enhanced RANK-L expression and reduced OPG expression. This resulted in increased PDLF-mediated osteoclastogenesis.

NaCl seems to promote bone formation and EM remodelling and to influence the expression of IL-6 or secretion of prostaglandin-E2. Based on the enhanced RANK-L expression and up-regulated osteoclastogenesis, increased salt intake in-vivo could increase the rate of OTM, but also the risk for undesired root resorptions and periodontal bone loss, which has to be verified in further animal studies.

Funding: German Research Foundation DFG (SCHR1622/1-1, KI 2105/2-1), Faculty for Medicine Regensburg (ReForM-A, SCHR11/2017)

Poster 91:

Titel:
Impact of hypoxia versus mechanotransduction on the biological regulation of orthodontic tooth movement

Autoren:
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Abstract:
For orthodontic tooth movement (OTM), mechanical forces are applied to teeth triggering pseudo-inflammatory, osteoclastogenic and remodelling processes in the periodontal ligament (PDL), mediated by PDL fibroblasts via the expression of various signalling molecules. So far it is unknown, whether these processes are mainly induced by mechanical cellular deformation (mechanotransduction) or by concomitant hypoxic conditions via a compression of periodontal blood vessels.

Human primary PDL fibroblasts were randomly seeded onto conventional 6-well cell culture plates with O2-impermeable polystyrene membranes and on plates with gas-permeable membranes (lumox®, Sarstedt), enabling an experimental separation of mechanotransductive and hypoxic effects, which occur concomitantly during OTM. To simulate physiological orthodontic compressive forces, PDL fibroblasts were stimulated mechanically at 2g/cm2 for 48h after 24h of pre-incubation. We quantified cell viability by MTT assay, gene expression by RT-qPCR and protein expression by Western-Blot/ELISA. In addition, PDL-fibroblast-mediated osteoclastogenesis (TRAP+ cells) was determined in 72h coculture with RAW264.7 cells.

Expression of HIF1α, COX2, VEGF, COL1A2, ALPL, the RANK-L/OPG ratio at the mRNA/protein level as well as PDL-fibroblast-mediated osteoclastogenesis were significantly elevated by mechanical loading irrespective of the oxygen supply present, whereas hypoxic conditions had no significant additional effect.

The cellular-molecular mediation of OTM by PDL-fibroblasts is expected to be predominantly controlled by the force application itself (mechanotransduction), whereas hypoxic effects seem to play only a minor role. During OTM the hypoxic marker HIF1α does not appear to be primarily stabilised by reduced O2 supply, but rather mechanically.

Funding: German Research Foundation DFG (KI 2105/1-1).

Poster 92:

Titel:
ECM targeting to improve the immune environment in desmoplastic tumors

Autoren:
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Abstract:
Elevated collagen-synthesis and -crosslinking leads to physical protection from infiltration of immune cells in breast cancer (BC) and pancreatic ductal adenocarcinoma (PDAC). Targeting collagene synthesis, we evaluated the effects of prolyl-4-Hydroxylase (P4HA) and procollagen-lysine 5-dioxygenase (PLOD2) inhibition on the extracellular matrix (ECM) and consequently the ability of lymphocytes to infiltrate the tumors.

The effects of P4HA and PLOD2 inhibition were tested in syngeneic murine BC- and PDAC-models. Immune cell infiltration post treatment was evaluated by FACS analysis and 3D-light sheet fluorescent microscopy. To better understand underlying molecular mechanisms, RNA-expression profiles for a range of cytokines and growth factors were monitored.

Inhibition of collagen synthesis by targeting either P4HA or PLOD2 reduced Treg accumulation and increased various TH-populations and CD8+ cytotoxic T cells in the treated tumors. Moreover, distribution of lymphocytes post treatment was more homogeneous throughout the tumors with T cells penetrating deeper into the tumor tissue from supplying blood vessels. P4HA or PLOD2 furthermore reduced expression of immunosuppressive cytokines in the tumor microenvironment.

These results support our hypothesis, that modulation of the ECM can allow immune cells to penetrate deeper into the tumor. The demonstrated positive effects of these drugs on the immune environment have the potential to increase the efficacy of concomitant immunotherapeutic approaches.

Poster 93:

Titel:
Choosing the Right Person for the Job - Personality Traits of Teacher Assistants in Innsbruck

Autoren:
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Abstract:
The importance of noncognitive factors for performance in medical practice is widely recognized, and this concept also applies to teacher assistants in Anatomy. The current selection process in Innsbruck emphasizes on practical (applicants are assessed during their dissection course and in a specific, additional laboratory course) and on social skills. The latter is estimated by the supervisor, based on their impression of each applicant during the dissection course. The optimal teacher assistant should be open, agreeable, and conscientious, but her or his competitiveness mitigated by the interest in helping the near-peers. We believe that administering a personality test before or during application leads to bias in answering the questionnaire. Therefore, we sought to assess the personality of our current teacher assistants.

During a general meeting, we invited all present teacher assistants to complete the International Personality Item Pool (IPIP) NEO-120 anonymously in paper form. All items were represented in English and German on the same row to minimize language and inventory validity bias.

Of the 60 questionnaires, 58 were returned. The study population comprised of 34 males, 23 females, and 1 not specified. All results were compared to the publicly available data set from John A. Johnson. Our teacher assistants are lower in neuroticism and higher in extraversion, openness, agreeableness, and conscientiousness (this trait unequally distributed) then the comparison group.

We show that our selection process is valid but needs to be improved to select a more homogenous group of applicants in the trait of conscientiousness.

Poster 94:

Titel:
Validation of a mouse model for experimental orthodontic tooth movement in wild-type mice

Autoren
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Abstract:
Animal experiments are essential for the elucidation of biological-cellular mechanisms in the context of orthodontic tooth movement (OTM). So far, however, there are no studies that assessed available mouse models regarding their relative validity and experimental suitability.

OTM was induced in C57BL/6 mice (upper jaw) either with a nickel-titanium (NiTi) spring (0.125N) between the first left molar (M1) and incisors or a separating elastic (Ø 0.2mm or 0.3mm) between M1 and M2 for 3, 7 or 12 days (contralateral side: control). We determined the rate of appliance loss, extent of OTM (µCT) and periodontal-alveolar expression of OTM-related genes (RT-qPCR).

After 7 days 53% (0.2mm) and 87% (0.3mm) of the elastics were in situ, after 12 days only 13% and 27%. Survival rate for NiTi springs was 100% for all time intervals, but 8.9% of the animals with spring died prematurely. Significant OTM was induced in all groups within 3 days with a maximum for NiTi springs after 12 days and for 0.3mm elastics after 7 days, the latter, however, with significant variation. In analogy, springs at 12 days and 0.3mm elastics at 7 days elicited a significant increase in gene expression of cyclooxygenase-2, RANK-L and cathepsin-K.

To induce OTM in mice for more than 7 days, a NiTi spring should be preferred, whereas a separating elastic due to high loss rate and variation is only suitable at a Ø of 0.3mm and for qualitatively assessed OTM (yes/no) of up to 7 days.

Funding: German Research Foundation DFG (KI 2105/2-1).

Poster 95:

Titel:
Inspecting human 3D histology in virtual reality. Mesoscopic models of the splenic red pulp microvasculature computed from immunostained serial sections.

Autoren:
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Abstract:
In human microanatomy, immunostained serial sections are essential for visualising mesoscopic structures in three dimensions. We create 3D models from 100 to 300 serial paraffin sections. Virtual reality (VR) is used to inspect the models and to control their quality.

The complete workflow consists of a non-rigid registration, inter-section interpolation, volume and mesh processing, using the marching cubes algorithm and mesh analysis methods, and of the actual VR application. We first focus on computational analysis such as connectivity studies and, second, on visual analytics in VR for discovering new morphological details.

We present 3D models in VR visualising arterioles, sheathed capillaries, and the connected capillary network of the human splenic red pulp together with the location of B-lymphocytes and follicles. We superimpose each single immunstained section for quality control. Although the model is self-occluding, front-plane clipping guarantees that any structure may be freely inspected from all sides. The user "cuts into" the model and can freely observe severely occluded regions. Subsequently, numbers and volumes of capillary sheaths, connections of different vessel types, and many other variables may be analysed.

Human microanatomy in 3D is realistic, because affordable VR headsets have recently become available. 3D models from immunostained serial sections can be inspected and rigorously controlled in VR. Thus, a unique level of information is reached in diagnosis and teaching. Participants of the congress will have the opportunity of using VR headsets on site to practically test the methods.

Poster 96:

Titel:
Teacher Assistant Training - Example from Innsbruck

Autoren:
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Abstract:
Despite different training approaches of anatomy teacher assistants worldwide, there is consensus on cultivating competencies for professional life. Therefore, we aimed to assemble and describe a training program with an emphasis on practical and social skills.

We implemented two supervised laboratory (dissection) courses (basic anatomical dissection and advanced anatomical dissection, totaling 22 contact hours) in which tutors acquired practical skills to support the students in the two dissection courses. Additionally, one lecture to convey the basics of different didactics. Teaching assistants are required (besides attending the dissection courses) to attend and support the anatomy lecture of the first year on four occasions and hold autonomous, but supervised, near-peer teaching units in two elective courses (totaling 30 hours).

The program was evaluated with a 34-item questionnaire (Likert-scale, ordinal data) and free-text comments.

Of the 60 questionnaires, 58 were returned. The study population comprised of 58.6% males, 39.7% females, and 1.7% not specified.

Tutors assessed item 2 (I learned something valuable), item 32 (I have improved my practical skills) and item 4 (I have understood and learned the contents of the course) in median with “strongly agree”. Item 31 (My critical thinking has improved due to the program) and item 3 (My interest in Anatomy has increased due to the program) in median with “agree” (Cronbach’s Alpha = 0.853).

We demonstrate that teacher assistants appreciate our concept and provide an example of how such a program is implemented.

Poster 97:

Titel:
How to Preserve Specimens for Vascular- and Enteroanastomosis Suturing Technique Training

Autoren:
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Abstract:
A proper suturing technique for vascular- and enteroanastomosis is a vital part of surgical training. One of the main challenges is the availability of realistic models because typical preservation solutions for human specimens are either inapt or too expensive. The use of fresh or frozen specimens is limited by the timeframe of natural decomposition and storage logistics.

Hence, we aimed to develop an easy and inexpensive preservation technique for vessels and intestines for anastomosis suturing training.

We harvested vessels and intestines from human bodies. Individuals donated their bodies to the Division of Clinical and Functional Anatomy of the Medical University of Innsbruck after written informed consent was obtained. Vessels were rinsed three times in hot water for five minutes to remove any blood clots and rinsed two times with cold distilled water for five minutes. Intestines were also rinsed in hot water until all fecal residues were removed and then rinsed four times with cold distilled water for five minutes. Specimens were then stored in small containers with 2000 ml solution made up of 1000 ml distilled water, 970 ml 96% denatured alcohol, and 30 ml 85% glycerin.

We stored vessels for six months and intestines for three months in small containers at room temperature (18° - 24°), without signs of decomposition.

Our described solution enables longer storage of donated vessels and intestines in an uncomplicated and cost-efficient way. Therefore, surgeons can practice sophisticated suturing techniques independent from natural decomposition.

Poster 98:

Titel:
Development of an ultrathin sheet plastination protocol for experimental morphology research in a rat osteoarthritis model

Autoren:
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Abstract:
Ultrathin sheet plastination has been used to study the morphology of structures, with strong application in anatomical education and research. Injection with monosodium iodoacetate (MIA) is widely used to produce osteoarthritis (OA). The aim of the present study was to carry out an ultrathin sheet plastination protocol in rats humeral joints in order to observe morphological changes provoked by OA.

We injected 0.1 mL of MIA into the left humeral joints of 10 Sprague-Dawley rats. Right shoulders of the same rats were used as control. Sixteen weeks after the injection, the animals were euthanized and were given an immediate epoxy red resin injection through the thoracic aorta. Humeral joints and surrounded tissue were fixed in 10 % formalin, prior to the plastination process, without decalcification. Samples were dehydrated with acetone (100 %) at -25 ºC, for ten days. Later, for degreasing, samples were immersed in methylene chloride at room temperature during one week. Forced impregnation was performed inside a stove within a vacuum chamber. Plastinated blocks were cut with a los speed diamond blade saw. Slices were placed in curing chambers to achieve the polymerization and final tissue transparentation.

230 µm thickness slices were obtained and analyzed under magnifying glass and microscope, achieving visualization of OA morphological changes. Cartilage affected by OA loses its ability to remain avascular, and blood vessels invade it from the subchondral bone to the calcified and uncalcified cartilage.

Ultra-thin sheet plastination is useful to observe articular cartilage neovascularization, caused by OA induced with MIA in humeral rat joint.

Poster 99:

Titel:
Acute and protracted neuronal correlates of single dose in vivo ethanol intoxication

Autoren:
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Abstract:
In the brain, the molecular and cellular consequences of acute ethanol exposure are still incompletely understood. We reasoned that characterization of molecular changes would also help to describe various cellular and behavioral correlates of acute ethanol stimulation.

Quantitative mass spectrometry to screen over 2000 hippocampal proteins identified 72 candidate proteins which changed their synaptic abundance up to two-fold and more; about a third had been described in the context of ethanol exposure before. We validated a few of the candidate proteins in vitro and importantly, also in vivo by immunofluorescence.

Some of the candidate proteins are important for neuronal morphology. Indeed, following a single dose of ethanol, both dendritic spines and the length of the axon initial segment showed subtle but significant changes in vivo. Surprisingly, some molecular and cellular changes occurred hours after ethanol was metabolized suggesting slow compensatory mechanisms of the brain. These protracted mechanisms were also reflected on a cognitive level as naïve mice showed impaired decision making in behavioral tests after the ethanol stimulus had subsided.

Our results extend the effective window of one-time ethanol exposure from transient to more lasting molecular, morphological, and cognitive changes which potentially could link the transition from acute to chronic ethanol consumption.

Poster 100:

Titel:
Enteric neurogenesis in human fetal gut wall

Autoren:
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Abstract:
To study the enteric neurogenesis in human fetal gut wall in stomach, small intestine and colon.

It is an observational descriptive study of PhD research. Prior ethical approval and consent were taken for the collection of human still birth fetuses. Fetuses of gestational ages from ten weeks to till birth were collected and grouped into eight groups by using Sturge's rule.

Sections were taken from the paraffin embedded blocks of stomach, small intestine and colon. Location and shape of enteric neurons, their nuclei and processes, confirmation of the neurons and neuroglia and migration of the neurons through the connective tissue pathway were studied by using haematoxylin and eosin, Bielschowsky's silver, Phosphotungstic acid haematoxylin and Masson's trichrome stains.

This study will be carried in hundred human fetuses sampled by using convenience sampling method.

To date thirteen human fetuses were studied. Enteric neurons were found in serosa, muscularis externa and submucosa in fetal gut wall of different gestational ages. As the fetal age increases neurons were more dense and mature with round or oval or pyramidal in shape having round or oval euchromatic nuclei with prominent nucleoli. Migrations of enteric neurons through the connective tissue pathway were more pronounced in the wall of stomach in comparison to the small intestine and colon.

Understanding of enteric neurogenesis provides new insights for the diagnosis and preventions associated with the disorders of gastrointestinal tract. This research will determine the enteric neurogenesis in stomach, small intestine and colon of human fetal gut wall.

Poster 101:

Titel:
Early affection of the vascular endothelium after experimental middle cerebral artery occlusion

Autoren:
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Abstract:
Stroke-related blood-brain barrier (BBB) breakdown exacerbates the cerebral edema, which increases intracranial pressure and detrimentally impacts on the clinical outcome. The impaired vascular integrity is further associated with the risk of intracranial bleeding, especially after therapeutic recanalization. Therefore, we aimed to investigate early vascular alterations in the setting of stroke.

For this purpose, early time points from 30 minutes to 4 hours after experimental middle cerebral artery occlusion (MCAO) were analyzed at the level of fluorescence and electron microscopy in mice. BBB breakdown was visualized by application of the permeability marker FITC-albumin.

Thereby, an extravasation of FITC-albumin became detectable in animals which underwent 2 hours and 4 hours of MCAO. Here, BBB breakdown correlated with alterations of the endothelial surface, indicated by a discontinuous isolectin-B4 staining, while tight junction strands remained detectable using electron and immunofluorescence microscopy. Noteworthy, already 30 minutes after MCAO, up to 60% of the ischemia-affected vessels showed an endothelial edema, paralleled by edematous astrocytic endfeet, clearly preceding FITC-albumin extravasation. With increasing ischemic periods, scores of vascular damage significantly increased with up to 60% of the striatal vessels showing loss of endothelial integrity. Remarkably, comparison of permanent and transient ischemia did not provide significant differences 4 hours after ischemia induction.

As the endothelial alterations also involved penumbral areas of potentially salvageable tissue, adjuvant approaches of endothelial protection may help to reduce the vasogenic edema after ischemic stroke.

Poster 102:

Titel:
Differential recruitment neuronal networks during cue-induced drug and natural reward-seeking behaviour

Autoren:
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Abstract:
Cue-reward associations form distinct memories that can drive appetitive behaviours and are involved in craving for both drugs and natural rewards. However, it is presently unclear how cue-induced reinstatement of previously learned reward-seeking behaviour recruits functional neuronal ensembles in different brain regions and whether the activation pattern is generalized or specific for the cue-associated reward.

We trained rats on a concurrent operant self-administration protocol for two different rewards: alcohol as a drug and a sweet saccharin solution as a natural reward. This paradigm allows for the matching of the reinforcement values of the two rewards, thereby avoiding potential biases due to difference in motor activity as well as such introduced by different values assigned to the rewards by the animal. Reinstating for either of the rewards led to the activation of reward-related neuronal networks and consequential reward-seeking behaviour.

The Reinstatement-induced neuronal activity levels in 12 distinct, reward-seeking associated brain regions were determined by by cFos-stainings. Both rewards activated neuronal populations of similar size in all brain regions examined. However, alcohol decorrelated the activity levels in between, brain networks. Specifically, the network of the medial prefrontal cortex, which is crucially involved in the control of reward-seeking behaviour, was affected by the alcohol induced decorrelation.

Decorrelated networks are less stable and possess lower communication efficiencies, rendering them less well suited to control the extent of reward-seeking behaviour.

Poster 103:

Titel:
Bacterial pathogenic factors in synaptic damage during meningitis

Autoren:
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Abstract:
Bacterial infections of the brain lead to high lethality and substantial disability in survivors. Among them, Streptococcus pneumoniae ranks as the most common cause, especially in Third world countries. Synapses and dendritic spines represent the anatomical substrate of learning and memory. The aim of our work was to clarify whether synapses are affected in meningitis and if yes - which are the critical pathogenic factors and underlying mechanisms.

We utilized acute brain slices and animal meningitis models, combined with live imaging and various staining approaches to study synaptic and dendritic dynamics in models of meningitis.

Our works int he last years demonstrated that pneumococcal meningitis leads to a massive loss of dendritic spines and synapses in pyramidal neurons of the neocortex in a glutamate-dependent manner. The major pathogenic factor of S. pneumoniae - the protein cytolysin pneumolysin, played a key role in this process, stimulating glutamate release from the astrocytes, but bacterial capsules (especially in lysed bacteria) contributed to these changes as well.

Our findings that synapses and dendritic spines are affected in meningitis answer a long-standing question in the meningitis field about the cause of long-term sequelae after successful disease recovery. The novel knowledge of the underlying molecular mechanisms provides new therapeutic possibilities.

Poster 104:

Titel:
Vitamin D in the vomeronasal organ and possible behavioural consequences

Autoren:
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Abstract:
Steroids are important olfactory signals in most mammalian species. The vomeronasal organ has been suspected

to be the primary target of pheromones. It is known that some steroids can affect the social behaviour in mammals. In recent studies we could demonstrate that the vitamin D receptor and the vitamin D binding protein are expressed in rat vomeronasal organ. Furthermore we studied behavioural effects of provitamin D in wolves.

We studied the expression of the vitamin D receptor and of the vitamin D binding protein with immunofluorescence, in situ hybridization and with reverse transcriptase PCR.

Behavioural tests were performed with three concentrations of provitamin D. We studied behavioural changes and sniffing durations on vitamin D samples compared to the control.

Sensory neurons of the vomeronasal organ contained immunoreactivity for vitamin D receptor in nuclei, cytoplasm, and apical protrusions. Vitamin D binding protein was observed in the sensory apical protrusions and a portion of vomeronasal glands. Both proteins were also found in single cells within the non-sensory epithelium.

In the behavioural tests we found longer sniffing durations of male and female wolves especially on the highest concentration of provitamin D compared to the control.

Our morphological findings suggest that the rat vomeronasal organ is a vitamin D target. Furthermore provitamin D induces fast behavioural responses in wolves suggesting that non-genomic steroid actions are involved. Our behavioural observations are in accordance with our histochemical results and in line with previous findings in reptiles, supporting a pheromonal role of vitamin D in mammals.

Poster 105:

Titel:
How to deal with (cross)-resistant retinoblastoma cells?

Autoren:
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Abstract:
Retinoblastoma (RB) is the most common malignant intraocular tumor in early childhood. Chemotherapy resistance diminishes the clinical-therapeutic options and emphasizes the necessity for new therapeutic approaches. The study presented focused on investigating cross-resistances of etoposide-resistant RB cell lines to the anticancer agents paclitaxel and vincristine, commonly used after failure of standard RB therapy.

Effects of chemotherapy resistance on RB cell viability, proliferation and apoptosis were revealed by WST-1 assays, growth curve analyzes and trypan blue exclusion tests. Tumorigenicity of RB cell lines was analyzed using the in vivo chorioallantoic membrane (CAM) assay and colony formation assays.

We could show that etoposide-resistant RB cell lines display altered proliferation and apoptosis levels as well as tumor formation capacity in vivo, resulting in increased aggressiveness of the tumor cells. Compared to their parental counterparts etoposide-resistant Y-79, WERI-Rb1 and RB-355 RB cell lines generated significantly increased numbers of CAM tumors with higher tumor weights

Aggressive, etoposide-resistant RB cells co-treated with paclitaxel showed significantly higher growth kinetics and viability, lower apoptosis rates and increased colony formation capacity, indicating a cross-resistance for paclitaxel in WERI-Rb1 but not in Y79 etoposide-resistant RB cells. By contrast, no cross-resistance for vincristine was observed in WERI-Rb-1 and Y79 etoposide-resistant cell lines.

Etoposide-resistant RB cells behave more aggressively than the tumor cells of origin and potentially bare the risk for local relapses. With regard to the detected cross-resistance, a future follow-up treatment of etoposide-resistant cells with vincristine instead of paclitaxel seems to be more reasonable.

Poster 106:

Titel:
The neuronal cell adhesion molecule L1CAM - a novel target for human retinoblastoma therapy?

Autoren:
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Abstract:
The neuronal cell adhesion molecule L1CAM (L1) is involved in the embryonic development of the nervous system but can also be found differentially expressed in a variety of human cancers such as glioblastoma and neuroblastoma. The present study focuses on the function of L1 in the progression of human retinoblastoma (RB), the most common malignant intraocular tumor in early childhood.

Effects of L1 after lentiviral knockdown and transient overexpression in RB cells on proliferation and cell viability were revealed by growth curve analysis, BrdU immunostaining, DAPI cell counts and WST-1 assay. Anchorage independent growth and tumorigenicity were analyzed by soft agarose assay and in vivo chicken chorioallontoic membrane (CAM) assay.

We investigated L1´s function with regard to changes in proliferation, cell viability, anchorage independent growth as well as tumor formation in vivo. L1 overexpressing Rbl30 and RB247 RB cells show significantly increased growth and proliferation rates as well as decreased apoptosis levels. Fittingly, L1 depleted RB355 and WERI-Rb1 RB cells show opposite effects on growth kinetics and cell viability. Anchorage independent growth significantly decreases after L1 depletion and is induced in L1 overexpressing cells. In vivo CAM assays revealed significantly reduced tumorigenicity after L1 depletion of RB355 and WERI-Rb1 cells. Additionally, the sensitivity against the commonly used RB chemotherapeutics etoposide, cisplatin and vincristin significantly increases after L1 knockdown in WERI-Rb1 cells.

L1CAM may have the potential of a novel therapeutic target in retinoblastoma therapy, as it affects tumor formation and progression as well as the susceptibility towards commonly used chemotherapeutics.

Poster 107:

Titel:
Cultivation of hippocampal neurons of BDNF knockout mice

Autoren:
Laura Plantera (Neuroanatomy, Institute of Anatomy and Cell Biology, Greifswald), Ina Eiffler (Neuroanatomy, Institute of Anatomy and Cell Biology, Greifswald), Oliver von Bohlen und Halbach (Neuroanatomy, Institute of Anatomy and Cell Biology, Greifswald); laura.plantera@uni-greifswald.de

Abstract:
The neurotrophin brain-derived neurotrophic factor (BDNF) is an essential differentiation factor in key neuronal processes like neurogenesis, survival and differentiation of neurons (Vilar & Mira 2016), neuronal plasticity, learning and memory (Leal et al. 2017).

We isolate and cultivate hippocampal embryonal neurons from C57BL6/N wild type mice (+/+), heterozygous BDNF knockout mice (+/-) and homozygous BDNF-knockout mice (-/-).

Because of the early postnatal lethality of mice with a homozygous BDNF-knockout (-/-), heterozygous BDNF knockouts with a conditional knockout of BDNF in neurofilament L expressing neurons (-/fl) were used for the cultivation of adult hippocampal neurons. After 4, 7 and 10 days

cells were fixed with formaldehyde and immunostained for differentiation markers (doublecortin, NeuroD), connectivity markers (spinophilin, MAP-2), and cell type markers (NeuN, GFAP). Neuronal culture were also treated with BDNF to investigate alteration in viability and plasticity of the cell culture.

We will examine whether the neuronal culture display changes in parameters attributed to adult hippocampal neurogenesis in-vivo. Hence, we will compare the ratio of neurons to glia cells and measure the length of dendrites. Currently, we noted an increase in neuron numbers expressing terminal differentiation markers and a decrease of neurons expressing early differentiation factors over the time of cultivation.

We analyzed the phenotypes of the different genotypes of primary embryonal and adult hippocampal neurons based on specific characteristics like growth, survival, cell structure and synaptic integrity to get insight in the role of BDNF.

Poster 108:

Titel:
Detailed analysis of adult neurogenesis in the telencephalon of domestic pigeons

Autoren:
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Abstract:
Adult neurogenesis occurs in a variety of species including birds. In birds, adult neurogenesis is more widespread compared to mammals and was reported in several brain structures. Only a few studies investigated adult neurogenesis in the domestic pigeon, although comprehensive analyses in various species will lead to a more complete understanding of the fundamental biology and evolution of adult neurogenesis and will provide a better framework for testing hypotheses regarding the functional significance of this trait.

Here, free flying homing pigeons (Columba livia f.d.) were treated with 5-bromo-deoxyuridine (BrdU) and sacrificed three months after injection. Brains were immunohistochemically processed with several markers to examine different stages of cell proliferation quantitatively and qualitatively. We used GFAP and SOX2 for precursor cell stages and doublecortin (DCX), Tbr2, NeuN and Calbindin for intermediate progenitor cells and postmitotic stages. We analyzed and compared pallial and subpallial regions and included an analysis of proliferating cells along the rostro-caudal axis of the pigeon forebrain.

Proliferating cells were widely distributed in the telencephalon. Generally, the quantity of DCX-positive cells exceeded BrdU-positive cells and the number of newborn glial cells exceeded the number of newborn neurons significantly. We further detected different levels of adult neurogenesis in pallial and subpallial regions and individual structures (e.g. hyperpallium apicale, hippocampal formation) showed variations along the rostro-caudal axis.

Our findings complete the knowledge about adult neurogenesis in birds, reveal a high level of plasticity in the pigeon’s telencephalon and suggest an important function of this dynamic process in the pigeon brain.

Poster 109:

Titel:
HspB5/alphaB-crystallin enhances dendritic complexity of hippocampal neurons in vitro and in vivo

Autoren:
Britta Bartelt-Kirbach (Institute of Anatomy and Cell Biology, University of Ulm, Ulm), Samuel Birk (Institute of Anatomy and Cell Biology, University of Ulm, Ulm), Christoph Wiegreffe (Institute of Cellular and Molecular Anatomy, University of Ulm, Ulm), Stefan Britsch (Institute of Cellular and Molecular Anatomy, University of Ulm, Ulm), Nikola Golenhofen (Institute of Anatomy and Cell Biology, University of Ulm, Ulm); britta.bartelt@uni-ulm.de

Abstract:
The dendritic arbor is crucial for building neuronal networks and rarefaction of dendritic complexity leads to neuronal dysfunction as observed in neurodegenerative disorders. Previously, we showed that the small heat shock protein HspB5/alphaB-crystallin, which is upregulated and phosphorylated in neurodegenerative diseases, regulates dendritic complexity in cultured hippocampal neurons. Here, we intended to evaluate this HspB5 function in dependence of its phosphorylation in vitro and in vivo.

HspB5 phosphomimics simulating or preventing phosphorylation at serine 19, 45 or 59 by exchange to alanine (A) or glutamate (E) were generated in all combinations. Rat hippocampal neurons were transduced at DIV7 with lentivirus carrying the different expression constructs and dendritic trees analysed at DIV14 by Sholl-analysis. For in vivo evaluation, we employed in utero electroporation of the expression constructs HspB5-wt, HspB5-AAA and HspB5-AEE to the hippocampus of mice at E15.5 and analysed dendritic tree complexity at P14.

In cultured hippocampal neurons HspB5-wt and HspB5-AEE significantly increased dendritic complexity in contrast to the non-phosphorylatable HspB5-AAA and the other phosphomimics. Enhanced dendritic complexity was also observed in vivo in CA1 hippocampal neurons overexpressing HspB5-wt and HspB5-AEE compared to controls.

HspB5 overexpression has a positive effect on the dendritic tree of hippocampal neurons not only in primary neuronal cultures but also in the hippocampus in vivo, dependent on phosphorylation at serine 45 and 59. Thus, upregulation and phosphorylation of HspB5 in neurodegenerative diseases may be part of a damage-response mechanism counteracting the pathological rarefaction of the dendritic architecture aiming at maintenance of neuronal function.

Poster 110:

Titel:
Characterization of mice models with different forms of BDNF knockouts

Autoren:
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Abstract:
Neurotrophins, such as brain-derived neurotrophic factor (BDNF), play a role in central functions of the brain like learning and memory (Leal et al. 2017, Monteggia et al. 2004), eating behaviour (Rios et al. 2013, Lebrun et al. 2006) and anxiety-related behaviour (Hashimoto 2007). Loss of BDNF or impairments in the BDNF-signalling are therefore linked with brain dysfunction. However, its role in the postnatal brain has remained difficult to assess, since the BDNF-null mutation is lethal (Rauskolb et al. 2010).

We therefore used different knockout mice with a C57Bl6/N background displaying a lack of BDNF, i.e. a heterozygous knockout, a heterozygous knockout with a conditional knockout in NFL expressing neurons (pyramidal neurons, projection neurons, purkinje cells, motor neurons) on the other allel and their control wildtype littermates. We performed several behavioural tests (van Gaalen and Steckler 2000) with female and male mice at the age of three, six and nine months to cover possible gender and age effects, respectively. At the age of 12 months Novelty induced hypophagia was performed to detect anxiety-related behaviour more specifically. Furthermore, we measured food intake and weight of the mice weekly.

Significant differences could be detected in most of our tests of basic behaviour as well as in Novelty induced hypophagia between the three genotypes and the three ages but not between the two genders.

Interestingly, changes in behaviour of BDNF knock out mice increased with age. This may be due to the fact, that BDNF expression is highest immediately after birth and decreases with age. We suggest that young mice may cope better with a loss of central BDNF than old mice (i.e. through a higher expression of BDNF by glial cells).

Poster 111:

Titel:
Phosphorylation-dependent localization of HspB5/AlphaB-crystallin in the mouse hippocampus

Autoren:
Hartmut Raisch (Institut für Anatomie und Zellbiologie, Universität Ulm, Ulm), Samuel Birk (Institut für Anatomie und Zellbiologie, Universität Ulm, Ulm), Britta Bartelt-Kirbach (Institut für Anatomie und Zellbiologie, Universität Ulm, Ulm), Nikola Golenhofen (Institut für Anatomie und Zellbiologie, Universität Ulm, Ulm); hartmut.raisch@uni-ulm.de

Abstract:
The small heat shock protein HspB5/alphaB-crystallin is known to be upregulated and phosphorylated in the brain by cellular stress conditions and in neurodegenerative diseases. It exhibits neuroprotective functions by its chaperone-like, anti-inflammatory and anti-apoptotic properties. The cytoprotective activity of HspB5 is known to be regulated by phosphorylation at serine 19, 45 and 59. In this study, we investigated the localization of these phosphoforms of HspB5 in the mouse hippocampus.

Mouse brains were dissected, fixed with 4% formaldehyde, cut with a vibratome and sections were further processed for immunolabelling. Double labelling using antibodies against HspB5, phospho-HspB5 (S19), phospho-HspB5(S45) or phospho-HspB5(S59) and markers for different cell types or subcellular structures was performed. Sections were analyzed by fluorescence and confocal microscopy.

HspB5 was found in mouse hippocampus in granule cells of the dentate gyrus as well as in pyramidal cells of the cornu ammonis (CA) but not in glia. Interestingly, phospho-specific antibodies against the three phosphorylation sites showed a different staining pattern. Anti-phosphoHspB5(S19) showed strong labelling of the hilar region of the dentate gyrus, the mossy fibers and of CA1 pyramidal cells. PhosphHspB5(S45) showed a similar distribution pattern with additional localization in some interneurons. In contrast, anti-phosphoHspB5(S59) showed diffuse strongly demarcated staining in the hilum of the dentate gyrus and the stratum lacunosum of the CA1 region.

In mouse hippocampus HspB5 is expressed in various types of neurons but not in glia. It shows a cell-type specific phosphorylation pattern with characteristic subcellular localizations dependent on which of the three sites was phosphorylated.

Poster 112:

Titel:
Botulinum Neurotoxin-A Injected Intrastriatally into Hemiparkinsonian Rats Improves the Initiation Time for Contralateral Forelimb in Both Forehand and Backhand Directions

Autoren:
Veronica Antipova (Gottfried Schatz Research Center for Cell Signaling, Metabolism and Aging, Macroscopic and Clinical, Medical University of Graz, Graz), Carsten Holzmann (Institute of Medical Genetics, Rostock University Medical Center, Rostock), Alexander Hawlitschka (Institute of Anatomy, Rostock University Medical Center, Rostock), Andreas Wree (Institute of Anatomy, Rostock University Medical Center, Rostock); veronica.antipova@medunigraz.at

Abstract:
In Parkinson's disease (PD), bradykinesia and postural and gait impairments are leading symptoms that are based on delayed movement initiation. Forelimb stepping is a widely used test for the assessment of forelimb akinesia in hemiparkinsonian (hemi-PD) rats. The initiation time (IT) is considered the most sensitive parameter in the stepping test procedure.

We propose a novel, reliable, and simple method for the measurement of IT of both forelimbs in both forehand and backhand directions in rats. Evaluating the same videos taken for quantifying adjusting steps (AS), IT measurements were done without additional experiments. This is in contrast to the classical approach introduced by Olsson et al. (1995), in which separate experiments are necessary. We successfully applied our approach to hemi-PD rats intrastriatally treated with botulinum neurotoxin-A (BoNT-A).

In naïve rats, an IT of about 0.62 s was found, and in right-sided hemi-PD rats the IT of the left forepaw increased to about 3.62 s. These hemi-PD rats showed reduced ITs of the impaired left forepaws 1 month following injection of 1 ng BoNT-A into the ipsilateral striatum. However, this effect depended on post BoNT-A survival time.

The method described offers the possibility of a precise and animal-friendly evaluation of IT in rats, including the beneficial effect of BoNT-A treatment in hemi-PD rats. Correlating our present results of AS and IT of movements from the stepping test with the apomorphine-induced rotation behavior we conclude that AS, IT, and apomorphine-induced rotations represent widely independent parameters.

Poster 113:

Titel:
Anatomical, behavioral and physiological variations between wild and laboratory rat strains -Does domestication affect predator odor induced innate fear behavior in rats? -

Autoren:
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Abstract:
Rats are among the most frequently used species in laboratory research. They have been bread for generations in laboratories which led to alterations in physiology and anatomy compared to wild rats.

In this project we exposed rats of the wild strain Warsaw Wild Captive Pisula Stryiek (WWCPS) and rats of the laboratory strain Lister Hooded (LH) to the predator odor, 2,3,5-trimethyl-3-thiazoline (TMT), a component of fox feces, in an open field paradigm.

Afterwards we analyzed behavioral differences as well as alteration of neuronal activation in specific brain regions involved in processing aversive olfactory stimuli and fear responses using immunhistochemical staining for c-fos.

Our previous results show significant differences between wild and laboratory rats during habituation session, prolonged freezing responses to TMT exposure and increased corticosterone levels in the wild rat strain. In the present study we additionally show (neuro)anatomical variations between the two rat strains.

Ourdata show clear differences between wild and laboratory rat strains only on a behavioral and physiological level. The results indicate that innate defensive strategies are affected by domestication effects.

Poster 114:

Titel:
Neuroligins mediate presynaptic maturation through brain-derived neurotrophic factor signaling

Autoren:
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Abstract:
Maturation is a process that allows synapses to acquire full functionality; failures in synaptic maturation may contribute to neurodevelopmental disorders. Neuroligins are postsynaptic cell-adhesion molecules essential for synapse maturation, but the underlying pathways are incompletely understood. Here, we show that maturational increases in active zone stability and synaptic vesicle recycling rely on the joint action of Neuroligins and brain-derived neurotrophic factor (BDNF).

Applying BDNF to neuronal cultures mimicked the maturation-promoting effects of overexpressing the Neuroligin isoforms NL1 or NL2. Inhibiting BDNF signaling reduced the effects of NL1 and NL2 on presynaptic maturation and of NL2 on synapse formation.

Applying BDNF to NL1-knockout mouse cultures rescued defective presynaptic maturation, indicating that BDNF acts downstream of NL1 and can restore presynaptic maturation at late stages of network development.

Our data introduce BDNF as a novel and essential component in a transsynaptic pathway linking Neuroligin-mediated cell adhesion, neurotrophin action and presynaptic maturation.

Poster 115:

Titel:
Immunocytochemical demonstration of the TSH receptor (TSHR) in transfected NIH/3T3 cells, mediobasal hypothalamus and thyroid gland of C3H mice

Autoren:
Horst-Werner Korf (Institut Anatomie I, Heinrich Heine Universität Düsseldorf, Düsseldorf), Wienand Anne (Institut Anatomie I, Heinrich Heine Universität Düsseldorf, Düsseldorf), Hanna Bellert (Institut Anatomie II, Heinrich Heine Universität Düsseldorf, Düsseldorf), Charlotte von Gall (Institut Anatomie II, Heinrich Heine Universität Düsseldorf, Düsseldorf); korf@uni-duesseldorf.de

Abstract:
TSH derived from the pars tuberalis (PT) is an important messenger of the "retrograde" output pathway. PT-derived TSH targets TSH receptors (TSHR) in the ependymal layer of the infundibular recess and drives the intrinsic hypothalamic T4/T3 system which controls seasonal function. Until now, the the hypothalamic TSHR was exclusively demonstrated by means of in-situ hybridization. To characterize the mode of action of PT-derived TSH we have investigated the subcellular location of TSHR protein by immunocytochemistry.

At first we tested the specificity of an antibody against the TSHR with NIH/3T3 cells which do not express the TSHR in their native state. The antibody revealed an immunosignal only in NIH/3T3 cells transfected with a TSHR pcDNA4, but not in native cells. This antibody was then used for immunocytochemical investigations of the thyroid gland and the mediobasal hypothalamus (MBH) of melatonin-proficient C3H mice.

In the thyroid the TSHR signal was located in thyrocytes and appeared concentrated at the cell membrane. Some follicles did not show any immunoreactive thyrocytes indicating a locally regulated expression of the TSHR which may depend on the functional activity of single follicles. In the MBH, TSHR immunoreactivity was demonstrated in tanycytes which were also vimentin-immunoreactive and can thus be classified as BETA-tanycytes. The immunoreaction was found in the apical pole of the cells facing the third ventricle, and in processes and terminals reaching the portal capillaries.

This distribution suggests that PT-derived TSH can directly act upon the tanycyte terminals exposed to the portal vasculature. Notably, TSHR immunoreactivity was also found in neurons of the arcuate nucleus, which may thus be an additional target of the TSH signal.

Poster 116:

Titel:
Comparisons of cortical association pathways across species using diffusion MRI tracography

Autoren:
Alpen Ortug (Division of Newborn Medicine, Department of Medicine, Boston Children's Hospital, Harvard Medical School, Boston), Christine J Charvet (College of Health and Behavioral Sciences, Department of Psychology, Delaware State University, Dover), Emi Takahashi (Division of Newborn Medicine, Department of Medicine, Boston Children's Hospital, Harvard Medical School, Boston); alpen.ortug@childrens.harvard.edu

Abstract:
The neural structures and architecture that underlie brain connectivity of different species are a basis of differential brain functions across species. Because white matter appears homogeneous in conventionally acquired structural MRI images, it is difficult to appreciate detailed fiber pathways within the white matter. As a result of recent technical advances in diffusion MRI, we can now study white matter neuronal fiber connections across the entire brain of different species.

The aim of this study is to compare the area/volume of major cortical association pathways across primates in proportion with the whole brain size in order to scale them in the course of brain evolution.

We obtained diffusion MRI data in adult humans, macaques, marmosets, rats, and mice. We reconstructed fiber pathways with a Q-ball model to resolve high angular resolution of the fiber pathways.

We identified and quantified diffusion MRI-based multiple cortical association pathways (e.g., cingulate bundle, superior longitudinal fasciculus), and explored the functional significance of detected white matter tracts that showed not only resemblance but also significant differences across species.

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Ortug A., Charvet C. J., Takahashi E. *Boston and Dover (USA)*
Comparisons of Cortical Association Pathways across Species using diffusion MRI tractography

Poster 117:

Titel:
VEGF receptor expression in the enteric nervous system

Autoren:
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Abstract:
The enteric nervous system (ENS) belongs to the PNS and consists of the plexus myentericus (Auerbach plexus) and plexus submucosus (Meissner plexus). In many neurodegenerative diseases, pathological changes such as Lewy bodies in Parkinson's can be found early in the ENS. These alterations lead to disorders of basic functions like peristalsis and secretion. In CNS and PNS, the vascular endothelial growth factor (VEGF) mediates neuroprotective and neuroregenerative effects in glial cells and neurons via VEGF receptor 2 (VEGFR2). Our group has already demonstrated the promoting effects on somato- and dendritogenesis in neonatal CNS neurons and on axonal growth in the PNS. As the ENS is suspected as a manifestation site for neurodegenerative diseases, it seems to be important to explore the impact of VEGF on its neurons. Therefore, a detailed knowledge of the receptor expression pattern is indispensable.

First, we optimized the preparation of the rat intestine at the age p0, p9, p15 and p30. Using laser microdissection, we were able to isolate the plexus myentericus for receptor analysis on mRNA level by RT-qPCR. Additional assays were performed to verify protein-expression by immunofluorescence staining and western blots.

VEGFR-2 and the co-receptors NRP1 / NRP2 are more strongly expressed than VEGFR-1 and 3, but in contrast to the CNS, no age-dependent regulation was detectable.

VEGFR-2 as the predominant receptor probably mediates neuroprotective and neuroregenerative effects within the ENS. Studies with cultured myenteric plexus will show how noxae affect neurons and VEGFR-2 expression and whether administration of VEGF can reduce these changes.

Poster 118:

Titel:
Progesterone receptor expression in the ENS

Autoren:
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Abstract:
The enteric nervous system (ENS) is an intrinsic network of neuronal ganglia in the wall of the intestinal tube. The majority of the intestinal motility is regulated by the plexus myentericus, which is located between the inner and outer muscle layers.

Neurodegenerative diseases, like Parkinson's (PD), seem to influence neurons of the ENS well before the CNS is impacted e.g. in form of Lewy pathology. Therefore, one could assume that an impairment of ENS cells may affect cells in the CNS via the brain intestinal axis and promotes the development of the diseases.

The neurosteroid progesterone shows promising potential for neuroprotection in the gut as this has already been shown in the CNS and PNS. These effects are mostly mediated by the classical progesterone receptors.

We optimized the preparation of the plexus myentericus from rat (p0, p9, p15, p30) for high quality cryosections to isolate single ganglia with aid of laser microdissection. In these samples, different progesterone receptors were examined by means of RT-qPCR. Additionally, immunofluorescence and western blots were used to characterize receptor expression on protein level.

We demonstrate for the first time that all examined subtypes of progesterone receptors are consistently expressed in the ENS during development, with increased expression levels of PGRMC1 and mPRA.

The strong expression of PGRMC1 and mPRA could lead to the assumption that these receptors are major mediators of progesterone effects in the ENS. Whether these receptors are capable to mediate neuroprotective effects in the ENS has to be checked in further studies.

Poster 119:

Titel:
Gut microbiota-brain changes in an animal model of anorexia nervosa

Autoren:
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Abstract:
It has recently been discovered that variations of the bacterial composition in the intestinal tract are causally involved in a variety of somatic diseases as well as in psychiatric disorders such as depression and anxiety. Current research supports that alterations in the gut microbiota composition are likewise involved in the pathophysiology of anorexia nervosa (AN), where brain volume changes are well-documented, but the gut-brain axis remains unclear.

The activity-based anorexia model (ABA) is well-established to mimic symptoms of AN in animals. Rats with access to a running-wheel and food restriction show body weight loss, hyperactivity, brain volume loss, glial changes and amenorrhea similar to patients with AN. Fecal samples from these animals were longitudinally analyzed using 16s rRNA gene amplicon sequencing. Additionally, brain sections were examined.

We showed that ABA rats display an altered microbiota composition compared to controls similar to that of patients with AN. We found an increase in alpha-diversity as well as significant differences in beta-diversity after chronic starvation. Moreover, relative abundance of Lactobacillus, Bifidobacteria, Akkermansia, Ruminococcus and Roseburia were increased in ABA animals, while Prevotella was reduced. Initial analyses indicate an inverse association between hippocampal volume and the genera Roseburia and Akkermansia. Further associations between gut microbiota and brain parameters will be presented.

The ABA paradigm appears to be an interesting translational model to study the gut-brain axis. Understanding the relationship between gut microbiota and the brain could lead to nutritional therapies with pro-/prebiotics as new therapeutic options for patients with AN.

Poster 120:

Titel:
Do casein-derived peptides modulate central and enteric gliosis?

Autoren:
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Abstract:
Gliosis is defined as an altered expression of inflammatory mediators and differentiation markers in glial cells, such as central astrocytes and enteric glial cells (EGC). While differentiation is usually perceived to have positive impacts on the development of a disease, inflammation is rather associated with negative effects. In consequence, therapeutic approaches may aim to inhibit inflammation without impairing differentiation. Previous research revealed that hydrolysates of the milk protein casein inhibit inflammation in cell lineages of human embryotic kidney cells and promote differentiation in EGC. Therefore, this study aimed to evaluate the impact of casein-derived peptides on central and enteric gliosis.

EGC primary cultures were prepared from intestines isolated from 14 days-old mouse embryos. Astrocyte primary cultures were prepared from brains extracted from newborn mice. The cells were incubated with two different casein-derived peptides after stimulation with lipopolysaccharide. The expression of inflammatory mediators and differentiation markers was measured using qPCR, ELISA and immunofluorescence.

We could not demonstrate any effect of casein-derived peptides on the expression of inflammatory mediators or differentiation markers in vitro.

This study suggests that casein peptides have limited impact on central and enteric gliosis in vitro. In addition, the peptides used do not reproduce the gain of differentiation in EGC observed in previous studies using whole casein hydrolysates. Further work, involving alternative combination of milk-derived peptides, is required to better characterize the processes involved in the regulation of central and enteric gliosis, and to further evaluate the potential impact of bioactive peptides on these processes.

Poster 121:

Titel:
Gephyrin expression in the hippocampus after traumatic brain injury in rodents

Autoren:
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Abstract:
Patients surviving traumatic brain injury (TBI) often suffer from long-term cognitive malfunction and epileptic seizures significantly impairing their quality of life. Aiming to unravel further therapeutic strategies for amelioration of cognitive recovery after TBI, we study the structural composition of inhibitory synapses in the hippocampus in TBI animal models. In addition to a delayed loss of neurons due to secondary damage mechanisms, for example inflammation, adult hippocampal neurogenesis is enhanced after TBI. Inhibitory GABAergic synapses arise early after neuronal birth and act as regulators in subsequent functional integration of newborn neurons into existing networks. Gephyrin, the main inhibitory postsynaptic organizer, mediates inter alia synaptic recruitment of GABAA receptors, which is mandatory for effective inhibitory neurotransmission. We hypothesize that experimental TBI leads to changes in expression and synaptic localization of gephyrin.

In a pilot project cryo-sectioned hippocampal slices of lateral fluid percussion (LFP) injured and sham-treated control adult male Sprague Dawley rats are immune-stained with antibodies against gephyrin and other (synaptic) markers. We then image and analyze gephyrin expression and its (synaptic) localization in both conditions with confocal microscopy and ImageJ.

First results show an increased abundance and changed synaptic localization of gephyrin in the hippocampus after experimental TBI.

Preliminary results are well in line with our hypothesis and encourage us to continue our project. Further replication of data, additive western blot analyses and studies in controlled cortical impact (CCI) injured mice are essential to establish a basis for developing innovative therapeutic agents to reduce cognitive impairment in TBI patients.

Poster 122:

Titel:
Subnuclear neuroarchitecture of dopaminergic afferents in the mouse central amygdala

Autoren:
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Abstract:
The central nucleus of the amygdala (Ce) is composed of subnuclei with distinct hodological and functional features. Plasticity of microcircuits formed by peptidergic inhibitory neurons underlies Ce functions in emotional behavior. Midbrain dopaminergic (DA) neurons provide a dense, heterogeneous innervation of Ce subnuclei. Subpopulations of these neurons co-express glutamatergic markers and/or peptides such as vasoactive intestinal polypeptide (VIP), and optogenetic studies in mice suggest that DA/glutamate co-transmission mediates subnuclear circuit plasticity via differential effects on specific peptidergic neurons. In order to assess the structural basis for DA-mediated effects and for possible co-release of glutamate and/or peptides, the present study aims to provide a detailed analysis of the subnuclear neuroarchitecture of DA afferents in the mouse Ce.

Serial vibratome sections from perfusion-fixed brains of male adult C57BL/6 mice were immunolabeled and analyzed by light, fluorescence, confocal, and transmission electron microscopy (TEM).

DA fiber plexus overlap with vesicular glutamate transporter(vGlut2)- and VIP-immunoreactive(ir) terminal fields particularly in the lateral Ce (CeL). Confocal analysis shows association of vGluT2-ir terminals with subsets of DA axons, and indicates partial colocalization of VIP. Preliminary TEM investigations document differential morphology and numerous mostly symmetric synaptic contacts of narrow DA axons with target neurons in Ce subnuclei. Dense core vesicles and subcompartimentalization with the formation of asymmetric synapses at large terminals are detected in some DA terminal axons.

Our findings indicate that Ce subnuclei are differentially innervated by subpopulations of DA neurons, some of which may co-release glutamate and/or VIP.

Poster 123:

Titel:
LPA1, LPA2, LPA4 and LPA6 receptor expression during mouse brain development

Autoren:
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Abstract:
LPA is a small bioactive phospholipid that acts as an extracellular signaling molecule and is involved in cellular processes, including cell proliferation, migration, and differentiation. LPA acts by binding and activating at least six known G-protein-coupled receptors: LPA1-6. In recent years, LPA has been suggested to play an important role in both normal neuronal development and under pathological conditions in the nervous system. However, only few systematic analyzes of LPA receptor expression during mouse organogenesis have been performed yet. Therefore, our study examined the expression pattern of LPA1-6 receptors in different mouse brain areas from late embryonic developmental stages to adulthood.

We analyzed the expression pattern by using qRT-PCR, in-situ hybridization and immunocytochemistry in developing mouse brain tissue and in primary cell culture.

Only LPA1, LPA2, LPA4, and LPA6 mRNA transcripts were detected throughout development stages from embryonic day 16 until postnatal day 30 of hippocampus, neocortex, cerebellum, and bulbus olfactorius in our experiments, while expression of LPA3 and LPA5 genes was below detection level. In addition to our qRT-PCR results, we also analyzed the cellular protein expression of endogenous LPA receptors, with focus on LPA1 and LPA2 within postnatal brain slices and primary neuron differentiation with and without cytoskeleton stabilization and destabilization.

The expression of LPA receptors changes depending on the developmental stage in mouse brain and in cultured hippocampal primary neurons. Interestingly, we found that commercially available antibodies for LPA receptors are largely unspecific.

Poster 124:

Titel:
Catecholaminergic properties of the efferent parasympathetic pathway via the ciliary ganglion

Autoren:
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Abstract:
Parasympathetic, cholinergic, preganglionic Edinger-Westphal nucleus (EWpg) neurons control lens accommodation and pupil constriction via the ciliary ganglion (CG). Pupil dilation is mediated by sympathetic pathways. Recent studies suggest that a noradrenergic input from locus coeruleus (LC) modulates the parasympathetic activity in pupillary neurons for example during arousal. Our aim was to verify these connections by using tract-tracing methods in monkey and to investigate the EWpg and CG for possible tyrosine hydroxylase (TH)-input.

retrograde tract-tracing in macaque monkey, immunofluorescence, immunohistochemistry

Tracer-injections into the oculomotor nucleus/EWpg region in three macaque monkeys revealed retrogradely tracer-labelled neurons within LC confirming the connection between EWpg and LC. The analysis of a rostro-caudal series of brainstem sections through the EWpg of two monkeys, immunohistochemically stained for choline acetyltransferase (ChAT) and TH showed that the cholinergic EWpg neurons receive dense TH-input. Surprisingly a subpopulation of ChAT-positive EWpg neurons also expressed weak TH-immunoreactivity. The systematic analysis revealed that at caudal levels almost all EWpg neurons were double-labelled, while at more rostral levels double-labelled neurons are intermingled with neurons expressing only ChAT. In the CG a subpopulation of postganglionic, cholinergic CG-neurons expressed strong TH-immunoreactivity. Distinct TH-positive terminals contacting CG-neurons were not detected.

These results suggest that the parasympathetic and sympathetic system are more closely interconnected than previously thought, although the functional significance of TH in EWpg neurons needs to be further investigated. In future studies it needs to be clarified, whether the TH-immunostaining is confined to either accommodation- or pupil-neurons.

Poster 125:

Titel:
The inositol-1,4,5-trisphosphate-3-kinase-A is a putative regulator of motor function

Autoren:
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Abstract:
The Inositol-1,4,5-trisphosphate-3-kinase-A (Itpka) is a neuron-specific actin binding protein that regulates dendritic calcium transients and controls actin dynamics in dendritic spines. It is highly expressed in the murine cortex, hippocampus and cerebellum. Previous studies showed defects in memory performances and differences in emotional behavior in Itpka deficient mice.

In this study we analyzed the functional role of Itpka in social interaction and motor function by performing a set of behavioral experiments on an Itpka transgenic mouse model.

While Itpka deficient mice showed no difference in social behavior so far, we found defects in motor performance. Morphological investigations are now on the way to unravel the underlying circuits of Itpka dependent motor function.

Taken together, Itpka is a novel putative player in the complex network of motor control both in health and disease.

Poster 126:

Titel:
Does shiftwork affect connectivity of the default mode network in the human brain? A pilot study

Autoren:
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Abstract:
Shiftwork has a major impact on the human circadian system that controls numerous body functions. Further, circadian rhythms affect functioning of brain networks, such as the default mode network (DMN) involved in diverse cognitive processes. Therefore, we investigated whether shiftwork affects the functional connectivity (FC) of the brain regions composing the DMN.

Out of the population-based cohort of the 1000BRAINS study datasets were selected from 9 current shiftworkers and 9 randomly selected non-shiftworkers. Both groups were matched according to gender, age and education. For each participant, functional magnetic resonance imaging data at rest, i.e. without doing a specific task, were acquired. Applying graph-theoretical models, FC at rest was calculated as: (i) “within”-FC, reflecting connection strength between brain regions of the DMN; (II) “inter”-FC, reflecting the connectivity of the whole DMN to all other regions of the cortex; (iii) “between”-FC, reflecting the connectivity of the DMN to other functional brain networks. Non-parametric tests were applied to test for significant differences between the two groups in all three parameters. Sleeping behavior was estimated from sleep questionnaires.

Our data reveal no significant differences between shiftworkers and non shiftworkers with regard to the “within”-, “inter”- and “between”- FC of the DMN.

Thus, our pilot study suggests that shiftwork per se does not influence the integrity of functional brain networks such as the DMN. These results need to be corroborated in further studies with larger cohorts also considering shiftwork duration or type of shiftwork.

Poster 127:

Titel:
Microglia regulate neuronal network formation and synaptogenesis

Autoren:
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Abstract:
Microglia are the resident immune cells of the central nervous system (CNS) and play essential roles under physiologic and pathologic conditions. Next, to their well-established role in the progression of neurodegenerative diseases such as Alzheimer´s disease (AD) and Parkinson´s disease (PD), recent reports underline the involvement of microglia during postnatal CNS maturation. The main objective of the present study was to examine the influence of microglia on neuronal network formation and synaptogenesis in cortical/midbrain neuron cultures.

Primary mouse E14 cortical/midbrain neuron cultures were used to determine the development of functional synapses in the presence and absence of microglia. Microglia depletion was performed using the Csf1r inhibitor BLZ945. Neuronal network formation and maturation of synapses were analyzed using the Microelectrode Array Technology (MEA) and immunocytochemistry labeling of synaptic markers such as Synapsin and PSD95.

Electrophysiology using MEAs indicated that microglia regulate the abundance of functional synapses during a time course of 28 days in vitro (DIV). Using a Csf1r inhibitor, we were able to show that the efficient depletion of microglia abrogated the inhibitory effects on synaptogenesis.

Taken together, our data demonstrate that microglia are effective regulators of neuronal network formation and synaptic connectivity further broadening the portfolio of versatile functions of these resident immune cells of the central nervous system.

Poster 128:

Titel:
Polycystin-1 and polycystin-2 in neural progenitor cell differentiation

Autoren:
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Abstract:
Mutations in the Pkd1 and Pkd2 genes (encoding polycystin-1 and -2, respectively) are causative for the development of autosomal-dominant polycystic kidney disease. A prominent feature of this disease is an unbalanced cell proliferation. Polycystin-1 and polycystin-2 expression have also been found in radial glial cells (RGCs). RGCs are neural progenitor cells (NPCs) that have to balance proliferation for expansion, or for self-renewal and differentiation to generate neurons. It is unknown whether polycystin-1 or polycystin-2 plays a role in this process.

The expression of polycystin-1 and polycystin-2 in mouse embryonic neocortex was analyzed by immunohistochemistry. We performed loss-of-function experiments in primary NPCs in culture to assess NPC proliferation, apoptosis, and neuronal differentiation. We conducted cell cluster and cell pair analysis to dissect the mode of NPC division. We evaluated Notch activity and STAT3 signaling to ascertain whether these proteins were functionally connected to polycystin-1 and polycystin-2.

We found that during active neurogenesis polycystin-1 and polycystin-2 were expressed prominently in nestin-positive RGCs at the ventricular zone. Reduced polycystin-1 or polycystin-2 expression led to increased NPC proliferation, while the differentiation to neurons became impaired. The increased NPC proliferation was preceded by enhanced Notch signaling and accompanied by a rise in the number of symmetric cell divisions. The transcription factor STAT3 was shown to be mechanistically important for polycystin-1 and polycystin-2 signaling in NPCs.

We conclude that a signaling complex of polycystin-1 and polycystin-2 drives NPC differentiation towards a neuronal cell fate.

Poster 129:

Titel:
Presenilin / y-Secretase – mediated signaling is crucial for the development of laminated brain structures

Autoren:
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Abstract:
Objectives: In the cerebral cortex, Cajal-Retzius pioneer neurons (CR cells) are highly critical for the control of neuronal migration. We have previously reported that a “knock-out” of presenilin 1 (PS1), the main active site of the □-secretase complex, causes a lissencephaly type 2 – like neocortical lamination defect by interfering with the survival of CR cells. As many CR cells are generated from transition zones between cortical primordia and choroid plexus, we have started to systematically investigate the development of these structures and their derivatives in murine and piscine knockout-out and knock-down models.

Methods: By immunohistochemical co-stainings with neuronal and glial markers of cells in the developing brain, migration events in WT- and PS1-deficient mice and zebrafish were monitored.

Results: In WT mice, CR cells emigrate from the hippocampal anlage into the medial cortical wall, being accompanied by a hitherto unrecognized BLBP – positive glial cell population. In PS1 deficiency, CR dendrites fail to fully differentiate already prior to their premature death, preceding the generation of cortical ectopia. In the cerebellar anlage, PS1 deficiency causes a striking developmental arrest of the expansion of the external granular layer beyond E14, prohibiting early foliation, but without discernible loss of specific cell types.

Conclusion: PS1 is crucial for cellular interactions of CR cells and stem cells of the cerebellar EGL, influencing neuronal migration and cell differentiation. Knockdown models in Danio rerio are currently established for a further genetic and (sub-)cellular dissection of these developmental events.

Poster 130:

Titel:
B-Catenin differentially affects proliferation & differentiation of early and late born LGE neural progenitors

Autoren:
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Abstract:
The striatum is mainly composed of a morphologically homogenous population of medium-sized spiny projection neurons (MSNs), however a subdivision into striosome and matrix compartments can be observed. Although all MSNs derive from the lateral ganglionic eminence (LGE), striosomal neurons are born in a discrete time-window preceding most matrix neurons. In order to look for molecular cues involved in lineage specification, here we investigate intrinsic properties of neural precursor cells isolated from E13 and E19 rat LGE focusing on the activity of ß-Catenin-signaling for proliferation and differentiation in vitro.

E13- and E19-LGE derived NPCs were characterized in respect to proliferation and differentiation using immunocytochemistry and volumetric analysis by neurosphere formation assays. The contribution of β-catenin-signaling in NPC proliferation and differentiation was evaluated using CHIR99021 (activator) and XAV-939 (inhibitor) as specific molecules interfering with this signaling pathway.

Qualitative and quantitative analysis of nestin-, vimentin-, sox-2- and Ki67-expression shows higher proliferation rates for E13 NPCs compared to that of E19, and E13 NPCs preferentially differentiate into neurons, while E19 NPCs additionally give rise to GFAP-positive astrocytes under control conditions. B-Catenin-signaling inhibition significantly enhances proliferation in both E13 and E19 NPCs, whereas activation inhibits proliferation only in E13. Concerning differentiation, activation of β-Catenin-signaling induces differentiation in E13 and E19 NPCs, while inactivation has no effects on neurogenesis.

Our data show β-Catenin signaling to be differentially involved in proliferation and differentiation of striatal NPCs in a developmentally regulated manner, indicating distinct intrinsic properties of early and late gained NPCs.

Poster 131:

Titel:
Site-specific expression of the phospholipase PLA2G2A in the human colon and its potential implication for intestinal inflammation and Parkinson's disease.

Autoren:
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Abstract:
Neuroinflammatory processes not only play an important role in the pathogenesis of Parkinson's disease (PD) but also occur within the enteric nervous system of PD patients. Phospholipase of the PLA2 family are important regulators of glia-mediated neuroinflammation in the central nervous system and are increased in inflammatory bowel disease (IBD). Here we aimed to characterize the expression of the PLA2G2A protein in the human colon and to further investigate its potential involvement in enteric inflammation in PD.

Expression of PLA2G2A was characterized within the colonic intestinal wall in control specimens using immunohistochemistry. Expression of PLA2G2A in response to inflammatory stimuli was assessed in enteric glial cell line in culture, using quantitative PCR (qPCR). Expression of PLA2G2A was further determined in deep colonic biopsies of 12 PD patients and 12 controls using qPCR.

PLA2G2A is expressed in the mucosa, the enteric musculature and in enteric neurons and glial cells within the adult human colon. Expression of PLA2G2A is induced by acute inflammatory stimuli in cultured enteric glial cells, whereas its expression is significantly down-regulated in colonic biopsies of PD patients.

These results confirm that PLA2G2A may be involved in intestinal neuroinflammatory processes in the adult human colon. Surprisingly, whereas PLA2G2A was increased by pro-inflammatory stimuli, its expression was decreased in colonic biopsies of PD patients suggesting that regulation of its expression and the related neuroinflammatory events observed in IBD and PD patients are more complex than previously appreciated. Further work is required to fully decipher the role of PLA2G2A in intestinal inflammatory disorders.

Poster 132:

Titel:
Ischemia-induced neurofilament reduction in different models of experimental stroke

Autoren:
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Abstract:
In the setting of stroke, neurofilaments have been suggested as serum markers reflecting the course and severity of cerebral ischemia, but their role during disease progression still remains poorly understood.

Using three different models of middle cerebral artery occlusion (MCAO) in mice, rats and sheep as well as human autoptic stroke tissue, we addressed ischemia-induced alterations of the four neurofilament subunits: neurofilament light (NF-L), medium (NF-M) and heavy (NF-H) chain and α-internexin (INA). Multiple immunofluorescence labeling and Western blot analyses were used to explore regional characteristics and protein levels.

Immunofluorescence intensities of the neurofilaments subunits were found to decrease after ischemia in the applied animal models and in human stroke tissue, with the exception of NF-L, which shows an increase of fluorescence intensity. These neurofilament alterations also extend to areas of the ischemic penumbra, which is indicated by a concurrent upregulation of the heat shock protein 70 in neurons. Importantly, Western blotting revealed a significant reduction of the NF-L and INA protein level in the ischemia-affected cortex after MCAO in mice. Moreover, the binding of NF-L degradation products was identified as underlying cause for the observed increase of NF-L fluorescence intensity in ischemic areas.

The neurofilament network was found to be highly sensitive to ischemia. Therefore the immunolabeling of NF-L serves as excellent histological marker for ischemic brain tissue. As the described alterations included potentially salvageable tissue of the ischemic penumbra, neurofilaments may turn out as promising targets for neuroprotective strategies.

Poster 133:

Titel:
Downregulation of inhibitory synapses by the CDK5 activator p35; an affected pathway in Alzheimers disease?

Autoren:
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Abstract:
In virtually all inhibitory synapses, the protein gephyrin is the core postsynaptic scaffolding protein. Gephyrin self-assembles into a submembraneous lattice that ‘captures’ glycine and GABAA receptors, thereby allowing for their clustering at postsynaptic sites. Multiple phosphorylation sites on gephyrin have been shown to affect its clustering ability and subsequently the strength of inhibitory transmission. Previously, we have shown that the kinase CDK5 phosphorylates gephyrin and positively regulates its clustering potential. Interestingly, both Gephyrin itself as well as CDK5 and its main activator p35 are downregulated in a mouse model of Alzheimers disease (AD). Here we follow up on our initial experiments by studying the effect of p35 knockdown on inhibitory synapses.

Therefore, we virally co-expressed GFP and a p35-specific siRNA in hippocampal dissociated cultures and monitored the subsequent effect on synaptic clusters of Gephyrin and GABAA receptors within developing hippocampal neurons.

Our siRNA infection led to a significant reduction in p35 levels in infected hippocampal pyramidal neurons. Importantly, p35 knockdown resulted in a strong (> 60%) reduction of both the numbers and the size of synaptic gephyrin clusters within infected neurons. Similarly, also synaptic expression of the GABAAR gamma2 subunit was strongly reduced.

Here, we could show that downregulation of the p35-CDK5 pathway results in a strong downregulation of inhibitory postsynaptic sites. Intriguingly, gephyrin and p35/CDK5 have also been found to be coregulated in symptomatic AD-like mice, suggesting that the reduced gephyrin levels in an AD context could also be caused by a dysregulated p35-CDK5 pathway.

Poster 134:

Titel:
Neuropathic pain on a chip

Autoren:
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Abstract:
Unlike physiologic pain, neuropathic pain does not originate from adequate stimulation of distal nerve endings. The causes include macro- or microscopically lesions resulting from trauma, surgery, chemotherapy or systemic diseases.

The pathologic mechanisms of neuropathic pain are poorly understood. It is assumed that neuropathic pain involves the sensitization of the nervous system. Upregulation of voltage-gated sodium channels in dorsal root ganglia (DRG) fibers leads to increased neuronal activity and a reduced threshold potential, which could cause peripheral sensitization. In contrast, central sensitization might result from increased glutamate release from DRGs into the extracellular matrix. This presumably induces degeneration of GABAergic interneurons in the superficial dorsal horn, which modulate synaptic transmission from mechanoreceptive and nociceptive fibers to second order spinothalamic neurons.

Our aim is to investigate altered signaling between DRG neurons and second order spinothalamic neurons under pathologic conditions. For this purpose, 2-compartment microfluidic silicone devices designed by Xona will be used. In a fist attempt, rat primary cultures of DRGs and the dorsal horn are established separately. Quantitative PCR is performed to detect altered levels of voltage-gated sodium receptors after incubation of DRGs with chemotherapeutics. Second, chemotherapeutic treatment will be compared to axotomy-induced changes.

First immunocytochemistry and qPCR results will be presented and discussed. Furthermore, the preliminary set-up of the 2-compartment microfluidic device for investigating neuropathic pain-related alterations in vitro will be shown.

This approach could improve the understanding of the connection between damaged peripheral nerve fibers and alterations within the spinal cord leading to the manifestation of neuropathic pain.

Poster 135:

Titel:
Structural and functional characterization of neuron populations in glioblastoma multiforme

Autoren:
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Abstract:
Glioblastoma comprise a group of aggressive tumors characterized by cellular heterogeneity and resistance to therapeutic intervention. Recent studies have implied that active neuronal networks within glioblastomas may contribute to the therapeutic resistance and high rate of recrudescence of glioblastoma. Potential mechanisms that support such therapeutic resistance include a trophic factor-secreting environment that exerts significant stability to the tumor and stable neuronal networks within the tumor that provide a permanent feedback loop for growth.

Therefore, we aimed at analyzing functional neuronal networks in primary human tumor tissue from surgery. Using multi-channel immunofluorescence and confocal microscopy, we identified pockets of neurons within the tissue as indicated by NeuN staining.

We then focused on the characterization of functional markers. Here, we were able to identify molecular components of axon initial segments (AIS) in these tumor network neurons. The AIS constitutes the site of action potential generation in neurons and therefore is a key component of functional cells. AIS were located at the proximal axon of NeuN-positive neurons within tumors as indicated by positive immunostaining against the AIS scaffolding proteins ankyrin-G and βIV-spectrin. Further analysis now focuses on the expression of the adequate channel architecture at the AIS, namely voltage-gated ion channels (Na, K and Ca) that cluster at the AIS and are the basis of action potential initiation at the proximal axon.

We conclude that within the tumor environment of primary human glioblastoma, neurons exist within subfields of the tissue and express the adequate cellular machinery for action potential generation.

Poster 136:

Titel:
Proteolytic processing of cell adhesion molecule L1 (L1CAM) in nervous system development and regeneration

Autoren:
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Abstract:
The cell adhesion molecule L1 (L1CAM) does not only „keep cells together“ by homophilic and heterophilic interactions, but L1 can also promote cell motility when cleaved by several proteases into fragments. L1 fragments are generated at the plasma membrane. Some of these fragments are released into the extracellular space, whereas other membrane-bound fragments are internalized via the subcellular traffic circuits and enter the nucleus, thus conveying extracellular signals to the cell interior. The present work aims at studying the effect of L1-proteolysis on morphogenic events during nervous system development and regeneration.

Genome-wide editing via zinc finger nucleases was used to generate mice with a mutation that abolishes cleavage of L1 by proteases targeting L1’s third FNIII-like repeat.

Mice with a mutation that abolishes cleavage of L1 develop congenital ventriculomegaly characterized by improperly anchored ependymal cell cilia and altered distribution of the axonal projections along the nigro-striatal axis. Viral re-introduction of proteolytic L1 fragments into L1 mutants in utero at critical neurodevelopment stages led to partial rescue of the congenital ventriculomegaly due to correction of the the ependymal cell cilia anchorage.

Stimulation of proteolysis of L1 via injection of L1-fragments or proteases active on L1 or L1 mimetics is beneficial for development and regeneration of the diseased nervous system.

Poster 137:

Titel:
PRG3 C-term: a major player in early neuronal differentiation

Autoren:
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Abstract:
Neuronal plasticity and the establishment of neuronal circuitries are essential features of the developing nervous system. Recently, the functional importance of the endogenous neural transmembrane proteins Plasticity-related gene 3 (PRG3) and 5 (PRG5) on neuronal differentiation has been shown. However, although the sequence of PRG3 is highly related to PRG5, both promote different morphological changes in neurons. While PRG3 was shown as a novel molecule promoting neuritogenesis in neuronal and non-neuronal cells, PRG5 promotes formation of filopodia in non-neuronal cell lines and contributes to spine induction in immature neurons as well as to regulation of spine density and morphology in mature neurons. Among the PRG family, PRG3 and PRG5 are the smallest members and exhibit carboxy terminal (C-term) domains which are located intracellularly. Varying C-term domains between both proteins gave rise to the assumption that these domains shape neuronal morphology differently and prompted us to investigate the involvement of the C-term of PRG3 and PRG5 in neuronal differentiation.

To approach the role of PRG3 and PRG5 C-term domains in early stages of neuronal development, we generated mutant constructs in which C-terms are exchanged between PRG3 and PRG5.

We analyzed the influence after overexpression in immature, not yet polarized, hippocampal neurons

Indeed, our results point to a critical role of PRG3 C-term domain in shaping early neuronal morphology.

Poster 138:

Titel:
Inflammasome expression in the hippocampus under normal light conditions and circadian disruption

Autoren:
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Abstract:
Objective: An intrinsic daily physiological rhythm, known as circadian rhythm, has been shown to affect the innate immune system and its related diseases. Neurodegenerative diseases, including Parkinson's and Alzheimer's disease are strongly associated with disruption of the circadian system and activation of the innate immune system. The link between circadian rhythms and neurodegeneration is not yet fully understood, but changes in immune and inflammatory function are proposed mechanisms. However, the effect of time of the day and circadian disruption on inflammasome expression in the hippocampus is not known.

Methods: The daily expression patterns of inflammasome components were investigated in C57BL6 mice maintained under a 12-h light/12-h dark (LD; 12h:12h). Tissues were collected from euthanized mice at Zeitgeber time (ZT) 2, 6, 10, 14, 18 and 22. To study the effect of circadian disruption on inflammasome expression, C57BL6 mice were kept either under LD conditions or constant light (LL; 24h) for two weeks. As a genetic model for circadian disruption, Bmal1 knockout (KO) mice were studied.

Results: Our results show a clear diurnal expression of inflammasome components in the hippocampus. Under LL conditions, increased caspase 1 activation was observed. Furthermore, NLRP3 inflammasome expression was increased in Bmal1 KO mice.

Conclusions: Taken together, our work highlights that circadian disruption is associated with inflammasome activation and may contribute to the development and/or progression of neurodegenerative diseases.

Poster 139:

Titel:
Impaired Function of the Cells Powerhouses – New Insights into ALS Research

Autoren:
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Abstract:
Amyotrophic lateral sclerosis (ALS) is the most common motoneuron disease in adults and the third-leading neurodegenerative disease worldwide. While 5–10% of patients inherit the disease (fALS), up to 95% of patients are diagnosed with the sporadic form (sALS). In the sporadic form, more than 80% of cases remain without a cause being discovered. Pathological processes that point to malfunctions in the cells powerhouses, the mitochondria, are increasingly coming to the fore. A recently published study shows that different functional parameters of mitochondria are altered in ALS patients. In this line, the aim of this project was to study potential morphological alterations of the mitochondrial network in detail and the molecular causes of abnormal mitochondrial function.

Here we used the wobbler mouse as an animal model for sporadic ALS, since the homozygous mice show typical hallmarks of this disease.

Various imaging techniques, such as confocal light microscopy, conventional electron microscopy and 3-dimensional electron microscopy (TEM tomography), were used to study mitochondrial morphological parameters. The imaging results were further supplemented by molecular biological methods to characterize the expression of certain genes.

The imaging results show an altered mitochondrial network structure. Modified expression of fission and fusion relevant genes provide an explanation for the altered morphological parameters.

Many important molecules responsible for mitochondrial function are located within the inner mitochondrial membrane (IMM). Since we could show that the IMM is disturbed in motoneurons of wobbler mice, it is obvious that the physiological processes cannot function properly and lead to a pathological phenotype.

Poster 140:

Titel:
High speed ventral plane videography is more sensitive to detect motor-deficits in experimental autoimmune encephalomyelitis (EAE) compared to conventional evaluation protocols

Autoren:
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Abstract:
Experimental autoimmune encephalomyelitis (EAE) is the most commonly used multiple sclerosis animal model. EAE mice typically develop motor symptoms in a caudal-to-rostral pattern. Subjective evaluation of such motor symptoms is the most frequently used method to assess treatment efficacy. Because of the subjective nature of this method, objective and reliable approaches are urgently needed. Here, we investigate whether gait analyses are superior to currently applied EAE evaluation protocols.

EAE was induced in 18 female mice by MOG35-55 peptide immunization. DigiGait™ (Mouse Specifics), consisting of a treadmill with a digital camera below a transparent belt, was used to record the gait pattern. Gait parameters were recorded daily from day 6 post-immunization. In parallel, mice were scored following a conventional grading protocol. Finally, brains and spinal cords were harvested and analyzed by (immune-) histochemistry.

Spinal cord brain motor-function areas showed inflammatory demyelination in EAE but not control mice. 10 out of 10 control mice (100%), and 14 out of 18 (77.8%) EAE mice could be evaluated using DigiGait™. EAE severity was not influenced by DigiGait™. Most gait parameters recorded from day 6 till the end of the experiment were found to be stable in control mice. During the subclinical disease stage, when conventional EAE score failed to detect any functional impairment, EAE mice showed increased hind limbs paw angle compared to control mice.

DigiGait™ is more sensitive than conventional scoring approaches to study motor deficits in EAE subclinical stage.

Poster 141:

Titel:
Defects in glutathione metabolism contribute to sporadic Amyotrophic Lateral Sclerosis

Autoren:
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Abstract:
Amyotrophic lateral sclerosis (ALS) is a progredient neurodegenerative disease, which is characterized by a degeneration of the first and second motor neuron. 90-95% of ALS patients suffer from sporadic ALS. Elevated levels of reactive oxygen species (ROS) have been reported in motor neurons of ALS patients and animal models, leading to cell death. The tripeptide glutathione (GSH) is part of an important defense mechanism against ROS. It is a cofactor of antioxidative enzymes and essential for remaining reducing conditions in the cell. The liver primarily determines plasma glutathione levels, which have a major influence on glutathione levels in the CNS. Decreased levels of glutathione in the CNS of ALS patients and animal models have been reported before. Thus, glutathione might play a role in the pathogenesis of ALS.

The wobbler mouse is used as an animal model for sporadic ALS. We investigate changes in glutathione metabolism of the CNS, liver and plasma of wobbler mice, using qPCR, Western Blot, immunolabeling and cell based assays.

Here we show for the first time an organ specific dysregulation of enzymes involved in glutathione synthesis and metabolism in wobbler mice.

Our study indicates that defects in glutathione metabolism might play a role in the pathogenesis of sporadic ALS. Thus, a therapeutic approach with antioxidant drugs, such as Edaravone, would be very useful.

Poster 142:

Titel:
The Role of Iron Metabolism and Ferroptosis in Amyotrophic Lateral Sclerosis

Autoren:
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Abstract:
Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease with yet unknown etiology. Possible mechanisms behind the loss of motor neurons in ALS have been described and involve the accumulation of iron in the central nervous system (CNS). Ferroptosis, a recently defined form of regulated cell death, requires iron and oxidative stress and has been observed in different neurodegenerative diseases. Since non-apoptotic forms of cell death have been described in motor neurons in ALS patients, the investigation of iron metabolism is a new option to verify whether the conditions for ferroptotic cell death are given.

The wobbler mouse, a rodent model showing strong symptomatic similarities to patients with the sporadic form of ALS, was used for the present investigations. To examine the presence of iron in the CNS as well as in liver and spleen, paraffin sections were used to visualize iron histochemically. To examine the genes and proteins involved in iron metabolism, quantitative Polymerase Chain Reaction and Western Blot were used.

Certain genes responsible for iron metabolism are deregulated which correlates with altered amounts of iron in different regions of the examined tissues.

The observed dysregulations in iron metabolism give a strong hint that ferroptosis is a present form of cell death in ALS. These findings may serve as a guidance to develop new treatments for ALS patients.

Poster 143:

Titel:
Role of aging in the development of vascular bagging and white matter lesions in the human brain

Autoren:
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Abstract:
Cerebral microangiopathies, also called small vessel disease (SVD), often lead to white matter lesions (WML) in the cerebral deep white matter and at periventricular sites. WML are associated with an enhanced risk of dementia, poor executive function, gait abnormalities and stroke with high mortality. Recently, we discovered vascular bags around microvessels that were filled with plasma proteins in individuals suffering from SVD with WML. The goal of the present study was to investigate the role of aging in the development of vascular bagging.

Vascular bags were studied in thick hemisphere sections by using double-label immunohistochemistry for the simultaneous visualization of collagen IV (COLL4)-positive membranous vascular bags and the endothelial glycocalyx.

Results showed that vascular bagging is a common finding in the white matter of a non-selected aged autopsy cohort. However, vascular bagging was less severe in the white matter of these aged individuals than in the WML or normal-appearing white matter of SVD cases. Quantitative analyses of vascular bagging in the frontoparietal white matter indicated a correlation between age and the degree of vascular bagging. Vascular bagging in SVD was accompanied by an increase in the density of hypertrophic IBA1-positive microglial cells and CD68-positive macrophages.

In conclusion, our findings support the view that vascular bagging increases with age. Moreover, severe forms of vascular bagging were associated with microglial activation and the manifestation of WML. Therefore, the chronic progression of vascular bagging seems to precede the formation of WML and might be a driver of SVD.

Poster 144:

Titel:
Altered miRNAs and their target genes in an ALS mice model

Autoren:
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Abstract:
MicroRNAs (miRNAs) are short, non-coding sequences with the ability to silence gene expression by complementary sequence binding which results in a great complexity of posttranscriptional regulation. miRNAs gained attention in amyotrophic lateral sclerosis (ALS) research, since ALS patients show altered miRNA expression profiles in affected tissues and blood serum. We were interested whether curtain miRNAs and their target gene(s) are affected in the M. gastrocnemius and lumbar spinal cord (lSC) of the SOD1(G93A) ALS mouse model.

To study miRNA expression profiles and their target genes, we combined a GeneChip and quantitative real-time PCR analysis at distinct time intervals (6-18 weeks). Additionally, the Western Blot approach was used for selected target gene evaluation.

We could show that miR-541-5p, -146a-5p, -193-3p and -181d-5p are altered in an age-dependent manner in wild type mice. In transgenic mice, we observed significant changes in the profiles of the latter three miRNAs in muscle tissue and lSC. In addition, target genes were either down-regulated, Traf6 (inflammatory signaling), or induced, such as Runx1 (differentiation or development of stem cells and neurons) and the chemokine CCL8, in muscle and lSC tissue.

In summary, we identified new players in ALS-affected muscle and spinal cord tissue which were not described in the context of ALS in the literature before. Future research has to pinpoint the particular roles of theses miRNAs and their targets in ALS development and disease progression.

Poster 145:

Titel:
In particular the entorhinal cortex layer III of human brain hippocampus showed reduced numerical abundance of peroxisomes with ongoing stages of Alzheimer´s disease

Autoren:
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Abstract:
Peroxisomal biogenesis disorders are inherited metabolic diseases with mutations in the PEX genes. In its most severe form, the Zellweger syndrome, patients have profound defects in the brain demonstrating the importance of this organelle for neuronal survival and function. Additionally, disturbed peroxisomal metabolism seems to be linked to Alzheimer disease (AD). Best known is the plasmalogen deficiency and increases of very long-chained fatty acids, which occur early in the disease course correspond well with cognitive dysfunction and which are associated more specific for AD than for age-related neurodegeneration.

To study the role of peroxisomes in AD, we analyzed their number and distribution in 15 different brain regions of autopsy material from patients grouped into 5 gender-matched groups based to the ABC-Score with different probabilities for AD dependent on the progress of ß-amyloid plaques and neurofibrillary tangles (NFTs). Double immunofluorescence was performed detecting either PEX14 to analyze the number of peroxisomes or ß-amlyoid plaques/NFTs in brain tissues.

First experiments (10 patients in total) showed a reduced number of peroxisomes with ongoing stages of AD especially in hippocampal areas. Thus, we extended the study by a total of 42 patients. In the entorhinal cortex layer III we found a negative correlation between the peroxisome number and AD stage, for the subiculum and CA3 the number varied with a first increase during stages II-III and a decrease at stage IV, whereas there were no changes in the gyrus dentatus.

Our data enhance the association between peroxisomes and AD progress.

Poster 146:

Titel:
Effect of astrocyte-specific hyperactivity of Nrf2-signaling on neuroinflammation after spinal cord injury

Autoren:
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Abstract:
Oxidative stress (OS) critically contributes to the pathogenesis of a variety of traumatic diseases including spinal cord injury (SCI). Astrocytes are essential regulators of oxidative homeostasis in the CNS. Dysregulation of astrocyte physiology contributes largely to oxidative damage. Nuclear factor erythroid 2-related factor 2 (Nrf2) is the main transcriptional regulator of cellular anti-oxidative stress responses.In this study, we study the effects of astrocytic Nrf2-activation on spinal cord inflammation and locomotor improvement after SCI.

Cells deficient for the Nrf2 repressor kelch-like ECH-associated protein 1 (Keap1) are characterized by a hyperactivity of Nrf2-signaling. To investigate the temporal and quantitative pattern of Nrf2/ARE-activation, we included transgenic ARE-Luc mice. Wild type-, Nrf2-deficent-, Luc-ARE-mice and mice with a GFAP-specific Keap1-deletion were subjected to SCI.

In ARE-Luc mice, a significant induction of luciferase-activity was observed as early as 2 days post injury, indicating a functional role of Nrf2-activity at the epicenter of SCI. Further, SCI-induced pronounced neuron and oligodendrocyte loss, demyelination and reactive gliosis were seen in wild type animals. In contrast, astrocyte-specific Nrf2-activation was sufficient to prevent neuronal loss, gliosis, improve locomotor function and ameliorate neuroinflammation by reducing the amount of pro-inflammatory chemokines such as IL-1b.

Our results highlight the potential of the Nrf2/ARE system for the treatment of neuroinflammation after SCI.

Poster 147:

Titel:
Differential susceptibility of hippocampal neurons in slice cultures to heat shock

Autoren:
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Abstract:
Hippocampal slice cultures are a well established in vitro model to study development, function and plasticity of hippocampal neurons during health and disease in a tissue context that is similar to the in vivo situation. Here, we used hippocampal slice cultures to study the susceptibility of different hippocampal neuronal cell types to heat shock.

To simulate fever in vitro, the incubation temperature for the slice cultures was increased to 41 °C for a period of several hours. Afterwards, the slice cultures were again incubated at 37°C for up to several days, stained with propidium iodide (PI) to evaluate cell viability, and subsequently fixed with paraformaldehyde. The slice cultures were then immunohistochemically stained with antibodies against cell type specific marker proteins, including Prox-1, a marker protein for dentate granule cells, and Reelin, which is expressed by Cajal-Retzius cells. Cell type specific damage was quantified by assessing the ratio of PI-positive and PI-negative cells.

Our results indicate that in particular principal neurons, i.e. dentate granule cells and pyramidal neurons, are susceptible to damage by heat shock, while Cajal-Retzius cells are surprisingly resistant and appear to be largely unaffected by the heat shock.

In future experiments we want to take advantage of our in vitro model to explore molecular mechanisms that underlie the differential susceptibility of different hippocampal neuronal cell types to heat shock. In particular we are interested in the mechanisms that underlie efficient protection against heat shock induced damage of specific neuronal cell types, such as Cajal-Retzius cells.

Poster 148:

Titel:
Microglial response in hippocampal slice cultures after heat shock

Autoren:
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Abstract:
Organotypic slice cultures of the hippocampal region are a well established in vitro model to study hippocampal neurons and glial cells in a tissue context that is similar to the in vivo context. Here, we used hippocampal slice cultures to study the response of microglial cells after heat shock.

To simulate a fever situation in vitro, the incubation temperature for the slice cultures was increased to 41 °C for a period of several hours. Nextt, the slice cultures were again incubated at 37°C for up to several days, stained in some cases with propidium iodide (PI) to evaluate cell viability, and subsequently fixed with paraformaldehyde. The slice cultures were then immunohistochemically stained with antibodies directed against the microglial marker protein Iba1 and with antibodies against neuronal marker proteins, in particular to identify dentate granule cells, and with an antibody against Reelin to identify Cajal-Retzius cells.

Our results indicate that the microglial cells respond to heat shock in a subregion-specific manner. Thus, the dentate granule cell layer, a region where numerous neurons were damaged by the heat shock, was invaded by microglial cells, while the adjacent dentate molecular layer containing Cajal-Retzius cells, which prooved to be remarkably heat shock resistant, was only sparsely invaded by migrating microglial cells.

Our findings suggest the that the microglial response to heat shock in hippocampal slice cultures is region- and layer-specific. In future experiments we want to take advantage of the hippocampal slice culture model to explore how the heat shock induced microglial response may be manipulated to promote neuronal survival and to reduce damage of surviving neurons.

Poster 149:

Titel:
Morphometric and transcriptomic analyses of a genetic mouse model (VPP) mimicking autosomal dominant retinopathia pigmentosa

Autoren:
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Abstract:
Hereditary retinal degenerations like retinopathia pigmentosa (RP) are amongst the leading causes of blindness in younger patients. To allow for testing of therapeutic strategies that could prevent retinal degeneration and to enable for in vivo investigation of cellular and molecular mechanisms responsible for photoreceptor cell death, animal models like e.g. the VPP mouse model have been created. Here we examined the degree of photoreceptor degeneration in the VPP model and analyzed its impact on the transcriptome level of the retina.

TdT-mediated dUTP-biotin nick end labeling (TUNEL) labeling was used to validate the number of apoptotic photoreceptors and morphometric analyses of the thickness of the outer nuclear layer (ONL) were performed to assess photoreceptor degeneration. Furthermore, immunohistochemistry and RNASeq analyses followed by weighted correlation network analysis (WGCNA) network analyses of retinal samples were performed from retinae of VPP and wildtype mice.

One-month old VPP mice showed a significantly higher number of apoptotic photoreceptor cells that resulted in a significantly thinner ONL in three-month old VPP mice compared to wildtype littermates. Following RNAseq analysis, we analyzed our data for dysregulated genes and performed WGCNA analysis to identify upstream regulators and involved pathways. We identified 9256 dysregulated genes and 5 significantly associated modules. Gene ontology enrichment showed amongst others involvement of apoptosis, photoreceptor loss and (micro)glia activation.

The VPP model is an appropriate genetic model mimicking RP. The retinal degeneration results in a massive dysregulation of the retinal transcriptome thus highlighting the molecular complexity of RP.

Poster 150:

Titel:
Mental retardation in PKU: The Link between Catecholamines and Microglia

Autoren:
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Abstract:
In Phenylketonuria (PKU), synaptic pruning is heavily disturbed and likely to be involved in mental retardation. Microglia activity, known to be essential for synaptic pruning, was consistently reduced in a PKU mouse model. Previous in vitro approaches questioned the role of elevated levels of phenylalanine (Phe) in PKU. Due to the inability to metabolize Phe and the presence of dopamine receptors on microglia, the role of reduced dopamine levels in PKU is in the focus of this study.

Long-term potentiation (LTP), paired-pulse facilitation (PPF) and synaptic density in electron micrographs were used as parameters of synaptic plasticity. In-vitro approaches included dissociated neuron and microglia cultures, as well as neuron-microglia co-cultures. Protein analyses were done by immunohistochemistry, ELISA and western blot.

In 12 weeks old Pahenu2 mice, a model of PKU, LTP and PPF were impaired in CA3-CA1 Schaffer collaterals as compared to controls. The activity of microglia was reduced, whereas the number of spines synapses and the expression of postsynaptic markers was significantly increased in these mice. Neuron-microglia interaction is mainly achieved by complement component 3 (C3). Remarkably, measurable amounts of C3 were solely found in co-culture systems. Elevated levels of phenylalanine, a hallmark of phenylketonuria, did not have any effect. Rescue experiments with dopamine, however, which is considerable reduced in the brain of Pahenu2 mice, resulted in a significant reduction of C3 secretion.

A perturbed catecholamine biosynthesis leads to disturbed synaptic pruning and reduced microglia activity. Dopamine is a highly promising factor that could mediate these impairments.

Poster 151:

Titel:
Interactions of an antiserum to Helicobacter pylori with synaptotagmin 5 correlates to reduced acetyl-choline dependent calcium responses and impaired vesicle recycling in SiMa human neuroblastoma cells

Autoren:
Aaron Kleine (Institute for Neuroanatomy, University Medical Center Göttingen, Göttingen), Abdul Rahman Asif (Institute for Clinical Chemistry, University Medical Center Göttingen, Göttingen), Bernhard Reuss (Institute for Neuroanatomy, University Medical Center Göttingen, Göttingen); breuss@gwdg.de

Abstract:
Early childhood infections with the gastric bacterium Helicobacter pylori (H. pylori) are assumed to be associated with an increased lifetime Schizophrenia risk.*

We hypothesize an immune mediated mechanism for this, and demonstrate now that an antiserum to H. pylori (α-HPy) on a human first trimester prenatal brain multiprotein array (HexSelect, Engine, Berlin, Germany), interacts with a set of 104 different proteins, including the intracellular calcium sensor synaptotagmin 5 (SYT5).

Interaction of the latter protein with α-HPy was confirmed independently by Western blotting, using SYT5 transiently overexpressed in HEK293 cells. We further demonstrate expression of SYT5 in the human neuroblastoma cell line SiMa, an established in vitro model for neuronal differentiation by Western blotting. On the functional level a 24h preincubation of this cell line with 10µg/ml α-HPy resulted in a significant reduction of acetyl-choline dependent intracellular calcium transients, and of vesicle recycling as revealed by the fluorescent dye FM1-43.

These results demonstrate for the first time immunological crossreactivity and also functional interference of α-HPy with the synaptic calcium sensor SYT5 due to molecular mimicry. Although highly speculative, regarding previous reports on altered expression of SYT5 in the thalamus of schizophrenic patients,** the present results could also be of importance for a better understanding of at least some of the changes in synapse functioning found in the brains of schizophrenic patients. * Yilmaz et al., 2008, Med Sci Monit 14:HY13-6; **Martins-de-Souza et al., 2010, J Psychiatr Res 44:1176-89.

Poster 152:

Titel:
Deletion of Ceacam1 promotes the accumulation of phagocytic active cells in the subretinal space

Autoren:
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Abstract:
Carcinoembryonic antigen-related cell adhesion molecule 1 (Ceacam1) is expressed in vascular endothelial cells during vascular development and in adult blood vessels that are activated by angiogenic proceses. To learn about its function in the eye, we analyzed its expression and studied the retinal and choroidal morphology of Ceacam1 knockout (Cc1-/-) mice.

Expression of Ceacam1 and members of the transforming growth factor (TGF) -β signaling pathway were analyzed by real time RT-PCR in retinal and choroidal tissue of wildtype and Cc1-/- mice at different postnatal time points. Ocular morphology of Cc1-/- mice was studied by light and electron microscopy and immunohistochemistry.

Ceacam1 is expressed in retinal and choroidal endothelial cells during development and in adulthood. Real time RT-PCR and immunohistochemistry confirmed the successful deletion of Ceacam1 in Cc1-/- mice. Cc1-/- mice showed a regular layering of the retina and no obvious morphological alterations of the choroid. Morphometric measurements of the thickness of the inner and outer nuclear layer did not show significant differences between Cc1-/- mice and controls. However, 3 and 9 months old Cc1-/- mice demonstrated a distinct accumulation of small cysts, partly surrounded by phagocytic active cells, in the subretinal space concomitant with significantly elevated retinal Tgfβ1 mRNA expression levels, compared to controls.

Our data revealed that Ceacam1 is expressed in developing and mature retinal and choroidal endothelial cells. Furthermore, its deletion promotes the accumulation of phagocytic active cells in the subretinal space, indicating that Ceacam1 contributes to the integrity of the outer blood retinal barrier.

Poster 153:

Titel:
Identification of key molecules involved in the development of B cell aggregates in the central nervous system in a mouse model of Multiple Sclerosis

Autoren:
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Abstract:
Multiple sclerosis (MS) is a neuroinflammatory disease of the central nervous system (CNS). The role of B cells as a pathogenic factor in MS has become increasingly important. In patients with secondary progressive MS, the occurrence of B-cell follicles in the meninges is associated with a higher disease severity. However, the formation of these follicles is poorly understood and requires further investigation. To gain a deeper understanding of this process, we use a B cell-dependent mouse model of MS, the MP4-induced experimental autoimmune encephalomyelitis (EAE). The model is characterized by B-cell aggregate formation during the course of the disease. We compared different stages of aggregate formation in the cerebellum of MP4-immunized mice to identify key molecules in the development of lymphoid structures.

We used Laser Capture Microdissection and RNA sequencing to identify relevant molecules that might be involved in aggregate formation.

The results of RNA sequencing showed higher expression of various genes compared to relevant controls, one of which is osteopontin (OPN). OPN is a secreted phosphoprotein expressed by several different cell types involved in modulating both innate and adaptive immune responses. Immunohistochemistry confirmed the presence of OPN in the CNS of our mouse model and revealed OPN expression by microglia, B cells and neurons.

OPN could be crucial for the development of B-cell aggregates in the CNS. Further research is needed to determine what exact role OPN plays in this process.

Poster 154:

Titel:
Intravertebral extradural end-to-side anastomoses between upper (T7-T9) and lower (L2, L4, S1) ventral spinal roots with inter-positional nerve grafts (IPNG): technical feasibility of a novel putative surgical treatment after spinal cord injury (SCI)

Autoren:
Svenja Rink (Department of Prosthetic Dentistry, School of Dental and Oral Medicine, University of Cologne, Cologne, Germany, University of Cologne, Cologne), Habib Bendella (Department of Neurosurgery, University of Witten/Herdecke, Cologne Merheim Medical Center (CMMC), Cologne), Aliona Wöhler (, Bundeswehrzentralkrankenhaus, Koblenz), Janna Feiser (, Praxis "Med 360 Grad", Remscheid), Andre Wilden (, Bundeswehrzentralkrankenhaus, Koblenz), Maria Grosheva (Department of Otorhinolaryngology, University of Cologne, Cologne), Hans-Jürgen Stoffels (Department of Anatomy, University of Cologne, Cologne), Maria Eleni Manthou (Department of Histology and Embryology, Aristoteles University, Thessaloniki), Doychin Angelov (Department of Anatomy 1, University of Cologne, Cologne); angelov.anatomie@uni-koeln.de

Abstract:
Severe spinal cord injuries cause permanent neurological deficits and are still considered as inaccessible to efficient therapy. Injured spinal cord axons are unable to spontaneously regenerate. Re-establishing functional activity especially in the lower limbs by reinnervation of the caudal infra-lesional territories might represent an effective therapeutic strategy. Numerous surgical neurotizations have been developed to bridge the spinal cord lesion site and connect the intact supra-lesional portions of the spinal cord to peripheral nerves (spinal nerves, intercostal nerves) and muscles. The major disadvantage of these techniques is the increased hypersensitivity, spasticity and pathologic pain in the spinal cord injured patients, which occur due to the vigorous sprouting of injured afferent sensory fibers after reconstructive surgery.

Using micro-surgical instruments and an operation microscope we performed detailed anatomical preparation of the vertebral canal and its content in five human cadavers.

Our observations allow us to put forward the possibility to develop a precisely targeted surgical approach, the so called "ventral root bypass" that avoids lesion of the dorsal roots and eliminates sensitivity complications.

The proposed kind of neurotization has been neither used, nor put forward. The general opinion is that radix ventralis and radix dorsalis unite to form the spinal nerve inside the dural sac. This assumption is not accurate, because both radices leave the dural sac separately. This neglected anatomical feature allows a reliable intravertebral exposure of the dura-mater ensheathed ventral roots and their damage-preventing end-to-side neurorrhaphy by interpositional nerve grafts.

Poster 155:

Titel:
The molecular mechanism behind the neuroprotection properties of nimodipine

Autoren:
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Abstract:
Multiple sclerosis (MS) is one of the most common neurological diseases in young adults. The disease is characterized by inflammation in the central nervous system (CNS) as well as demyelination followed by loss of oligodendrocytes and axons and neurodegeneration. MS generates high economical costs directly through medications and indirectly through early retirement. The existing drugs focus on immune modulation and suppression. There are currently no drugs that primarily facilitate neuroregeneration and prevent neurodegeneration in the CNS. Such drugs, however, would be needed to prevent MS progression over time. We have previously shown that the L-type calcium channel antagonist nimodipine triggers remyelination. The general question now is what molecular mechanisms, especially on the level of the oligodendrocytes, are altered trough nimodipine.

To investigate the molecular mechanisms of the mode of action of nimodipine, a myelinating co-culture system generated from primary mouse cells will be used, which allows to study the myelinating properties of nimodipine in vitro. Subsequently, RNA-sequencing will be performed to investigate gene alterations after nimodipine treatment. Knock-down and overexpression studies will then be performed to confirm the results.

Our preliminary data suggest that nimodipine alters myelination and enhances the expression of the myelin protein O4. We were also able to show that the calcium channels CaV1.2 and CaV1.3 are expressed in oligodendrocytes. Since these are the typical binding partner of nimodipine are expressed the main mode of action may be through the conventional L-type channel.

Overall, nimodipine may have the potential to become the first neuroprotective drug for MS treatment.

Poster 156:

Titel:
Interactions between adipose tissue derived mesenchymal stem cells (AdMSCs) and primary cell cultures of the substantia gelatinosa (SG) during co-cultivation

Autoren:
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Abstract:
Due to its wide dynamic range nociceptive-specific neurons, the substantia gelatinosa (SG) plays an important role in pain processing. In the case of neuropathic pain some structural changes were found in the SG. Chronic neuropathic pain is still refractory to common analgesics. Concerning to this, we investigated the interactions between AdMSCs and primary cell cultures of the SG during Co-cultivation under LPS-stimulation.

Rat AdMSCs and SG cells were isolated and cultured according to standard protocols. The SG cells were seeded on poly-L-lysin coated coverslips in 24-well plates together with AdMSCs, which were cultured on Sarstedt TC-inserts. After 24h of co-cultivation the LPS stimulation was implemented for 2h. Subsequently supernatants and cells were collected for implementing PCR, immunocytochemistry, MTT assay and bioassays for TNFα and IL-6.

In the MTT assay there was no effect of LPS-stimulation on AdMSCs detected. Either the co-cultivation showed no effect on neuronal and astroglial growth of SG cells as well as on the MTT assay of AdMSCS. However, there was an effect on the inflammatory response found. NFκB staining intensity in the nuclei of microglial cells caused by LPS stimulation was significantly reduced, when SG cells were cultured together with AdMSCs. Also TNFα concentration was decreased while co-cultivation.

The co-cultivation of SG cells and AdMSCs showed some hints for anti-inflammatory impacts of AdMSCs on SG cells. There were no obvious effects of SG cells on AdMSCs found. But it should be further investigated, whether SG cells enhance the potential for differentiation of AdMSCs towards the glial direction.

Poster 157:

Titel:
Influence of Empagliflozin on primary microglia in vitro

Autoren:
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Abstract:
Microglia can lead to neuroinflammation and neuronal damage, which gives them an important role in neurodegenerative diseases. For that reason possible drugs to modify neuroinflammatory processes could be a therapeutic option.

Empagliflozin is a SGLT2-inhibitor which showed a suprisingly good cardiovascular outcome that could not be explained only by the glucose-lowering action of the drug. Therefore direct cellular effects with an antiinflammatory mechanism of action can be suspected. This study examines the inflammatory response of LPS (5ng/ml)-activated rat microglia to Empagliflozin (0,5 and 50µM) in vitro.

MTT-proliferation-tests were performed with different concentrations to investigate a potential cytotoxicity of Empagliflozin on microglia. Nitric oxide (NO) as a proinflammatory component was measured photometrically (Griess reagent). Modulation of the inflammatory response of LPS-activated microglia was analyzed by quantification of mRNA-synthesis of IL-6, IL-1β, TNF-α and iNOS (qPCR). Western blots of ERK1/2 and NFκB-immunfluorescence were performed to detect potential influence of signaling pathways by Empagliflozin.

Empagliflozin showed no cytotoxicity on microglia but supports proliferation. NO-synthesis was slightly downregulated by Empagliflozin. qPCR-analysis showed a time- and concentration-dependent reduction of IL-6, IL-1beta, TNF-alpha and iNOS. Western blot- and immunfluorescence-analysis indicated an interaction with ERK1/2 as well as with NFκB in high concentrations (50 µM).

The results indicate that LPS-activated microglia decrease synthesis of proinflammatory mediators in the presence of Empagliflozin. However, this effect was clearly visible only after application of high concentrations. Therefore further investigations are needed to show if Empagliflozin might be a useful drug in the therapy of neurodegenerative diseases.

Poster 158:

Titel:
Are any lymphatics vessels in normal human placenta – podoplanin,LYVE-1 and VGFR3 immunohistochemistry expression – the preliminary study.

Autoren:
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Abstract:
The presence of lymphatic system in the placenta is a topic of interest for the scientific world. Scientific data indicate that, similarly to the vascular system, there should be very dynamic changes in the lymphatic system during pregnancy. The available studies, however, are very different and therefore the aim of the study is a preliminary evaluation of the microscopic anatomy of the human placenta using antibodies against podoplanin, LYVE-1 and VGFR3.

The study was carried out on 42 paraffin fragments taken from 14 mature human placenta of single pregnant women without significant pathology terminated by Caesarean section (38-40 weeks of pregnancy) with anatomical indications. The mean age of mothers was 31.24 years and the mean weight of newborns was 3634 g. Sections were taken from several placenta locations in accordance with the standard. Immunohistochemical reactions were performed using antibodies against D2-40, VGFR3 and LYVE-1 antigens. The immunohistochemical method was used to locate positive cells for selected antibodies and to assess the anatomical localization of cells expressing selected proteins.

Intensive expression of D2-40 was found in all types of placental villi in the stroma tissues. VGFR-3 expression was differentiated and no LYVE-1 expression was observed.

The achieved results do not confirm the presence of lymphatic vessels in the tissues of the placenta. It is necessary to continue research based on other scientific techniques

Poster 159:

Titel:
Improvement of assisted reproductive technology by combining classical histology and elaborate cell culture systems

Autoren:
Volker U. Buck (Institute of Molecular and Cellular Anatomy, Uniklinik RWTH Aachen, Aachen), Matthias Kohlen (Institute of Molecular and Cellular Anatomy, Uniklinik RWTH Aachen, Aachen), Florian Streuter (Institute of Molecular and Cellular Anatomy, Uniklinik RWTH Aachen, Aachen), Cendra Zajber (Institute of Molecular and Cellular Anatomy, Uniklinik RWTH Aachen, Aachen), Benjamin Rösing (Clinic for Gynaecological Endocrinology and Reproductive Medicine, Uniklinik RWTH Aachen, Aachen), Fariba Biazar (Clinic for Gynaecological Endocrinology and Reproductive Medicine, Uniklinik RWTH Aachen, Aachen), Bartlomiej Berger (Clinic for Gynaecological Endocrinology and Reproductive Medicine, Uniklinik RWTH Aachen, Aachen), Joseph Neulen (Clinic for Gynaecological Endocrinology and Reproductive Medicine, Uniklinik RWTH Aachen, Aachen), Rudolf E. Leube (Institute of Molecular and Cellular Anatomy, Uniklinik RWTH Aachen, Aachen), Irmgard Classen-Linke (Institute of Molecular and Cellular Anatomy, Uniklinik RWTH Aachen, Aachen); vbuck@ukaachen.de

Abstract:
Receptivity of the human endometrium for embryo implantation is restricted to a short phase during the menstrual cycle called window of implantation (WOI). Aim of this study was to individually determine the WOI of patients undergoing assisted reproductive technology (ART) by combining morphological assessment and functional in vitro tests.

Tissue samples were obtained from ART-patients who underwent a defined regime of hormonal treatment. To determine the WOI histologically the embedded paraffin samples were dated according to Noyes' criteria and by immunohistochemistry (IHC) against the proliferation marker Ki67 and progesterone receptor (PR). Functional tests on primary endometrial epithelial (EEC) and stromal cells (ESC) were established in order to mimic the hormonal treatment of ART in vitro.

In coculture experiments on transwell inserts paracrine effects of hormonally treated primary ESC on primary EEC could be shown by IHC against Ki67. For functional implantation studies 3D-cocultures of primary EEC and the invasive trophoblast cell line AC-1M88 were established in the extracellular matrix Matrigel.

By means of light sheet microscopy, we could observe and test individually the invasive interaction of the established AC-1M88 Dsc2a::mCherry reporter cell line with cell tracker green labeled primary EEC.

Conclusion: A combination of classical dating and functional in vitro assays was established in order to individually assess the receptivity of ART-samples. By performing hormonal substitution of primary cocultures we will optimize hormonal treatment of ART-patients in the future.

Poster 160:

Titel:
PARL deficiency interferes with gonadal function and pubertal maturation of the genital tracts in both female and male mice

Autoren:
Sarah Schumacher (Anatomisches Institut, Universitätsklinikum Bonn, Bonn), Jessica Lambertz (Anatomisches Institut, Universitätsklinikum Bonn, Bonn), Angelika Zoons (Anatomisches Institut, Universitätsklinikum Bonn, Bonn), Birgit Rau (Anatomisches Institut, Universitätsklinikum Bonn, Bonn), Dieter Hartmann (Anatomisches Institut, Universitätsklinikum Bonn, Bonn); sarah51@uni-bonn.de

Abstract:
The rhomboid protease PARL is part of a proteolytic machinery that controls mitochondrial crista neck topology but is also involved in mitochondrial transmembrane signalling and turnover e.g. via the pink1/parkin pathway. PARL deficient mice develop a multisystem atrophy involving mainly skeletal muscle and lymphatic organs as well as sterility in both sexes. Males show normal prepubertal development of external genitalia but an incomplete descensus testis terminating at the inner inguinal ring and a maturation arrest of spermatogenesis occurring during prophase I of meiosis. In females, uterine development does not progress beyond prepubertal morphology. Strikingly, the ovaries start follicular maturation up to early tertiary stages. This phenotype indicates a partial dysregulation of sex steroid hormones mainly affecting organ function while being largely compatible with most of their hormone – dependent development.

Here we investigated the influence of PARL on proteins involved in the biosynthesis of steroid hormones by qualitatively comparing the expression and processing of key proteins within the steroid pathway in testis, ovaries and adrenal glands of PARL wildtype and knockout mice using morphological and biochemical analysis (the authors are indebted to Prof Bart De Strooper, KU Leuven for providing PARL breeding mice).

Our data reveal significant changes in the expression level and distribution of proteins involved in (mitochondrial) steroid transport and synthesis mainly occurring around and shortly after puberty.

These findings can be interpreted as an additional effect of the role of PARL in the organization of the mitochondrial (inner) membrane now also affecting cholesterol and steroid metabolism.

Poster 161:

Titel:
Novel Sex-typing Strategies in Humans and Horses based on the NLGN4X/NLGN4Y gene pair

Autoren:
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Abstract:
Sex determination, “Sex-typing”, of DNA samples is a fundamental challenge in forensic DNA analysis but also when it comes to selective livestock breeding. PCR detection of the SRY gene (sex-determing region on Y) remains without an internal control to judge DNA quality and PCR performance of presumptive female DNA samples. The detection of AMELX and AMELY, sex chromosome specific copies of the ancestral amelogenin gene has been the gold standard within the last 20 years. We hypothesize that sex chromosome specific copies of genes encoding the neuronal cell adhesion molecule neuroligin-4, NLGN4X and NLGN4Y, should similarly serve as an alternative to AMELX/Y detection.

We have developed a simple PCR protocol for human and horse NLGN4X/Y genes, respectively, based on a length polymorphism and screened a total of 111 human and 29 horse samples. Sex determination was further complemented for human samples using rhAMP-genotyping strategies based on a SNP on exon 7 between both neuroligin-4 genes.

The sex of all human and horse samples were correctly identified using the employed detection methods, thus, enabling us with a simple protocol to infer the sex of unspecified DNA samples based on PCR screening. Furthermore, this is the first report on the application of rhAMP-genotyping as means for sex determination of human DNA samples.

The gametologuous genes NLGN4X and NLGN4Y emerge as a suitable and, therefore, alternative gene pair to infer sex based upon PCR analysis in horses and humans.

Poster 162:

Titel:
The expression of Hand2 in human endometrial stromal cells in vitro correlates with changes in gene expression and secretion of fibroblast growth factors FGF1, FGF2 and FGF18

Autoren:
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Abstract:
The transcription factor Hand2, which is induced by progesterone in endometrial stromal cells, is essential for acquisition of epithelial receptivity in mice. Since the expression of Hand2 correlates with decreased fibroblast growth factor (FGF) expression in murine endometrium, we wanted to find out whether this is also the case in human.

Human immortalized endometrial stromal cells (THESC) were treated with estradiol (E2), cyclic adenosine monophosphate (cAMP) or a combination of E2 together with either progesterone (P4) or medroxyprogesterone acetate (MPA) and/or cAMP for 15 days. Microscopical evaluation of the morphological transformation and immunofluorescence staining of Hand2 were performed at days 7 and 14. In addition, the secretion of prolactin, FGF1, FGF2 and FGF18 as well as the gene expression of Hand2, prolactin, FGF1 and FGF2 were investigated after 15 days of hormonal treatment.

The most significant morphological transformation to decidualized cells was observed under E2 + MPA + cAMP treatment. Decidualization was confirmed by detecting high amounts of prolactin. High expression of Hand2 was obtained which correlated with a reduction of gene expression and secretion of FGF1 and FGF2. In contrast, high levels of Hand2 correlated with an increase of FGF18 secretion.

We found out that increased Hand2 expression correlates with a decreased expression of FGF1 and FGF2 in human endometrial stromal cells. These findings suggest a role for Hand2 in inhibiting epithelial endometrial proliferation, which is essential for implantation. The positive correlation of Hand2 with FGF18 on the other hand indicates a different role in human endometrial cells.

Poster 163:

Titel:
Cyclic stretching of ACL-derived fibroblasts in 2D and 3D culture leads to synchronized cell orientation and activation

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Abstract:
Due to the fact that the mechanical stress of ligaments can constantly change, ligament fibroblasts must be mechanosensitive and possess sufficient adaptability to the new mechanomilieu to ensure the permanent load capacity of the tissue. When membrane mechanoreceptors are activated, the fibroblasts react with a specific signal transmission (mechanotransduction). The cellular response of fibroblasts of the anterior cruciate ligament (ACL) to cyclic mechanical stretching is still unclear. Hence, this study was undertaken to directly assess the mechanoresponse of ACL ligamentocytes under 2D and 3D conditions using cyclic stretching devices.

For the 2D (monolayer) and for the 3D (cell cluster: spheroid) cultures, functionalized silicon chambers were statically colonized for 24 h with lapine anterior cruciate ligamentocytes (LACL cells) and then cyclically stretched for 48 h. The second approach with the 3D cultures was to stretch functionalized scaffolds for ACL replacement. Scaffolds were precolonized dynamically with a LACL cell suspension and then stretched for 72 h. Cells were counted and cell survival was checked by a vitality assay. The orientation of the cytoskeleton was shown by immunocytochemical staining.

The results showed that the viability of the cells in stretched samples (2D, 3D, scaffold) was above 90 %. It was found that elongation resulted in an increase in cell counts and a significantly higher colonized area than in unstretched controls.

Cells oriented against the stretch direction in the 2D and 3D chambers, whereas a more compact cell arrangement was found on the scaffold threads than in unstretched cultures.

Poster 164:

Titel:
Phenotypic changes of SV40-Antigen expressing ACL ligamentocytes – applicable for ACL Tissue Engineering?

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Abstract:
Cultured human primary cells are limited available with restricted life span undergoing dedifferentiation or senescence. ACL tissue contains only few cells (5% of tissue volume) but Tissue Engineering (TE) approaches require high cell numbers. SV40 antigen expression could extend the life span of cells.

The aim of this study was to identify cellular changes induced by SV40 expression in human anterior cruciate ligamentocytes comparing them with non-transfected ligamentocytes and characteristics of the same donor tissue to evaluate their applicability as TE model.

Human ACL ligamentocytes (40 year-old female donor after ACL rupture) were either transfected with a simian virus SV40 plasmid or remained untreated (control) before monitored for SV40 expression, survival and proliferation. Protein expression of cultured ligamentocytes was compared with the donor tissue. Ligamentocyte spheroids were seeded on scaffolds embroidered either from polylactic acid (PLA) threads alone or combined PLA- and PLA-co-caprolacton-(P(LA-CL)) threads, further functionalized with fluorination and collagen. Cell distribution and survival was monitored for up to 5 weeks.

Transfected cells maintained SV40 expression throughout the whole observation time, but often showed chaotic and incomplete cell divisions, high DNA content and numerous nucleoli. ACL protein expression profile in situ was shared by control and SV40 expressing ligamentocytes. In comparison to controls, SV40 positive cells showed more dead cells, less vimentin and focal adhesions. Compared to the controls SV40 expressing cells formed instable spheroids and died on the scaffolds after 21 d.

SV40 expressing ligamentocytes share most properties with their non-transfected counterparts, however, applicability for TE is limited.