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Lecture Abstract Booklet

Saturday, 31th March 2007

MT I - Sessions I-III (O1-O17)
AG Immunobiology & Reprod. Biology (I1 – I7)

Sunday, 1st April 2007

MT II – Session I: Nucleus, Cytoskeleton (NC1-6)
MT II – Session II: Mitochondria (Mi1–4)
MT II – Session III: Peroxisomes (P1-P4)
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MT II – Session V: Cell-type Specific Organelles (Org7-10)
AG Neuroanatomy/Neurobiology (N1-13)
AG Cell Biology (C1-13)

MT I: O2

Hypoxic pulmonary vasoconstriction of intra-acinar pulmonary arteries in murine precision-cut lung slices depends on complex II of the mitochondrial respiratory chain

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Hypoxic pulmonary vasoconstriction (HPV) is a local reflex that shunts blood flow from poorly to well oxygenated regions of the lung. Our previous videomorphometric studies of small intra-acinar arteries in murine precision-cut lung slices (PCLS) with inhibitors of the individual complexes of the mitochondrial respiratory chain indicated a crucial role of complexes II and III in HPV. In view of the lack of specificity of any of the classical inhibitors, in this study the capacity to HPV was analysed in mice heterozygous for the complex II subunit SDH-D (SDH-D+/-). In isolated buffer-perfused and ventilated mouse lungs of SDH-D+/- mice the hypoxic increase in perfusion pressure was indistinguishable from that of wild type (WT) animals. In contrast, videomorphometric analysis of arteries in PCLS prepared from SDH-D+/- mice revealed complete loss of HPV: Whereas arteries of WT mice responded to hypoxia with distinct vasoconstriction (30% reduction of the luminal vessel area) neither the small intra-acinar arteries (mean diameter: 27 µm) nor immediate pre-acinar vessels (mean diameter: 67 µm) exhibited hypoxia-induced vasoconstriction. The responses of the vessels to KCl (depolarizing agent) and U46619 (thromboxane analogue) were unaffected in SDH-D+/- mice.
In summary, our data obtained by videomorphometric analysis of PCLS reveal an essential role of complex II of the mitochondrial respiratory chain for HPV at the pre- and intra-acinar level. However, further investigations are needed to explain why this does not translate into a deficiency of rising perfusion resistance in isolated hypoxic ventilated lungs of SDH-D+/- mice. Possible mechanisms include intact HPV in large vessels that are inaccessible to videomorphometric analysis in PCLS or a determining influence of intravascular shear stress that occurs in perfused lungs but is absent in superfused PCLS.

Main Topic I
vortrag

MT I / O3

Effect of bone marrow transplantation on the phenotype of SP-D deficient mice

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Surfactant protein D (SP-D) is a member of the lung s innate immune system. SP-D deficient (SP-D -/-) mice develop postnatal emphysematous alterations, abnormalities in surfactant homeostasis and alveolar type II cells and increased intracellular and intraalveolar surfactant pools. The structural abnormalities are associated with elevated levels of reactive oxygen species (ROS) and matrix metalloproteinases (MMP). Alveolar macrophages (AM) are a major source of ROS and MMP in the lung. The present study evaluates whether the replacement of AM by bone marrow transplantation (BM-Tx) from wildtype (WT) to SP-D -/- mice corrects these structural abnormalities and whether BM-Tx from SP-D -/- to WT mice induces a phenotype similar to SP-D deficiency. SP-D -/- mice (n=5) and WT C57BL/6 mice (n=5) were irradiated and reconstituted with bone marrow cells from age- and sex-matched SP-D -/- or WT mice, respectively. Untreated SP-D -/- (n=4) and WT mice (n=4) served as control. Using design-based stereology, the number and size of alveoli, type II cells and their lamellar bodies were estimated. Wildtype BM-Tx in SP-D -/- mice had no effect on alveolar number or lamellar body pool size but decreased the number of type II cells compared to untreated SP-D -/- mice. SP-D -/- BM-Tx in WT mice led to an increase in type II cell number and volume with an increased lamellar body pool size but had only little effect on alveolar number compared with WT mice. In conclusion, WT AM are necessary and sufficient for normal type II cell number and necessary but not sufficient for normal type II cell size. SP-D -/- AM in a WT environment are sufficient to cause type II cell hyperplasia and hypertrophy similar to SP-D deficiency. Our study demonstrates that emphysema alterations in SP-D -/- mice are independent of AM.

Main Topic I
vortrag

MT I / O4

Phosphodiesterase 1 Upregulation in Pulmonary Arterial Hypertension – Target for Reverse-Remodeling Therapy

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Pulmonary arterial hypertension (PAH) is a severe disease with still largely unresolved pathogenesis. It is characterized by increased pulmonary vascular resistance and thus right ventricular afterload, which in the further course of the disease leads to right ventricular failure and death. Phosphodiesterases hydrolyze the cyclic nucleotide second messengers cAMP and cGMP, which are known to play an important role in regulating vascular tone and smooth muscle cell proliferation. We investigated the expression of PDE1 in explanted lungs from idiopathic PAH (IPAH) patients and animal models of PAH, and undertook therapeutic intervention studies in the animal models. Strong upregulation of PDE1C in pulmonary arterial vessels in the IPAH lungs as compared to healthy donor lungs was noted on the mRNA level by laser-assisted vessel microdissection and on the protein level by immunohistochemistry. In chronic hypoxic mouse lungs and lungs from monocrotaline (MCT)-injected rats, PDE1A upregulation was detected in the structurally remodeled arterial muscular layer. Chronic infusion of the PDE1 inhibitor 8-methoxymethyl 3-isobutyl-1-methylxanthine (8MM-IBMX) in hypoxic mice and MCT rats with fully established pulmonary hypertension reversed the pulmonary artery pressure elevation, the structural remodeling of the lung vasculature and the right heart hypertrophy. Strong upregulation of the PDE1 family in pulmonary artery SMC is noted in human IPAH lungs and lungs from animal models of PAH. The PDE1 family may thus offer as new target for therapeutic intervention in pulmonary hypertension.

MT I / O6

Dopamine is an endothelium-derived vasodilator in hypoxic vessels

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Acute changes in the local concentration of oxygen have profound effects on arterial contractility. Arteries of the systemic circulation relax in response to a fall in pO_2. Dopamine (DA) is an important modulator of blood pressure by regulating vascular resistance. In most mammalian systemic arteries, DA acts as a potent vasodilator. Observations in sympathectomized rats caused us to investigate the putative existence of an intrinsic, non-neuronal dopaminergic regulatory system in the vascular wall of systemic arteries. Administration of the sympathetic neurotoxin, 6-hydroxydopamine, significantly reduced norepinephrine and epinephrine levels in the aorta and superior mesenteric artery (SMA), while dopamine content was unaffected. Employing real-time RT-PCR we detected the mRNAs for the complete enzymatic machinery necessary for catecholamine synthesis, i.e. tyrosine hydroxylase (TH), L-aromatic amino acid decarboxylase, dopamine-□-hydroxylase, and phenylethanolamine-N-methyl transferase in rat lung, aorta, and brain endothelial cells. Treatment of lung and aorta endothelial cells with hypoxia (1 % O_2, 6 h) caused an increase in TH mRNA and protein expression. Dopamine was detected in endothelial cells isolated from porcine pulmonary trunk by radioimmunoassay and secretion was stimulated by treatment with db-cAMP. Functional studies showed an endothelium-dependent vasodilation in U46619-precontracted SMAs under mild hypoxic conditions (125 mm HG), which could be blocked by the specific dopamine D_1 receptor antagonist SCH23990. However under severe hypoxic conditions (75 mm Hg), vasodilation was not affected by the D_1 receptor antagonist, although its onset developed slower.
In conclusion, our results demonstrate that dopamine is endogenously synthesized in endothelial cells from which it can release upon hypoxic stimulation and participates in regulation of vascular diameter.

MT I / O8

Direct visualisation of the effects of acute hypoxia, hyperoxia, hypercapnia and hypoxic hypercapnia on the activation of pulmonary neuroepithelial bodies (NEBs) in an in situ lung slice model

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Pulmonary NEBs are extensively innervated organoid groups of neuroendocrine cells, preferentially located at airway bifurcations. Although their physiological functions are still not elucidated, NEBs have been suggested to be receptor-effector units ideally positioned to respond to changes in air composition. Acute hypoxia is suggested to close background K+ channels in NEB cells, resulting in membrane depolarisation, voltage-gated Ca2+ entry and transmitter release. Our lab recently established an in situ live cell imaging model based on vibratome slices of live mouse lungs that allows the fluorescent visualisation of pulmonary NEBs with the styryl pyridinium dye 4-Di-2-ASP, and simultaneous monitoring of the intracellular Ca2+ concentration ([Ca2+]i) using Fluo-4. Aim of our study was to investigate the effects of bubbling the standard physiological solution with different gas mixtures on the [Ca2+]i of NEB cells.
Challenging lung slices with acute hypoxia (0% O2), hyperoxia (95% O2), hypercapnia (10% CO2) and hypoxic hypercapnia (0% O2, 10% CO2) never resulted in a [Ca2+]i rise in NEB cells. Hypoxia did, however, evoke bronchial smooth muscle contractions, while hypercapnia and hypoxic hypercapnia were observed to cause a [Ca2+]i rise in surrounding Clara- and Clara-like cells. Moreover, all NEB cells studied showed a fast and reproducible rise in Fluo-4 fluorescence after a short experimental application of high extracellular K+, confirming the viability and loading of the cells. Using identical protocols, hypoxia also resulted in a clear [Ca2+]i rise in a human small cell lung carcinoma cell line (H146) that was used as a positive control.
If indeed changes in gas concentrations, like acute hypoxia, would be key stimuli for NEBs in vivo, the absence of a detectable increase in [Ca2+]i in our in situ experimental conditions at least argues for a reconsideration of the proposed straightforward signal transduction pathway.

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MT I / O8

Sirt1 Modulates Foxo-dependent Transcription and Prevents Smooth Muscle Cell Apoptosis in Response to Oxidative Stress

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Background: Forkhead transcription factors represent an important physiological target of phosphatidylinositol-3 kinase/protein kinase B signaling and regulate genes that contribute both to longevity and resistance to various stresses in C. elegans. We previously demonstrated that phosphorylation of FoxO1a regulates vascular smooth muscle cells (VSMC) homeostasis. However, the effect of Foxo1a acetylation remains elusive. Therefore, in the present study we sought to determine the effect of Foxo1a acetylation on VSMC function.

Methods and Results: In cultured human VSMC, Foxo1a was detected in a deacetylated form only. Testing different deacetylase-inhibitors, Foxo1a acetylation was detected following the application of nicotinamide only, indicating the involvement of a NAD-dependent deacetylase. As demonstrated by co-immunoprecipitation, the class III histone deacetylase SIRT1 physiologically interacts with Foxo1a under native conditions, which was further confirmed by FRET-technique. Activation of Sirt1 by resveratrol increased DNA-binding and transactivation capacity of Foxo1a and further induced expression of Fox1a target genes including GADD45, known to regulate oxidative stress response. In contrast, siRNA-mediated knock-down of Sirt1 as well as the Sirt1 inhibitor sirtinol attenuated DNA-binding and transactivation capacity of Foxo1a, resulting in a reduced expression of Foxo1a target genes. Consistent with these findings, resveratrol significantly reduced VSMC apoptosis in response to oxidative stress, whereas incubation with sirtinol or knock-down of Sirt1 resulted in a significant increase in the apoptotic response. Moreover, embryonic fibroblasts (MEF) isolated from $SIRT^{-/-}$ mice were more resistant to oxidative stress-induced apoptosis compared to WT-MEF cells.

Conclusion: These results indicate that SIRT1 plays a pivotal role for FOXO function via NAD-dependent deacetylation, thereby contributing to stress resistance of VSMC in response to oxidative stress. Thus, SIRT1-dependent modulation of Foxo1a plays an essential role in apoptotic processes which regulate vascular homeostasis and remodeling.

MT I / O10

Free radicals and oxidative stress – Regulation of Peroxiredoxin Isoforms in the heart of mice with decreased MnSOD Proteinexpression and wild-type mice with and without physical training

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Background - Peroxiredoxins (Prx) are a family of multifunctional antioxidant thioredoxin- or glutathione dependent peroxidases. The major functions of Prx comprise in the cellular protection against oxidative stress as well as in the modulation of signaling cascades that apply hydrogen peroxide (H2O2) as a second messenger molecule. So far not much is known about the Proteinexpression and function of Prx with physical training. The hypothesis of the present study is that physical training changes the Peroxiredoxin expression at the myocardium and thereby improves the anti-oxidative protection.
Methods - We investigated the influence of physical training for protein expression of the Prx isoforms 1-6 in heterozygoten mice with decreased expression of the Mangansuperoxid Dismutase 2 (MnSOD(+/-))and increased myocardium stress.
Male mice of Wildtype(WT_T, n=6) and MnSOD (+/-) (MnSOD_T, n=11) were trained eight weeks for each 1h/day with 15 m/min and an upward gradient by 5 degrees. The control group were untrained animals (WT_K, n=7; MnSOD_K, n=9).
Results – Within the untrained WT and MnSOD-animals we did observe that the expression of the protein was equal for Prx 1 and 3, while the expression of Prx 2 was more highly and the expression of Prx 4, 5 and 6 was lower in the group of MnSOD mice than in WT. Between not trained and trained wildtyp mice the protein expression of Prx 1-2 was unchanged, while Prx 3-6 was down regulated after training. Between not trained and trained MnSOD mice the protein expression for Prx 2,5 and 6 was invariably, while Prx 3 was down regulated an Prx 1, 4 was high regulated.
Conclusion - Physical training causes a change of the expression of the enzyme Peroxiredoxin as a radical buffer and can possibly serve as a protection against reactive substances. The selective adjustment of the Prx Isoformen lets assume that in addition also different anti-oxidative systems are adjusted, which were not examined. That could mean that dependent on the basal anti-oxidative protection different adjustment samples of the individual Prx Isoformen are to be observed. Further studies have to show whether a change of the training extent or the intensity would have a similarly regulation.

Main Topic I
vortrag

MT1 / O13

Role of PHD2 in the interference of aryl hydrocarbon receptor (AhR) and hypoxia-inducible factor-1alpha (HIF-1alpha) signalling

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HIF-1alpha and the AhR both orchestrate a variety of cellular responses to exogenous stimuli in a broad range of tissues. To act as functional transcription factors each of them has to dimerise with ARNT (aryl hydrocarbon receptor nuclear translocator). While HIF-1alpha is activated by low oxygen concentrations, the AhR responds to xenobiotica, for example dioxins. Since both subunits share ARNT as a common dimerisation partner, we have analysed the interference between the AhR and HIF-1alpha pathway in the human breast carcinoma cell line MCF-7 following exposure to 10nM 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and hypoxia (5% O2). Specific attention was paid to prolyl hydroxylase domain containing protein 2 (PHD2), the main regulator of HIF-1alpha stability. HIF-1alpha amounts were quantified by Western blotting and correlated with PHD2 protein expression. Finally, interference of TCDD with the PHD2 promoter was analysed.
Whereas the AhR pathway was not sensitive to hypoxic conditions, combined treatment with TCDD and hypoxia clearly reduced the stabilization of HIF-1alpha and decreased hypoxia response element-mediated promoter activity if compared to exposure to hypoxia alone. AhR inhibition by alpha-naphthoflavone prevented these reductions. Lowered HIF-1alpha amounts were not caused by transcriptional down-regulation, indicating that the HIF-1alpha stabilisation was directly affected by TCDD in an AhR-depended mechanism. Therefore, the regulation of PHD2 by TCDD was studied next. Both, a distinct hypoxic stress or TCDD treatment increased PHD2 promoter activity, transcript and protein amounts. However, no such changes were found under simultaneous exposure to TCDD and hypoxia. After a 6 hour exposure, neither a TCDD mediated increase nor a TCDD/hypoxia mediated decrease in PHD2 amounts could be correlated with HIF-1alpha stabilisation, indicating that PHD2 is not the target for the TCDD-mediated decrease in HIF-1alpha stabilisation. Thus, the both mechanisms of TCDD-mediated HIF-1alpha destabilisation and the interference of AhR and HIF-1alpha pathways need further investigation.

Main Topic I
vortrag

MT I / O14

The role of trkB in hypoxia-induced vascular remodelling processes

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Hypoxia-induced vasoconstriction of the pulmonary arteries in the lungs results in vascular remodelling. These structural alterations are caused by hyperplasia and hypertrophy of vascular smooth muscle cells (VSMC) and the neighbouring fibroblasts. To gain a deeper understanding of the processes underlying hypoxic remodelling in the lung, we investigated the hypoxic mouse model of pulmonary hypertension (PH) in combination with whole genome microarray analysis. To determine differentially regulated genes, lung homogenates from mice subjected to 24h normoxia/hypoxia were analysed. Trk-B was one of most significantly up regulated genes (7 fold). Expression levels of Trk-B were confirmed by quantitative PCR and Western blot using lung homogenate of hypoxic and normoxic mice (1, 7 & 21days). Additionally, increased expression of the Trk-B ligand BDNF was detected throughout the remodelling process. Similar regulation of Trk-B and BDNF was evident in the rat monocrotalin model of PH. To establish the significance of Trk-B and BDNF in IPAH, quantitative PCR was performed. Both, in lung homogenates and laser-microdissected pulmonary arteries increased expression was observed when comparing IPAH patients to donor controls (n=5 per group). To examine the functional role of Trk-B and BDNF in remodelling, isolated PASMC were stimulated with BDNF ligand. PASMC proliferation was increased as analysed by 3H-thymidine incorporation and MTT assay, however, migration was not affected. BDNF induced proliferation was associated with activation of the ERK and upregulation of its downstream gene Cyclin D1. This finding allows us to conclude that Trk-B and BDNF are new mediators of hypoxic pulmonary vascular remodelling in pathogenesis of IPAH.

MT I / O15

Signaling to the antioxidant gene heme oxygenase-1 as a therapeutic target
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Heme oxygenase (HO)-1 is the inducible isoform of HO, which catalyzes the first and rate-limiting step of heme degradation. Enzymatic degradation of heme is protective, because excess heme can cause cellular injury. Moreover, the HO products carbon monoxide and biliverdin are of physiological significance as a signaling gas or as an antioxidant, respectively. Gene expression of HO-1 is induced by multiple stress stimuli and is primarily regulated at the transcriptional level. Overexpression of the HO-1 gene not only protects cells and tissues against oxidative damage, but also has anti-inflammatory effects. Anti-inflammatory effects of HO-1 have been demonstrated in a HO-1 knock out mouse model and a case of genetic human HO-1 deficiency. To ultimately apply HO-1 as a therapeutic target for the treatment of clinical conditions such as inflammatory disease, a detailed understanding of the signaling pathways that are involved in HO-1 gene regulation is essential. To achieve this goal, the molecular mechanisms of various stress-dependent and stress-independent stimuli that induce HO-1 gene regulation have been investigated in cell culture models of mononuclear cells (monocytes and macrophages). Here, data are presented, how activation of a casein kinase-2 (CK2)/ NF-κB signaling pathway mediates phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA)-dependent HO-1 gene induction in monocytes in a cell-type specific manner. Moreover, it is demonstrated that the NAD(P)H oxidase inhibitor 4-(2-aminoethyl) benzenesulfonyl fluoride (AEBSF), which is clinically applied as a protease inhibitor (Pefabloc®), up-regulates HO-1 gene expression in macrophages via activation of the Akt/ p38 mitogen-activated protein (MAP) kinase pathway.

MT I / O16

Kavalactones protects PC12 cells against amyloid peptide (1–42)-induced neurotoxicity via an ERK1/2-dependent Nrf2-activation

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One hallmark of Alzheimer\'s disease (AD) is the accumulation of amyloid beta-peptide (AP), which can initiate a cascade of oxidative stress that may result in neuronal death. Since Nrf2 is the major regulator for a battery of genes encoding detoxifying and antioxidative enzymes, it is of great interest to find nontoxic activators of Nrf2 render neuronal cells more resistent to AP toxicity.
In the present study, we show that the kavalactones (KL) methysticin, kavain and yangonin activate Nrf2 dose dependently in differentiated PC12 and C6 cells in an ERK1/2 dependent fashion, and thereby protect PC12 cells from AP induced neurotoxicity. Downregulation of Nrf2 by shRNA as well as ERK1/2 inhibition abolish the protective effects. KL activate Nrf2 at nontoxic concentrations via direct cystein modification of Keap1 rather then producing oxidative stress, since Nrf2 activation could be inhibit by DTT but not by antioxidants.
Our results demonstrate that the KL attenuate AP toxicity by inducing protective genes through Nrf2 activation. This implicates that kavalactones could potentially be of importance for prevention and therapy of AD.

Main Topic I
vortrag

I1

Nicotinic Receptors on Rat Alveolar Macrophages Dampen ATP-Induced Increase in Cytosolic Calcium Concentration

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Nicotinic acetylcholine receptors (nAChR) have been identified on a variety of cells of the immune system and are generally considered to trigger anti-inflammatory events. In the present study we set out first to determine the nAChR inventory of alveolar macrophages (AM) in the rat, and second to investigate the cellular events evoked by stimulation of AM with nicotine. Rat AM were isolated freshly by bronchoalveolar lavage (BAL). Inter-individually variable expression patterns of nAChR subunits investigated by RT-PCR were noted. Positive results were obtained for subunits alpha 3, 5, 9, 10 and beta 2. Most stable expression was noted for subunits alpha 9, alpha 10 and beta 2. Notably, mRNA coding for subunit alpha 7 which is proposed to convey the nicotinic anti-inflammatory response of macrophages from other sources than the lung was not detected. Subunits alpha 3, 5, 9 and 10 and beta 2 were also detected by immunohistochemistry on AM isolated by BAL as well as on AM in lung tissue sections. Changes in intracellular calcium concentrations were monitored in rat AM by fura-2. Nicotine (100 µM) had almost no effect upon intracellular [Ca^{2+}] whereas ATP (200 µM) induced a drastic increase with rapid onset and long duration. Nicotine (1 µM), given 2 min prior to ATP, significantly reduced the ATP-induced rise in intracellular [Ca^{2+}] by 30%. This effect was blocked by α-bungarotoxin. These data demonstrate the presence of functionally active nAChR that interact with ATP-induced rise in intracellular [Ca^{2+}] in rat AM.

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I2

Detection of the surfactant proteins A and D in the human eye and lacrimal system

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The human eye as well as the entire nasolacrimal apparatus has a variety of natural barrier defences against external stimuli, which could be pollutants, dust, microorganisms or infectious proteins. A normally functioning tearfilm and surface of the regarding epithelial cells is of exceeding importance to the eye's defence and moreover, ensures an optimal refractive surface. The surface active proteins A and D (SP-A and SP-D) are members of the collectin family of C-type lectins that include a number of molecules with known host defence functions. These proteins, amongst others might responsible for blocking microorganisms. Due to RT-PCR experiments it could be shown, that the mentioned proteins are not only expressed in the lacrimal glands but also in conjunctiva, cornea and the nasolacrimal ducts. Immunhistochemical staining confirmed the presence of SP-A and SP-D at the cellular surface of the epithelial cells for the investigated tissue. Also, Western blot and dot blot analysis showed distinct reactions for SP-A and SP-D in the tissue as well as in tear fluid, so expression could additionally be proven on protein level. Furthermore, different pathological tissues (eg. bacterial ulcer (*Staphylococcus aureus*) were investigated immunhistochemical for the presence of the both mentioned surfactant proteins. Therefore, a higher level of SP-A and SP-D expression could be estimated for the pathological samples in comparison to the healthy ones, especially for the investigated corneas. In conclusion, SPA as well as SP-D are present in human tear fluid and different tissues of the lacrimal apparatus. Moreover, both proteins might play an eminent role in protecting the eye against pathogens.

I3

Cytological characteristics of haematopoiesis in human prenatal thymus

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Introduction: Two stages of haematopoiesis were described during human intrauterine development (IUD) - extraembryonal (in yolk sac) and intraembryonal (mainly in hepatosplenic region and bone marrow). The foundation of thymus is colonized by stem cells that make origin of lymphocyte at 10th week of IUD. We studied specification, quantity and distribution of haematopoietic stem cells (HSC) and mature immune cells in human prenatal thymus.
Material and methods: We used 10 thymuses from foetuses ranging from 14th to 30th week of IUD. For the detection of apoptotic cells, TUNEL was applied. The detection and typisation of cells was made by standard indirect three-step immunohistochemical method. CD34 antibody and CD117 antibody were used for detection of HSC and SSEA-1 antibody for detection of embryonal stem cells was used. Macrophages were detected by CD64, CD68 and Ab-1. Detection of mature T- and B-lymphocytes was accomplished by CD3 and CD79b.
Results: CD117+ haematopoietic cells were detected in all sections sporadically, but surprisingly we discovered small group of CD117+ HSC too. Number CD34+ blood cells in prenatal thymus was great. CD34+ blood stem cells were spread uniformly in all content of prenatal thymus. Macrophages were found in all sections too and their role in cleaning of apoptotic bodies was proved. CD3+ lymphocytes were detected in all sections, but the level of CD3+ cells was not high, CD79+ cells were detected in all section too and in higher quantity than CD3+ cells.
Conclusion: HSC in thymus and their role in human prenatal haematopoiesis and immune system development are not well known. We detected small group of CD117+ cells in prenatal thymus. Any other similar group of CD117+ cells was detected in other organs we studied.
Acknowledgement: This work was supported by grant MSM 6198959205.

I4

Body weight dependent effects of ghrelin in a rat model of acute endotoxemia

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Obese patients with sepsis have higher morbidity and mortality rates than normal weight subjects, but exact pathomechanisms are largely unknown. Latest research showed growing evidence of the coupling of immune status to the metabolic system mediated by cytokines, hormones and neuropeptides. Ghrelin, a stomach-derived 28-amino acid peptide, is assumed to be one of the mediators, since apart from the potent orexigenic effects, ghrelin seems to play a role in the generation and control of immune interactions.
To examine a possible benefit of a single ghrelin application on acute endotoxemia, chronic i.v. cannulated lean and diet-induced obese male LEW rats were treated with a bolus injection of either ghrelin (10 nmol/kg) or vehicle, 10 minutes prior to a challenge with a sublethal bolus of endotoxin (100μg/kg) or vehicle.
Multiple blood samples were taken within a period from 24h before the experiment up to 24h after the endotoxin challenge to measure ghrelin and cytokine levels. Additionally, food consumption was recorded and ghrelin expression in fore- and glandular stomach was evaluated immunohistochemically.
Our data show that ghrelin was beneficial on the food consumption and herewith the well-being of normal weight rats in endotoxemia. Despite higher serum ghrelin levels, this effect was abrogated in obese subjects. Furthermore we could show an increase of anti-inflammatory IL-10 serum levels after ghrelin treatment of normal weight endotoxemic and an opposite effect in obese animals. In conclusion, the present study shows that the therapeutic effects of ghrelin were dramatically attenuated in obese endotoxemic subjects.

I5

Uropathogenic E.coli but not commensale E.coli infection activates TLR-4 MyD independent signaling pathways in rat testicular cells

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Immunological infertility due to infection, inflammation or autoimmunity accounts for at least 12-13% of all cases of male infertility. Uropathogenic Escherichia coli (UPEC) is the most frequent pathogen causing acute and chronic bacterial genital tract infections in men, however, the testicular defence to bacterial infection is poorly investigated. Therefore, we investigated the basal mRNA expression pattern of Toll-like receptors (TLRs) 2-10 in isolated testicular cells and peritoneal macrophages (PM) because TLRs function as sensors for conserved pathogen-related molecular patterns (PRMPs) such as LPS and petidoglycan. Using RT-PCR all somatic and germ cell types as well as PM expressed mRNAs for at least two TLRs. Expression of TLR4 protein, that is recognizing LPS as PRMP, was induced 2h and 6h after UPEC infection in primary TM and Sertoli cells (SC) or peritubular cells (PTC), respectively, whereas after infection with non-pathogenic commensale E.coli (NPEC) no TLR4 was detectable. TLR4 induction coincided with already macroscopically visible massive cell death due to apoptosis. When tracing the TLR signaling pathways activation of either p38, JNK and/or ERK1/2 was observed in all three cell types and PM, but only PTC and PM reacted with degradation of IkBa. Infection with UPEC induced expression of MCP-1, IP10 and IFNa/b in all cell types except TM indicating the induction of MyD88 independent signaling pathways in SC and PTC. Surprisingly, the proinflammatory cytokines IL-1 and IL-6 and TNFa were induced in PM only by LPS and NPEC, but not by UPEC. TNFa induction by LPS or NPEC could be abolished by co-infection with UPEC. We conclude from our results that UPEC but not NPEC infection is sensed by TLR4 in testicular cells. Moreover, UPEC infection seems to activate MyD88-independent signaling pathways.

I6

Expression of ZO-1, ZO-2 and ZO-3 in human testis with normal spermatogenesis and Carcinoma in-situ (CIS)

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Carcinoma in-situ (CIS) is the non-invasive precursor of most human testicular germ cell tumours. In normal seminiferous epithelium, specialized tight junctions (TJs) between Sertoli cells constitute the major structural component of the blood-testis barrier (BTB). Sertoli cells associated with CIS cells exhibit an impaired maturation status amongst others indicated by the reexpression of cytokeratin 18, but its functional significance remains unknown. Aim of the present study was to determine, if the BTB is morphologically and/ or functionally altered. For that purpose, we investigated the expression and distribution pattern of the tight junction proteins ZO-1, ZO-2 and ZO-3 in normal seminiferous tubules compared to tubules showing CIS. In normal tubules, ZO-1 and ZO-2 immunostaining was observed at the BTB region of adjacent Sertoli cells. Within CIS tubules, ZO-1 and ZO-2 immunoreactivity was reduced at the BTB region but spread to stain the Sertoli cell cytoplasm. ZO-3 was neither detectable in normal tubules nor in CIS tubules. Western blot analysis confirmed ZO-1 and ZO-2 and respective mRNA expression was shown by RT-PCR. Additionally, we assessed the functional integrity of the BTB in CIS tubules by lanthanum tracer studies. Lanthanum permeated the TJs in CIS tubules, indicating a disruption of the BTB. In conclusion, Sertoli cells associated with CIS cells show an altered distribution pattern of ZO-1 and ZO-2 and loose their BTB function.

I7

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MT II / NC1

Odyssee in the nucleus: Discovering nuclear substructures

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The nucleus not only contains the genetic material, but also harbours many important biochemical processes. Complexes of DNA, proteins as well as coding and non-coding RNAs are key players in this highly organized cellular structure. Recent work on the structure of chromatin domains, the nucleolus and nuclear bodies as well as other subcompartments will be summarized in the presentation.

Nuclear bodies are a distinct class of subnuclear structures. The survival of motoneuron (SMN) protein, which is either mutated or deleted in patients with the neurodegenerative disease spinal muscular atrophy (SMA), is a protein marker for one class of nuclear bodies denoted nuclear gems. Little is known about the regulation of nuclear body formation and stabilization. New data about factors influencing nuclear body assembly will be presented.

MT II / NC2

The permeability barrier of nuclear pore complexes

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Cell nuclei lack protein synthesis and therefore import all needed proteins from the cytoplasm. In return, the supply the cytoplasm with "nuclear products", such as mRNAs, tRNAs and ribosomes. Nuclear pore complexes (NPCs) constitute the sole site of nucleo-cytoplasmic exchange and function as giant and yet highly selective gates. They allow passage of material in essentially two modes: passive diffusion and facilitated translocation. Passive diffusion is fast for metabolites and small macromolecules, but becomes increasingly inefficient as the substrate approaches or exceeds a size-limit of 20-40 kDa. In contrast, facilitated translocation can accommodate the rapid transport of even very large objects. Facilitated translocation requires specific interactions between the translocating species and NPC-components. It is therefore a highly selective process and available only for "privileged" objects with "translocation-promoting" properties. These properties are normally conferred by nuclear transport receptors. Molecules that lack translocation-promoting properties are referred to as "inert" objects. Their NPC-passage is normally restricted by a permeability barrier. They can cross NPCs only in a facilitated manner when bound to a suitable nuclear transport receptor. The presentation will be focused on the nature of the permeability barrier, its reconstitution from defined components and on the molecular mechanism that accounts for the rapid passage of transport receptors through the barrier.

MT II / NC3

Actin filaments and microtubules cooperate to prevent chromosome loss in starfish oocytes

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The cytoskeleton gives the cells shape, organizes their internal structure, and allows them to move and divide. The cytoskeleton is built of three types of proteins: actins, tubulins and intermediate filament proteins. All three of these proteins polymerize to thin filaments, but the filaments they form have very different mechanical properties. Previously it was thought that these different types of filaments are specialized to perform distinct functions. Recent findings revealed, however, that cells often use these cytoskeletal elements in combination to build complex cellular structures – similar to bones, muscles and tendons that together give the stability and flexibility to our bodies.

As cells are preparing to divide, chromosomes are aligned at the cell center and the two copies of the genome are pulled apart towards opposite poles. Failure to align all chromosomes results in chromosome loss leading to aneuploidy and cancer. Chromosome capture and alignment was thought to be mediated exclusively by the microtubule spindle. However, the observed length of spindle microtubules and computer simulations of spindle assembly predicted that chromosome capture is efficient in small cells, but may fail in cells with large nuclear volumes such as animal oocytes. Indeed, when we investigated chromosome congression during the first meiotic division in starfish oocytes, we found that microtubules are not sufficient for capturing chromosomes. Instead, chromosome congression requires actin polymerization. After nuclear envelope breakdown, we observed the formation of a filamentous actin mesh in the nuclear region, and found that contraction of this network delivers chromosomes to the microtubule spindle.

Together, we showed that in starfish oocytes microtubules and actin filaments work together to align chromosomes on the meiotic spindle and to prevent chromosome loss. This unexpected finding exemplifies how cytoskeletal filaments are cooperate to perform specific cellular functions, and identifies a novel role for actin in chromosome capture and thus in preventing aneuploidy.

MT II / NC4

Impaired Actin Dynamics in CD2AP-Deficient Podocytes

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CD2AP (CD2-associated protein) knock-out mice die of renal failure at the age of 6-7 weeks. The renal phenotype can be rescued by podocyte-specific expression of CD2AP, confirming the critical function of CD2AP in podocytes. Since CD2AP localizes to specific F-actin structures in podocytes and since CD2AP interacts with F-actin, cortactin, and capping protein, CD2AP may play an important role in the regulation of the actin cytoskeleton in podocytes. We therefore examined the actin cytoskeleton in conditionally immortalized podocytes derived form CD2AP knock-out mice and in wild-type (WT) podocyte cell lines. CD2AP deficient ($CD2AP^{-/-}$) podocytes predominantly possess a polygonal shape with more stress fibers, larger focal adhesions and less lamellipodia as compared to WT podocytes. Upon treatment with cytochalasin, $CD2AP^{-/-}$ podocytes show an incomplete disruption of the actin cytoskeleton. In response to stimulation with epidermal growth factor (EGF), the formation and motility of ring-like actin structures (RiLiS) are markedly reduced in $CD2AP^{-/-}$ podocytes. The morphology of RiLiS is altered in $CD2AP^{-/-}$ podocytes as well. The phenotype of the actin cytoskeleton and of actin dynamics is rescued by transfection of $CD2AP^{-/-}$ podocytes with a GFP-CD2AP construct. In contrast to the N-terminal half of CD2AP, the C-terminal half of CD2AP, containing cortactin, capping protein and F-actin binding sites, was sufficient to confer localization into RiLiS, and to rescue RiLiS formation in $CD2AP^{-/-}$ podocytes. Our data demonstrate that CD2AP plays a non-redundant role in actin dynamics in podocytes, possibly representing a critical function of CD2AP in podocytes in vivo.

MT II / NC 5

Intermediate filaments: dynamic stabilizers of the cytoskeleton

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The 8-12 nm intermediate filaments are ubiquitous components of the cytoskeleton. They are composed of cell type-specific constituents that are encoded by several multigene families encompassing at least 65 functional genes in human and even 11 genes in the nematode *Caenorhabditis elegans*. In conjunction with specific adhesion sites they contribute to cell stability, most notably in mechanically challenged tissues such as the epidermis. Multiple diseases that are caused by intermediate filament gene mutations therefore present reduced mechanical resilience. Yet, accumulating evidence shows that intermediate filaments also contribute to numerous other cellular properties including cell size determination, proliferation, cell type-specific organelle transport, malignant transformation and stress response. The highly dynamic nature of intermediate filaments as revealed by live cell imaging supports these novel functions. The molecular regulatory mechanisms determining the specific assembly states and their interconversion, however, are poorly understood and differ profoundly from those of the other cytoskeletal systems given the lack of intrinsic polarity and spontaneous assembly capacity of intermediate filaments. Highly complex patterns of phosphorylation are major factors affecting intermediate filament formation and disassembly. Interference with specific signalling cascades therefore results in rapid intermediate filament restructuring. Furthermore, interactions with scaffolding proteins, with distinct structural platforms and with other cytoskeletal filament systems are involved in temporally and spatially-restricted intermediate filament formation in defined microenvironments and their subsequent dispersion. The mutual influence between cellular differentiation/functioning and specific forms of intermediate filament organization begins to become unfolded in transgenic organisms ranging from mouse to *Caenorhabditis elegans*.

MT I / NC6

Dendritic cytoskeletal dynamics of developing rat Purkinje cells

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Aim of this study was to check the correlation between dynamics of actin filaments, neurofilaments (NF-M) and tubulin and the development of dendritic organization and spines of rat Purkinje cells. It is well known that dendritic spines are dynamic components of neurons, which undergo changes in density and shape during development and learning in response to synaptic activity.
Slice cultures of rat cerebellar cortex of different age were obtained from newborn rat pups (P1) and at postnatal day 10. Dendritic development was monitored with histochemical techniques as calbindin staining and Golgi-impregnation. Distribution of cytoskeletal proteins was particularly analyzed by rapid-freeze deep-etch electron microscopy. In order to study dynamics of certain cytoskeletal proteins microinjection of YFP-tubulin, GFP-NF-M and YFP-actin vectors into individual Purkinje cells was done. Analysis of cytoskeletal movement was performed by means of “fluorescence recovery after photobleaching” (FRAP) with confocal laser scanning microscopy to check for cytoskeletal links.
Characteristic features for these Purkinje cells were slightly developed dendrites in P1 cultures that developed prominent dendritic arborization in the following weeks during cultivation. Unlike unconjugated GFP two days after microinjection of the vector into single neurons, YFP-tubulin, GFP-NF-M and YFP-actin filaments were clearly visible in the somata and dendrites of Purkinje cells. Time-lapse imaging displayed rapid movements of these cytoskeletal elements through bleached regions. Treatment with the microtubules depolymerizing drug colchicine stopped transport of tubulin and neurofilaments. Besides this highly dynamic dendritic spines filled with actin filaments could be observed in older slice cultures. This movement was blocked by treatment with cytochalasin D or latrunculin A.

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MT II / Mi1

Mitochondrial protein import and assembly

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Most mitochondrial proteins are encoded in the nucleus and transported into mitochondria after translation in the cytosol. Within the mitochondrion the precursor proteins need to be transported into various subcompartments, such as the outer membrane, the intermembrane space, the inner membrane and the matrix. Five multi-protein complexes mediate recognition and transport of the preproteins. The outer membrane translocase (TOM complex) transports proteins across and into the outer membrane. Some outer membrane proteins also require the SAM complex for their insertion into the outer membrane, in addition to the TOM complex. A set of small proteins of the intermembrane space utilizes the recently discovered MIA pathway.
Two translocases in the inner membrane (TIM complexes) accept precursor proteins from the TOM complex and mediate further transport steps across the inner membrane. The twin-pore carrier translocase (TIM22 complex) is specialized to insert multi-spanning inner membrane proteins with internal targeting signals into the inner membrane. In contrast, proteins that utilize a presequence as a means of targeting require the aid of the presequence translocase (TIM23 complex) for transport. While most precursor proteins of this group are destined for the matrix, some are directed to the inner membrane or the intermembrane space. Therefore, the TIM23 complex has to be highly dynamic in order to adapt to the needs of the different precursor proteins for transport or membrane insertion. Therefore, the presequence translocase consists of two functional modules: a pore forming membrane integral module that can insert proteins into the inner membrane and a presequence translocase-associated motor complex (PAM) that is required for complete translocation of the precursor.

MT II / Mi2

'Mitochondrial dysfunction in neurodegenerative diseases'

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MtDNA mutations are associated with a variety of progressive encephalomyopathies in which there is evidence of neurodegeneration. These include Kearns-Sayre syndrome, myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) and myoclonic epilepsy with ragged red fibres (MERRF) and Leigh's syndrome. In Leber's hereditary optic neuropathy (LHON), there is degeneration of retinal ganglion cells. Occasional reports have described mtDNA mutations in association with Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS).

Mutations in the nuclear gene for mtDNA polymerase gamma (POLG) have been found in a range of disorders that include Alpers' syndrome, progressive external ophthalmoplegia (PEO), sensory ataxia, neuropathy, dysarthria and ophthalmoplegia (SANDO) and parkinsonism. Although most cases of parkinsonism due to POLG mutations have been preceded by PEO, some have been described with only parkinsonism and neuropathy. The early onset form of hepatocerebral mtDNA depletion is associated with mutations in the deoxyguanosine kinase gene and thymidine phosphorylase mutations are a cause of mitochondrial neurogastrointestinal encephalomyopathy (MNGIE). Mutations have been identified in nuclear genes for mitochondrial proteins involved in the assembly and maintenance of cytochrome oxidase that usually present in early life with Leigh syndrome, myopathy, and encephalopathy, lactic acidosis and a progressive course with early death.

There is deficiency in complex I activity in PD substantia nigra and platelets. The pathogenesis of PD also includes protein aggregation (Lewy bodies). Mitochondrial dysfunction will contribute to dysfunction of the energy dependent ubiquitin proteasomal system (UPS) and oxidative stress will add to the substrate load. Several of the single gene mutations causing familial PD have been identified as mitochondrial proteins including PINK1, DJ1 and parkin. The cellular distribution of the latter appears to depend upon the stage of cell differentiation. A proportion of LRRK2 is associated with the outer mitochondrial membrane.

MT II / Mi3

The impact of estrogen on morphology and function of astrocyte mitochondria

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Mitochondria play an essential role in the regulation of cellular energy metabolism and the initiation of apoptotic processes. This makes these cellular compartments a perfect target for strategies of cellular protection against toxic compounds and hypoxic conditions. Steroid hormones, such as estrogen, are well-known to fulfill a neuroprotective function in the brain during ischemic and degenerative processes. In this project, we have analyzed the effect of estrogen on morphology and function of mitochondria in astroglial cells from different brain regions in vitro, since this particular cell type is of major importance for the energy supply in the brain. Firstly, we could demonstrate by gene expression studies that estrogen regulates the level of mitofusins which act as structural proteins being involved in fusion and fission of mitochondria. Secondly, we observed that catalytic subunits of respiratory chain complexes and the regulatory subunit IV isoforms of cytochrome c oxidase reveal a time- and brain region-specific regulation by estrogen.
Our results lead us to the conclusion that estrogen affects structural and functional properties of astrocyte mitochondria, thereby stabilizing astroglial cell function and promoting cell survival. This could represent an important indirect mechanism by that estrogen protects neurons from cell death under neurotoxic conditions.
Supported by the DFG (Emmy Noether-Program, SA) and the START-Program of the Faculty of Medicine, RWTH Aachen (SA)

MT II / Mi 4

Dynamics and division of mitochondria and peroxisomes in mammalian cells

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Organelle dynamics and especially fission and fusion events are important for the regulation of organelle number, volume, size and content, which plays an essential role in organelle inheritance and function. Mitochondria and peroxisomes are ubiquitous subcellular organelles, which fulfil an indispensable role in the cellular metabolism of higher eukaryotes. Moreover, they are highly dynamic and display large plasticity. Recent studies have led to the surprising finding that both organelles share components of their division machinery, namely the dynamin-related protein DLP1/Drp1, which belongs to the growing family of large GTPases, and hFis1, a tail-anchored membrane protein. hFis1 is supposed to recruit DLP1/Drp1 to the organelle membranes. Moreover, it is one of the few transmembrane proteins described so far that is targeted to both mitochondria and peroxisomes. We presented evidence that peroxisomal fission is a multistep process involving peroxisome elongation/growth, constriction and final scission. The transmembrane protein Pex11p-beta is specific for peroxisomes, and is required for peroxisome elongation/growth. Peroxisome elongation and constriction can occur independently of DLP1/Drp1, whereas the final fission step requires DLP1/Drp1 function. Growing evidence indicates that organelle dynamics including fission and fusion is linked to organelle function thus influencing cell and tissue physiology.

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MT II / P1

Protein Import into Peroxisomes

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Peroxisomal import receptors bind their cargo proteins in the cytosol and target them to a docking- and translocation-machinery at the peroxisomal membrane. The receptors release the cargo proteins into the peroxisomal lumen and, according to the model of cycling receptors, they are supposed to shuttle back to the cytosol. The peroxisomal protein import has been implicated with ubiquitination ever since the discovery of the ubiquitin-conjugating enzyme Pex4p[1], which has also been classified as Ubc10p and is anchored to peroxisomes by the integral membrane protein Pex22p[2]. Both proteins are required for the formation of functional peroxisomes but their detailed role in peroxisomal protein import and especially the molecular target for ubiquitination remained a mystery. Pex5p, the import receptor for peroxisomal matrix proteins harbouring a type I signal sequence (PTS1) is mono- and polyubiquitinated in yeast[3-5]. The import receptor cycles between the peroxisome and the cytosol with the ATP-dependent release from the peroxisomal membrane being performed by AAA-type ATPases[6,7]. We demonstrate that monoubiquitination of Pex5p depends on Pex4p (Ubc10p) and its peroxisomal membrane anchor and that either poly- or monoubiquitination is required for dislocation of Pex5p from the peroxisomal membrane to the cytosol. Pex4p does act upstream of the Pex1p/Pex6p complex, indicating that export of Pex5p requires two distinct ATP-consuming reactions, ubiquitination and dislocation. Polyubiquitinated Pex5p is then degraded by the proteasome as part of a quality control system while monoubiquitinated Pex5p is supposed to undergo deubiquitination and is made available for another round of peroxisomal protein import.

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MT II / P2

PEX1 Mutations and the Peroxisome Biogenesis Disorders

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Diseases of the Zellweger spectrum represent a major sub-group of the peroxisome biogenesis disorders, a group of autosomal recessive diseases characterized by widespread tissue pathology, including neurodegeneration. The Zellweger spectrum represents a clinical continuum, with Zellweger syndrome (ZS) the most severe phenotype, and neonatal adrenoleukodystrophy (NALD) and infantile Refsum disease (IRD) representing progressively milder phenotypes. Peroxisome biogenesis requires a group of peroxins (proteins encoded by PEX genes) for protein import and organelle assembly. Mutations in the PEX1 gene, which encodes a 143 kDa AAA ATPase protein, are the most common cause of the Zellweger spectrum diseases. More than 40 disease-causing PEX1 mutations have been identified to date from patients representing a wide spectrum of ethnic backgrounds. The types of mutations are wide-ranging, as would be expected for a large gene (24 exons), and comprise insertions, deletions, nonsense, missense, and splice site mutations. Mutations producing premature truncation codons (PTCs) are distributed throughout the PEX1 gene, whereas most missense mutations segregate with the two essential AAA domains of the PEX1 protein. Severity at the two ends of the Zellweger spectrum correlates broadly with mutation type and impact – the severe phenotype with PTCs on both alleles; the milder phenotypes with missense mutations. In addition, a number of promoter polymorphisms that appear to influence PEX1 expression have also been identified. These findings will be discussed in the context of predicting patient clinical outcome.

MT II / P3

PEX gene-knockout mice: Models to study the molecular pathogenesis of peroxisomal biogenesis disorders

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Peroxisomal biogenesis disorders are devastating autosomal-recessive metabolic diseases, characterized by general hypotonia of the children, severe neurological pathologies leading to neonatal seizures and chronic degeneration of a variety of organs, including liver, adrenals and testis. Children suffering from the most severe form of these disorders – the Zellweger syndrome – usually die within the first year of life. To date no functional and valid therapy is available to treat patients with these diseases. In addition, in most cases the underlying molecular mechanisms leading to organ defects are not understood. Therefore, our group and others have developed several knockout mouse models during the last few years to study the molecular pathogenesis of peroxisomal biogenesis disorders and to discover the yet unknown functions of peroxisomes in distinct organ systems.
Several knockout mouse lines (PEX2, PEX5, PEX11, PEX13) with peroxisomal deficiencies have been described, which exhibit similar phenotypes to the corresponding patients and are therefore of high value to develop new treatment strategies and to test different therapy forms in the near future.
With the help of these animal modelsour group was able to show that peroxisomal deficiency leads to severe disturbances of enzymes and metabolic pathways also in other cell compartments, such as the mitochondrial respiratory chain or several antioxidative enzyme systems. Accumulation of reactive oxygen species (ROS) in neurons and other cell types was observed promoting apoptotic cell death. Apoptotic cell death could be prevented by treatment of neuronal cultures with antioxidative compounds, suggestive for a possible new treatment strategy of patients in preventing intracellular ROS accumulation. In addition, severe alterations in signal transduction pathways regulating growth and differentiation in different organ systems were noted, further substantiating the importance of peroxisomal metabolism for intracellular homeostasis and the survival of the entire organism.

MT II / P4

Chaperone-mediated import of peroxisomal proteins lacking specific targeting signals

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Peroxisomes are cell organelles with important functions in the metabolism of lipids and of reactive oxygen species. Import of peroxisomal proteins relys on the function of a number of essential proteins called peroxins (Pex). Most peroxisomal matrix proteins are targeted to the organelles by specific peroxisomal targeting signals (PTS) located either at their C-terminus (PTS1) or at their N-terminus (PTS2). Usually peroxisomal matrix proteins are imported as a complex after interaction of their PTS sequences with concomitant import receptors Pex5 or Pex7.
However, a number of peroxisomal proteins lack specific tagrting signals and the import of these proteins is not understood. We have investigated the import of a peroxisomal superoxide dismutase (SOD) and could show, that import of this protein was mediated by a specific chaperone. The heterodimeric protein complex of SOD with its chaperone was imported in a Pex5-dependent fashion. Import of superoxide dismutase is the first physiological example for import of peroxisomal proteins in a “piggy back” fashion in higher eukaryotes. In contrast to the established pathways for targeting of proteins to subcellular organelles our data support the idea that proteins with physiological functions in several subcellular compartments could be directed to their location in a chaperone-mediated fashion.

EFEM – Lecture

**Intracellular Transport and Kinesin Superfamily Proteins, KIFs :
Structure, Dynamics, Functions, and Diseases**

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The intracellular transport is fundamental for cell morphogenesis, functioning and survival. To elucidate this mechanism we have identified and characterized kinesin superfamily proteins, KIFs, using molecular cell biology, molecular genetics, biophysics, X ray crystallography and cryoelectron microscopy. KIFs transport various membranous organelles such as mitochondria(KIF1Balpha/KIF5s), synaptic vesicle precursor(KIF1A/KIF1Bbeta), NMDA type(KIF17) and AMPA type(KIF5s) glutamate receptors and mRNAs with a large protein complex(KIF5s) in neurons and other cells along microtubule rails and play significant roles on neuronal function and survival including learning and memory and relate to human diseases. KIF2A, a unique middle motor domain KIF, plays a significant role in brain wiring by depolymerizing microtubules in growth cones and controlling extension of axonal branches. KIF3 is fundamental for left-right determination of our body through formation of monocilia in the node which rotate and generate leftward flow of extra embryonic fluid, nodal flow, conveying vesicular parcels containing Sonic hedgehog and retinoic acid toward left and determines left-right asymmetry. KIF3 also suppresses tumorigenesis by transporting N cadherin – beta catenin complex from cytoplasm to plasma membrane which works as a transcriptional factor with T cell factor and enhances cell proliferation as a signaling molecule. KIF4 controls activity dependent neuronal survival during development.
In terms of the mechanism of motility we identified the simplest monomeric motor, KIF1A and revealed that KIF1A can move processively along microtubules as a monomer by a biased Brownian motion and elucidated how KIF 1A moves on a microtubule using a single molecule biophysics, optical trapping nanometry, cryo EM, and X ray crystallography. Thus, KIFs play a number of significant roles not only on various cellular functions, but also on higher brain functions, brain wiring, activity dependent neuronal survival, and fundamental developmental events such as left-right asymmetry and tumorigenesis.

M1

Anatomical dissection improves students' spatial knowledge - First steps towards scientific evidence

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BACKGROUND: Discussions about the value of anatomical dissection as part of the medical curriculum are characterised by a lack of scientific evidence. One aspect not addressed in such educational research so far is students\' appreciation of the true size of anatomical structures, which is one important aspect of spatial anatomical knowledge. We hypothesised that studying and dissecting human cadavers will improve this appreciation of size.

METHODS: At the beginning and at the end of two parallel dissection courses, students were asked to give their appreciation of the size of 18 different anatomical structures by simply charting their estimation on a horizontal line. As part of a bigger research project, students were also asked each course day to report their activities (active dissection, prosection, etc.) on short questionnaires.

RESULTS: For 12 of 18 structures, appreciation of size improved during the course, for only 3 structures it worsened slightly. The mean of deviation of all size estimates from the respective norm value was considerably smaller at the end of the course. This reduction was statistically significant. For one structure, the sciatic nerve, a statistically significant relationship could be demonstrated between the improvement of size appreciation and the individual commitment to active dissection.

DISCUSSION: For the first time, we have demonstrated that an anatomical dissection course has a positive effect on students\' appreciation of size of anatomical structures. The comparatively high variability of our measured data is partly due to the natural variability of the size of anatomical structures, which is not sufficiently represented by a single norm value, but partly also due to methodological problems. Moreover, the accuracy of self-reported data may be limited. We are currently working on an improvement of our measuring methods and expect to be able to present more clear-cut results in the near future.

Methods/Teaching
vortrag

M2

Plastinated slices of the zonula fibers and the corpus vitreum after shrinkage reduction

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Plastination means to exchange the specimen's water against polymerizing resin by an intermedium, e.g. acetone. Increase in water content of the specimen parallels increase in shrinkage problems during the plastination procedure. The method to reduce shrinkage in the brain (90% water) is insufficient for delicate components of the inner eyeball, which consists of 99% water. We used eyeballs of the cow to improve shrinkage reduction. The zonula apparatus and the corpus vitreum were dissected under 0.9% NaCl solution. Shock-freezing was conducted at -85°C in 85% acetone and 15 % water followed by very slow dehydration with ascending acetone concentrations at -25°C. The dehydrated specimens were incubated with a mixture of Epoxid for Plastination (E12/E6) and xylene (1:7 at -25°C). Then we warmed the impregnated probes to 50°C to make acetone evaporate, whereas xylene and resin remained. This step enabled the mixture of xylene and resin to intrude into the very tender structures without vacuum. The mixture became gelatinous after one week, because the polymerized Epoxy was softened by xylene, which we extracted under low vacuum over days. The polymerised probes now looked like cotton wool. It meant that solely polymerized Epoxy stabilizes the probe. We finally plastinated the cotton-wool like specimen by use of pure acetone as intermedium with E12/E6. By our step-wise embedding procedure we obtained plastinated blocks to be finally cut into slices. They may give new insights into the close-to-native-behaviour of the zonula fibers and the corpus vitreum after having developed an appropriate staining procedure.

Methods/Teaching
vortrag

M3

Factors affecting the variation in the adult temporomandibular joint of archaeological human populations

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Although there is a wealth on literature about the morphology of the human temporomandibular joint (TMJ) components, little is known about the TMJ morphology in archaeological human populations. Since it is well known that the morphology of the craniofacial skeleton adapts to the environment, extinct human populations may be valuable for testing hypotheses and elucidating factors that are likely to be responsible for the functional morphology of the craniofacial skeleton. Thus, to elucidate factors that may affect the variation in the bony components of the TMJ, a preliminary study was conducted on the temporal articular surface of the TMJ of 30 skulls from an Iron Age and Middle Age populations from Lithuania and a mixed Neolithic and Bronze Age population from the Central Elbe-Saale region. Using 3D – photos of the skulls, length and width measurements of the TMJ were obtained and compared with external skull measurements. Distinct, random variation between the TMJ values from opposite sides of the cranium were identified as fluctuating asymmetry. ANOVA results suggest significant differences in the length of the TMJ between the population of the Central Elbe-Saale region and the two Lithuanian populations, but not between the two Lithuanian populations. Environmental factors, including geography, may be responsible for the variation in the TMJ form.

Gross Anatomy/Clinical Anatomy
vortrag

M4

Quantitative measurement of the androgen receptor in prepuces of boys with and without hypospadias

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Quantitative measurement of the androgen receptor in prepuces of boys with and without hypospadias.

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Hypospadias, a midline fusion defect of the male ventral urethra, is the most common congenital anomaly of the penis. Besides a higher incidence in affected families, identifiable causes are defects in the synthesis of testosterone or adrenal steroid hormones, receptor defects, syndrome-associated hypospadias, chromosomal anomalies, defects in other genetic factors, or exogenous forms. Nevertheless, most of the cases remain idiopathic. The aim of this study was to quantify the androgen receptor in prepuces of boys with and without hypospadias.

Forty prepuce specimens of circumcised boys, aged approximately 14 months, with (n=20) and without hypospadias (n=20), were deep-frozen and homogenized immediately after operation. The total RNA of the specimens was isolated and cDNA was written. With the help of real time PCR the probes were analysed for present androgen receptors and the amount was measured. Statistical analyses were performed.

Our observations show that the number of androgen receptors differs in healthy and affected boys in a high statistical manner ($p<0.01$). Therefore, our results provide evidence that the amount of androgen receptors influences the fusion of the male ventral urethra during early prenatal development in a crucial way. We conclude that the less the number of androgen receptors, the higher is the feasibility for developing a hypospadias.

M5

The role of smooth muscle in the pathogenesis of pelvic organ prolapse – a morphometric and immunohistochemical analysis of the uterosacral ligament

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Pelvic organ prolapse (POP) is a widespread disease with increasing occurrence which severely decreases the quality of life. Despite its epidemiological extent little is known about the pathophysiology. In the pelvic suspension system, the uterosacral ligaments (USL) form semicircular bands that stabilize the vaginal apex and the uterus to the dorsal body wall.
In paraffin sections of samples obtained from hysterectomy the smooth muscle component of the ligament was studied by morphometric determination of nuclear size as measure for the functional state of the cells, and by smooth muscle actin immunohistochemistry as indicator of structural abnormalities. USL from patients with POP were matched by age, parity, menopausal and hormonal state with ligaments from patients without POP.
In patients with POP the size of smooth muscle cell nuclei was significantly reduced in comparison with patients without POP of similar age, parity and hormonal state (28.87± 2.92µm² vs. 25.45±1.92 µm², $p<0.01$). Immunohistochemistry of smooth muscle actin showed a granular patchy distribution of reaction product as compared to homogenous staining in the control group.
We conclude that the functional state and the structural integrity of the smooth muscle component of the pelvic floor are impaired in POP patients, indicating a decisive role of smooth muscle in the pathogenesis of POP.

M6

Implications to back muscle function by the distribution of muscle fibre types

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Based on their supposed function, human back muscles have been classified as stabilisers and mobilisers (Gibbons & Comerford, ODR Mar/Apr 2001, 21-27). According to this, the m. multifidus is referred to as stabiliser and the m. sacrospinalis (mm. longissimus thoracis et iliocostalis lumborum) as mobiliser. Stabilisers are deep, mono- or oligosegmental, continuously active muscles controlling motion. Mobilisers are superficial, multisegmental muscles connecting thorax and pelvis and producing movements. This implies that stabilisers should be mainly composed of slow contracting, fatigue resistant fibres (type I) and mobilisers should mainly contain fast contracting, forceful fibres (type II). Whereas our studies on quadrupedal mammals support this concept as a widespread principle not exclusive to human, human muscle biopsy results are controversial.
To test this concept, we investigated the muscle fibre type distribution in the human back musculature using immunehistochemical methods. In the m. multifidus of a donated male cadaver, the percentage of type I fibres ranged between 30% and 90% which confirms its stabilising function. In the m. sacrospinalis, the percentage of type I fibres was only slightly lower (25%-75%) implying a more stabilising role as expected. Thus, the distribution of fibre types does not fully support the suggested concept for human back muscle function.

Experimental Morphology
vortrag

D1

Normal gastrulation and neurulation of rabbit embryos during prolonged ex-utero culture in the extraembryonic coelom of chick embryos

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Analysis of the effects of experimental manipulations on mammalian embryos often requires the use of whole embryo ex-utero cultures facilitating normal embryonic development. While short-term culture of six day-old rabbit blastocysts can be performed in cell culture medium supplemented with fetal calf serum (FCS) up to late primitive streak stages (about 18 hours), methods allowing prolonged ex-utero culture are presently not available. Here we tested whether the coelomic cavity of chick embryos can be used for prolonged ex-utero culture of rabbit blastocysts. Rabbit embryos were isolated from the uterus on day 6.2 post conceptionem. Blastocysts were kept in Ham's F10 supplemented with FCS until transplantation into the extraembryonic coelom of chick embryos (stages 24 to 27 according to Hamburger and Hamilton (1951)). Chick eggs were prepared as follows: the egg shell was windowed above the embryo. The extraembryonic coelom was opened in the tail region of the embryo by cutting the vitelline and chorionic membranes. The rabbit blastocysts then were pushed through this opening into the coelomic cavity near to the head region of the chick embryo. The window in the egg shell was resealed with tape and eggs were reincubated for 19 to 72 hours. Cultured rabbit embryos where fixed for morphological examinations, immunohistochemistry and in situ hybridization. After culture in the coelomic cavity for 19-24h, 43-48h and 70-72h, an almost normal morphology was found in 42% (n = 5/12), 28% (n = 12/43) and 25% (n = 2/8) of the transplanted rabbit embryos, respectively. Morphological, immunohistochemical and molecular comparison between cultured and regular in vivo developed rabbit embryos will be presented here. Our data show that explanted day six rabbit embryos can continue proper development for up to 72 hours when cultured in the extraembryonic coelom of day 5 chick embryos.

D2

The development and function of cartilage canals in the mice femur

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The development and function of cartilage canals in the mice femur
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A crucial step in epiphyseal bone development appears to be the formation of cartilage canals which run through the hyaline cartilage clearly prior to the establishment of a secondary ossification centre (SOC). In this study, we examined the femur in an age series of mice postnatal stages and various approaches (3D reconstruction, light microscopy, electron microscopy, immunohistochemistry and in situ hybridization) were performed to investigate both the fate of resting chondrocytes and the function of cartilage canals in endochondral bone formation.
Cartilage canals appear the first time at D5 and only a few are present at this stage. At D 8 the number of canals has increased some of which penetrating deeper into the chondroepiphysis exclusively made up from resting cartilage until this point of time. Macrophages are present at the canal blind ends, and several resting chondrocytes are either released as viable cells into the canal cavity or undergo programmed cell death. At D 10 cartilage canals are highly branched within the hypertrophic zone and the first signs of endochondral bone formation are detectable. Canals mesenchymal cells express type I collagen, and several small ossification centres are formed in this stage. At D18 these ossification nuclei have coalesced into a large SOC and short cartilage canals are still present.
Our results provide evidence that in mice the ossification of the epiphysis proceeds quickly and that canal mesenchymal cells have an osteogenic potential. During canal formation several resting chondrocytes undergo programmed cell death but others are released into the canal cavity and may differentiate in bone-forming cells.

D3

Multipotent progenitor cells from the apical human wisdom tooth germ region

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Human tooth germs are covered by neural-crest (NC) derived ectomesenchymal progenitor cells residing in spatial different niches. We focussed on apical parts (apical pad, AP) of these tissues, which probably remain in a more undifferentiated state compared to other parts of the developing periodontium or the dental papilla.
80 surgically removed human immature wisdom teeth were investigated histologically. The distribution of 30 stem cell and tissue differentiation markers was studied by using immunocytochemistry. The expression of selected markers was investigated in native tissues by using RT-PCR. AP cells from other teeth were cultured, expanded and passaged. Neurogenic, chondrogenic and osteogenic differentiation was achieved by culturing the cells in different media and analyzed by FACS and RT-PCR.
AP tissue could clearly be differentiated histologically from other parts of the developing periodontium or dental papilla by its loose connective tissue character. In contrast to the papilla, AP cells showed stronger immunostaining for stem cell and NC markers like AP-2, CD49e, msx2, runx2 or BMP-3 and stronger expression for twist1 or nanog. The osteogenic marker osteocalcin was expressed in both tissues. In vitro, we observed fibroblastic cells showing a specific growth pattern, expansion into passage 20 and expressing a typical CD subset (e.g. CD9+, CD44+, CD49b,d,e+, CD105+). Chondrogenic, osteogenic and neurogenic differentiation could be induced.
The AP region of the human developing periodontium represents a dental progenitor niche, which is easily to obtain performing surgery. Our data indicate not only the multipotency of NC derived AP cells but also their high potential for bone formation which may be useful for craniofacial tissue engineering.

MT II / Org1

A Novel Class of Membrane Proteins Shapes the Tubular Endoplasmic Reticulum

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How is the characteristic shape of a membrane-bound organelle achieved? We have used an in vitro system to address the mechanism by which the tubular network of the endoplasmic reticulum (ER) is generated and maintained. Based on the inhibitory effect of sulfhydryl reagents and antibodies, we demonstrate that network formation in vitro requires the integral membrane protein Rtn4a/NogoA, a member of the ubiquitous reticulon family. Both in yeast and mammalian cells, the reticulons are largely restricted to the tubular ER and excluded from the continuous sheets of the nuclear envelope and peripheral ER. Upon overexpression, the reticulons form tubular membrane structures. The reticulons interact with DP-1/Yop1p, a conserved integral membrane protein that also localizes to the tubular ER. The simultaneous absence of the reticulons and Yop1p in S. cerevisiae results in disrupted tubular ER. We propose that these proteins stabilize ER tubules by utilizing a common hairpin structure to partition into and stabilize highly curved membranes.

MTII / Org2

The LYSOSOME: more than a waste basket of the cell? Insights into lysosomal (patho) physiology from knockout mouse studies

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In the past years we have established a number of different mouse mutants with deficiencies in selected genes for lysosomal membrane proteins, sorting receptors and lysosomal hydrolases. Using these mouse models we were able to decipher functions of the corresponding proteins in lysosomal storage disorders, biogenesis of the lysosomal compartment, autophagy and phagocytosis.

MTII / Org3

Normal and disordered biogenesis of lamellar bodies, lysosome-related organelles in type II alveolar epithelial cells involved in surfactant storage and secretion

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The pulmonary surfactant system has biophysical and immunomodulatory functions that are essential for normal lung function. Surfactant consists of about 90% lipids and about 10% proteins, including the surfactant-associated proteins SP-A, -B, -C, and -D. Only at the EM level does surfactant show its morphological complexity. Surfactant is synthesized, stored, secreted, and to a large extent recycled by type II alveolar epithelial cells. The vast majority of intracellular surfactant material (all lipids and the hydrophobic SP-B and -C) is assembled in specific storage organelles, lamellar bodies (LB), prior to secretion.
LB ultrastructure varies between species. In the human, LB consist of a surrounding limiting membrane, concentrically arranged phospholipid lamellae, a matrix, and a projection core where SP-B and -C are localized. Due to their lysosomal features (enzyme equipment, acidic pH), LB are now considered as members of the family of lysosome-related organelles.
The detailed mechanisms of LB biogenesis are still incompletely understood. Steps involved in LB biogenesis include lipid and SP synthesis, processing, trafficking and fusion into composite bodies, maturation into LB, and finally secretion via exocytosis.

Morphological tools (EM, immuno-EM, stereology) are essential to characterize disorders of LB biogenesis. Recent data from human patients and animal models with genetic alterations clearly demonstrate that disordered LB biogenesis results in severe lung disease. In turn, these studies help elucidating normal LB biogenesis by identifying novel genes and pathways. Deficiency of SP-B or the ATP binding cassette protein ABCA3 results in underdevelopment of LB (too few and/or too small) leading to severe perinatal respiratory distress syndrome. On the other hand, deficiency of the hydrophilic lung collectin SP-D results in overdevelopment of LB (too many and/or too big) associated with alveolar lipidosis, type II cell hyperplasia and hypertrophy, and emphysema. Here, we give an overview on normal and disordered LB structure and biogenesis.

MTII / Org4

Exosome biogenesis and retrovirus budding

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MTII / Org5

Mechanisms of Endocytosis and Signalling revealed by Functional Genomics

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Endocytosis is an essential process in eukaryotic cells, which not only serves the purpose of internalizing molecules from extracellular space, but is also one of the key mechanisms for intracellular processes such as establishment of cell polarity, intracellular signaling and protein sorting. In receptor-mediated endocytosis, for instance, the internalized cargo can be destined for degradation – thereby shutting down an extracellular signal; on the other hand, the cargo can be recycled to the cell surface via the recycling pathway, which would lead to a prolongation of the extracellular signal. One of the central questions is to unravel the molecular mechanisms underlying endocytic uptake and sorting of cargo in the cell and most importantly, to identify the molecular players regulating this complex process.
Here we report a genome wide screen for the identification of proteins involved endocytosis. For this purpose we use a reverse genetic approach using RNAi and an image-based assay that captures a number of parameters indicative of the distribution of cargo upon disruption of the endocytic pathway. To distinguish between recycling cargo and cargo that is destined for degradation, we use two differentially sorted cargo molecules: EGF, as an example of cargo destined for degradation and Transferrin, as an example of cargo destined for recycling. Various parameters are measured for endosomes from the acquired images (size, average number, shape, subcellular localization) and any significant differences from control experiments are scored. Clustering of the scored phenotypes along with the determination of statistically relevant functional domains and classifications allows for annotation and grouping of the hits.

MTII / Org6

Cryo-EM tomography to analyze subcellular compartments

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Over the past decades, cryo-electron microscopy of vitrified specimens has yielded a detailed understanding of samples reassembled /in vitro/ from purified components and from small bacterial cells and organelles. However, our knowledge of whole eukaryotic cells and tissue /in vivo/ remains limited by the chemical treatments commonly used to observe cellular architecture using electron microscopy. We use cryo-electron tomography of vitreous sections to investigate the ultrastructure of microtubules and desmosomes in their cellular context. Vitreous sections were obtained from organotypic neuronal cells derived from rat hypoccampus, and from Chinese hamster ovary cells in culture. Microtubules revealed their protofilament ultrastructure, polarity, and in the most favorable cases, molecular details comparable to those visualized in three-dimensional reconstructions of microtubules reassembled /in vitro/ from purified tubulin. In the desmosomes characteristic features could be visualized. The resolution of the tomograms can thus be estimated at app. 4 nm, which enabled the detection of luminal material that is connected to the microtubule wall. This work provides a first step towards a description of organelles and macromolecular complexes in their native structure.

MTII / Org7

Primary cilia – multiple roles of a re-discovered organelle in physiological and pathophysiological processes

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For a long time, primary cilia have been considered evolutionary relicts but that may only have been so because no function could be attributed to them. This has changed dramatically in the recent past when it was discovered that primary cilia are probably involved in polycystic kidney disease and left-right differentiation.

Primary cilia can be found on many, if not most, cell types in our body. Ultrastructurally they are composed of nine peripheral doublets of microtubules, in contrast to kinocilia the two central microtubules are lacking and therefore they are thought to be immotile in most circumstances. One exception are the cilia found in the primitive node, of which one subpopulation has been observed to be rotating. By such a rotating movement a so far hypothetical morphogen is believed to be distributed extracellularly in an asymmetrical fashion, and the thus generated concentration gradient of the morphogen is sensed by a second population of primary cilia. Such a break of symmetry could elegantly explain the establishment of the left-right symmetry axis during development. Indeed several proteins which have been localized to primary cilia are mutated in patients and in rodent models of situs inversus.

A sensory role for primary cilia has also been suggested in the case of tubular epithelial cells in the kidney and this would explain the combined appearance of situs inversus and cystic kidney diseases. In the kidney, primary cilia are found as extending from the apical plasma membrane into the tubular lumen where they may act as mechano- and/or chemosensors. Exposing kidney epithelial cell lines in culture to a flow of medium will cause an increase in the intracellular calcium concentration. By such a mechanism tubular epithelial cells could sense the flow velocity and indirectly the diameter of the renal tubules. Looking at the amazingly regular dimensions of the different structures in the kidney, one has to wonder how the diameter of the tubular lumina is determined and maintained. Many of the genes mutated in cystic kidney diseases have been identified and except for the one mutated in medullary cystic kidney disease they have been localized to primary cilia.

Polycystin-2, one of the two proteins mutated in patients suffering from ADPKD, represents a member of the TRP family of cation channels. We have been able to determine the tissue distribution and the intracellular location of the rat and human polycystin-2 proteins. As most other cyst-associated proteins, polycystin-2 is present in primary cilia. By establishing transgenic animals we were also able to demonstrate that a truncated polycystin leads to polycystic kidney disease and retinal degeneration. In cell culture synthesis of the mutant polycystin-2 protein causes shorter cilia thus again hinting at the pathogenetic role of primary cilia.

MTII / Org8

The axon initial segment and its role in structural and functional compartmentalization of neurons

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The axon initial segment (AIS) is a unique neuronal compartment, which is considered crucial for the initiation of action potentials. In addition, the AIS provides a membrane-associated diffusion barrier which controls the sorting of membrane proteins into the axon. Structurally, the AIS is characterized by a membraneassociated dense undercoating, fasciculated microtubules, and the presence of a cisternal organelle (CO). Recently, we have identified the following novel compartment-specific features of the AIS, which suggest that it is involved in additional biological functions:

(1) The AIS was found to be enriched in proteins, which function as activators of NF- κB, a ubiquitous transcription factor which is crucial for the regulation of cell survival. These proteins included a phosphorylated form of the inhibitory protein IκBα and the activated form of the IκB-Kinase. These findings suggest that the AIS is involved in neuronal NF-κB activation.

(2) The role of the AIS-specific diffusion barrier was studied in the cerebellum of mice deficient for the membrane adaptor protein ankyrinG, a crucial component of the diffusion barrier. Interestingly, axons of ankyrinG-deficient mice exhibited several dendritic features, including cytoplasmic protrusions, which closely resembled dendritic spines. These findings demonstrate that disruption of the AIS-specific diffusion barrier leads to impairment of the axonal phenotype.

(3) The actin-binding protein synaptopodin – previously identified in the dendritic spine apparatus – was found to be associated with the CO. A lack of the CO was noted in AIS of synaptopodin-deficient mice, demonstrating that synaptopodin is an essential component of the CO.
Taken together, our studies point to novel functions of the AIS, including NF-κBrelated signalling and maintenance of axo-dendritic polarity.

MT II / Org9

Molecular organization of the pre- and postsynaptic specialization

Böckers T, Ulm

Glutamatergic synapses in the central nervous system are morphologically characterized by a pool of neurotransmitter filled synaptic vesicles (SV) in close apposition to the presynaptic membrane and an electron dense web underneath the postsynaptic membrane, called the postsynaptic density (PSD). Docking and priming of synaptic vesicles to the membrane is organized by proteins of the so called active zone (CAZ). Up to know only a few multidomain molecules are known that localize specifically to the synaptic active zone serving different functions like scaffolding, channel clustering and/or SV priming. Among these CAZ proteins are Munc13s, Piccolo, Bassoon and RIMs that are essential for synaptic function. The cytomatrix of the PSDs is composed of a dense network of several hundred different proteins. Prominent PSD proteins like members of the MaGuk or ProSAP/Shank family build up a dense scaffold that creates an interface between clustered membrane bound receptors, cell adhesion molecules and the cytoskeleton. Small GTPases and regulating proteins are also enriched in PSDs being the molecular basis for regulated structural changes of cytoskeletal components within spines and synapses in response to external or internal stimuli, i.e. synaptic activation. The undisturbed interplay of molecular components of the pre- and postsynapse is a prerequisite for the rapid synaptic rearrangement (structural plasticity) that is believed to underlie learning and memory formation.

MTII / Org10

Cofilin may play an important role in stopping migrating neurons in response to Reelin

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Recently we have shown that Reelin, an extracellular matrix protein in the marginal zone of the cerebral cortex and hippocampus, is a positional signal for radially migrating neurons. In wild type (wt) animals, the marginal zone is almost cell-free but is full of neurons in reeler mutants lacking Reelin. Thus, Reelin is also believed to function as a stop signal for radially migrating neurons. However, the underlying signaling cascade is still unclear. Here, we suggest that cofilin, a small actin-binding protein, may play an important role in stopping migrating neurons in response to Reelin. Cofilin binds to actin monomers and filaments and regulates actin filament dynamics and reorganization by stimulating the depolymerization and severance of actin filaments. The activity of cofilin is reversibly regulated by phosphorylation and dephosphorylation at Ser-3 with the phosphorylated form being inactive. Our Western Blot analysis shows that in dissociated neurons prepared from reeler mice, the treatment with recombinant Reelin results in a high level of phosphocofilin when compared to controls. Immunostaining shows that in wt mice, high expression of phosphorylated cofilin is mostly observed in close vicinity of reelin-positive regions. We suppose that, once the tip of the leading process of a migrating neuron has reached an area of high Reelin concentration, cofilin will be phosphorylated and migration will be stopped.

N1

Accumulation of activated IKK and phosphorylated I□B□ in nodes of Ranvier

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Nuclear factor-κB (NF-□B) is a ubiquitously expressed transcription factor that regulates inducible gene expression crucial for the regulation of immunity, inflammatory processes and cell survival. The classical NF-□B heterodimer p50/p65 is mainly controlled by the inhibitory protein I□B□. Phosphorylation of I□B□ at Serine 32/36 by the activated IKappaB Kinase (IKK) leads to activation of NF-□B. Recently, we demonstrated a striking enrichment of activated IKK and phosphorylated I□B□ (pI□B□) in the axon initial segment (AIS). Since the AIS shares fundamental structural and functional features with nodes of Ranvier (NR), we examined whether enrichment of activated IKK and pI□B□ also occurs in NR. Double immunofluorescent labeling of rat brain sections and rat dorsal root ganglia (DRG) was carried out using antibodies against activated IKK, pI□B□ and □IV-spectrin, an established marker for AIS and NR. Interestingly, both activated IKK and pI□B□ accumulated in central and peripheral NR. Immunolabeling for activated IKK and pI□B□ extended from the nodal domain into the adjacent paranode. Laser scanning microscopy demonstrated that activated IKK preferentially clustered beneath the cytoplasmic membrane of NR. By comparison, pI□B□ predominantly accumulated within the cytoplasm. This cytoplasmic distribution was further studied using preembedding immunoelectron microscopy. Anti-pI□B□-labeling mainly decorated fasciculated microtubules of the NR. In conclusion, the coincident accumulation of activated IKK and pI□B□ in AIS and NR suggests that these specific axonal compartments contribute to neuronal NF-□B regulation.

N2

Analysis of connexin expression during mouse Schwann cell development: Connexin29 as a novel marker for the transition of neural crest to precursor cells

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Connexins are transmembrane proteins forming gap junction channels for direct intercellular and, for example in myelinating glia cells, intracellular communication. In mature myelin-forming Schwann cells, expression of multiple connexins, i.e. connexin (Cx) 43, Cx29, Cx32, and Cx46 (after nerve injury) has been detected. However, little is known about connexin protein expression during Schwann cell development. We used histochemical methods on wildtype and Cx29lacZ transgenic mice to investigate the developmental expression of connexins in the Schwann cell lineage. Our data demonstrate that in the mouse Cx43, Cx29, and Cx32 protein expression is activated in a developmental sequence that is clearly correlated with major developmental steps in the lineage. Only Cx43 was expressed from neural crest cells onwards. Cx29 protein expression was absent from neural crest cells but appeared as these cells generated precursors (embryonic day 12) both in vivo and in vitro. This identifies Cx29 as a novel marker for cells of the defined Schwann cell lineage. The only exception to this were dorsal roots, where the expression of Cx29 was delayed four days relative to ventral roots and spinal nerves. Expression of Cx32 commenced postnatally, coinciding with the onset of myelination. Thus, the coordinated expression of connexin proteins in cells of the embryonic and postnatal Schwann cell lineage might point to a potential role in peripheral nerve development and maturation.

N3

Morphological correlates of recovering vibrissal whisking after manual mechanical stimulation of denervated whiskerpad muscles*

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Collateral branching of injured axons and their subsequent regrowth to incorrect targets have been considered for long time as major reasons for the poor recovery of motor function after peripheral nerve lesion. Surpisingly, using the facial nerve transection paradigm and a combination of triple retrograde neuronal labeling plus video-based motion analysis of vibrissae movements, we recently showed that a restoration of function was associated with a diminished polyneuronal innervation of the motor end-plates, rather than with less collateral branching of axons at the lesion site. Since polyneuronal innervation of muscles is activity-dependent and can be manipulated, these findings raised hopes for clinically feasible and effective therapies. Accordingly, our subsequent studies demonstrated that a mechanical stimulation of vibrissal muscles (gentle strokes by hand for 5 minutes daily) after facial nerve transection and suture resulted in full recovery of vibrissal motor performance. In the present report we show that restoration of function after mechanical stimulation is associated with (i) inhibited bridge-formation by the terminal Schwann cells that attract and direct intramuscular axonal sprouts towards denervated end-plates, (ii) reduced post-lesional intramuscular (terminal) axonal sprouting and (iii) reduced degree of polyneuronal re-innervation of the motor end-plates. Mechanical stimulation had no effect on collateral axonal branching at the lesion site, or on the dynamic reorganization of the motor cortex after facial nerve transection. This therapy was not effective under conditions of nerve resection (removal of 1 cm nerve length from all facial nerve branches), after sensory depletion of the whiskerpad (extirpation of the infraorbital nerve) and after a delay with two months. These findings have immediate potential for enhancing clinical rehabilitation strategies to restore function following periphery nerve injury. *Supported by the Köln Fortune Programm (E.S.), Jean-Uhrmacher Foundation (O.G.-L., M.S., S.A.), Sokrates/Erasmus European Programm (A.A., M.C.), DFG (AN 331/3-1, AN 331/5-1) and BMBF (N.S. 01GN01111).

N4

First three-dimensional reconstruction of „synaptic bodies“ in cones, rods and bipolar cells of the human retina

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To gain a realistic functional picture of the visual process, it is important to understand both morphology and spatial arrangement of the ribbon synapses being the basis for the ultra-fast signal transduction in the visual pathway. The synaptic terminals of the involved rods, cones and bipolar cells, are characterized by electron-dense synaptic bodies (SBs). To date few of these presynaptic transmitter-vesicle-binding organelles were reconstructed in man (rods only). Therefore we investigated the three-dimensional (3D) shape and the exact placement of the appropriate SBs. Further we visualize the postsynaptic elements of rod and cone synapses. The reconstructions base on digital images taken of 10 different regions of a complete series of 55 electron microscopic sections from one human retina analysed. Yet, a total of 10 rod-, 18 cone- and 22 bipolar-cell-SBs were visualized in 3D. By far most SBs were attached to an electron-dense plaque anchored to the cell-membrane. In rods about 2/3 of the SBs had a C-shaped, eventually bent or slightly twisted, electron-dense centre with attached vesicles, two parallel flat major surfaces and a constant thickness of 35 nm. Some bifurcated rod ribbon synapses showed two SBs. In cones, ribbon-like organelles were predominant and rarely involved in two different synapses. Some SBs showed stronger torsions or deviations in multiple planes. Partly they had short, strongly bent sections, and only few were virtually straight. Some cones showed several finger-like lateral processes terminating either basally on neighbouring cones or laterally on different rods at non-ribbon synapses. Unlike others, bipolar cell SBs mostly were slightly twisted plates with considerably minor volume resp. surface area. Our results for the first time demonstrate the complicated morphology of human cone-terminal-synapses in 3D. The SBs seem essential for the high transmitter output of the first two neurons of the visual pathway possibly by retaining ready-to-release vesicles close to the active zones.

N5

The role of CLOCK and BMAL1 for photic induction of Per1 and Per2 mRNA in SCN and retina

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Circadian rhythms control many aspects of mammalian physiology and behavior. The suprachiasmatic nuclei (SCN) in the hypothalamus contain the circadian pacemaker which generates circadian rhythms and synchronizes peripheral clocks. Circadian oscillations are based on transcription-translational feedback loops of clock genes which encode for transcriptional regulators. The positive regulators CLOCK and BMAL1 activate transcription of the negative regulators PER and CRY through E box enhancer elements. PER and CRY interact with the BMAL1:CLOCK complex to inhibit their own transcription thus shutting down the feedback loop. The circadian clockwork in the SCN can be entrained to the environmental light/dark cycle by light received through the retina. A nocturnal light pulse leads to an increased expression of Per1 and Per2 in the SCN and thus resets the clock with consequent changes in behavioral rhythms. Light also induces expression of Per1 in the retina which represents another circadian oscillator. In the SCN expression of Per1 in response to a light pulse is known to be mediated by CREB-responsive elements (CRE) in the promoter region. In the retina, however, CLOCK is thought to play an important role in photic induction of Per1. We tested the hypothesis whether BMAL1 and CLOCK are dispensable for the light induction of Per1 and Per2 in the SCN but not in the retina by comparing expression levels of both genes in BMAL1/CLOCK-deficient and wildtype mice after light pulses presented during early (CT14) or late (CT22) subjective night. Our data show that CLOCK and BMAL1 are indeed not required for the photic induction of Per1 and Per2 in the SCN.

N6

The foetal mouse circadian system contains a central oscillator

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The circadian system contains a central biological clock in the suprachiasmatic nucleus of the hypothalamus (SCN) that regulates circadian rhythms of behaviour, metabolism and physiology. Even in the foetal SCN of rodents, rhythms in electrical activity, metabolism, and vasopressin release have been demonstrated. At the molecular level, the mature endogenous clock is based on transcriptional/translational feedback loops of clock genes (Clock, Bmal1, mPer, mCry) which encode for positive and negative transcriptional regulators. However, little is known about the ontogeny of the molecular mechanisms underlying the endogenous rhythm generation. Therefore, we analyzed the levels of clock gene proteins in the mouse SCN on embryonic day 18 (E18), postnatal day two (P2) and postnatal day 21 (adult) at four different time points using immunohistochemistry. At E18 we found a low amplitude rhythm in the negative regulators mPER1 and mPER2 with the same phase as in P2 and adult mice. In addition we observed constitutive levels of the positive regulators CLOCK and BMAL1 in the SCN of E18 mice consistent with P2 and adult mice. However, in contrast to adult mice we found constitutive levels and the absence of the negative regulators mCRY2 and mCRY1, respectively, in the SCN of E18 mice. To test whether the observed oscillation of the PERs in combination with constant levels of CRY2 was sufficient for driving a rhythm of an SCN-output gene in E18 mice, we analyzed the vasopressin mRNA levels at different time points. We found an oscillation of vasopressin expression in the SCN of E18 mice suggesting the existence of a molecular oscillator in the foetal mouse that is sufficient for driving rhythmic output.

N7

Effects of melatonin injections on type 2 deiodinase mRNA levels in the mediobasal hypothalamus of male golden hamsters

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For many species living in temperate zones, the timing of reproduction is regulated by the photoperiod. In mammals, the photoperiod is transmitted by a daily cycle of melatonin secretion from the pineal gland. However, the molecular basis of the action of melatonin on the gonadal axis remains unknown. A previous study has shown that daily melatonin injections given for 8 weeks at late afternoon to male Djungarian hamsters kept under long day condition suppresses the expression of type 2 deiodinase (Dio2) in the mediobasal hypothalamus. This enzyme is known to play an important role for the photoperiodic response by converting thyroid hormone T4 into bioactive T3. In this study, we determined the time course of the suppression of Dio2 mRNA levels by melatonin in golden hamsters. Melatonin was injected intraperitoneally in the late afternoon for 10 days into golden hamsters kept under long day condition. Thereafter, the animals were killed, their brains were dissected, cut into frontal sections and used for in situ hybridization. Dio2 mRNA was observed in the ependymal cell layer lining the basolateral walls of the third ventricle (EC) and in the zone that overlies the tuberoinfundibular sulcus and does not contain any cell bodies (TIS). In the EC, Dio2 mRNA levels were shown to be suppressed by melatonin already at the next day of the first treatment, which means 19 h after the injection. In the TIS, the suppression was found at the second day after the onset of treatment. The suppressive effects were found to persist for the total period of the investigation (10 days). These results suggest that the inhibition of Dio2 expression by melatonin occurs rather rapidly.

N8

Increased MAP Kinase activity in the hippocampus after targeted motor skill learning task in the rat

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MAPkinase activation is an indicator of central nervous activity and can be visualized by immunohistochemistry. We asked whether a difference between unspecific and targeted learning led to distinct patterns of MAPkinase activation in the brain.
To distinguish between unspecific and targeted learning, juvenile rats were trained to grasp a food pellet with a dominant right forelimb (targeted motor skill learning) after release via a touch sensor on the opposite side of the cage (unspecific conditioning). The animals were sacrificed within 15 minutes after the training session and the brains immediately processed for histology.
We performed immunohistochemistry of the phosphorylated MAPkinase in serial paraffin sections to depict activated areas in the rat brain. Three groups of rats were studied:
1. The experimental group of rats releasing the pellet by touching the sensor and subsequent targeted grasping of the pellet.
2. Motivated rats releasing the pellet by touching the sensor without additional grasping.
3. Naïve rats that received the pellet without touching and grasping.
In the experimental group, activated MAPkinase immunoreactivity was increased in the hippocampus with pronounced higher activity on the contralateral side. In the motivation-group, there was an elevation of the MAPkinase activity with no side difference. In the naïve-group, only basal levels of MAPkinase could be detected. The results indicate that the contralateral side of the hippocampus is activated during a targeted motor skill learning task.

N9

Reelin induces membrane motility in cortical neurons

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Reelin, an extracellular-matrix protein, regulates neuronal positioning during neurodevelopment and modulates synaptic plasticity in the adult brain. Binding to its receptors, the very low-density lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (apoER2), activates nonreceptor tyrosine kinases of the Src family resulting in the tyrosine phosphorylation of the cytoplasmic adaptor protein Disabled-1 (Dab1) and induction of phosphatidylinositol 3-kinase (PI3K) activity. By controlling the stability and assembly of the actin cytoskeleton PI3Ks regulate neuronal morphogenesis and migration. We report here that Reelin increases membrane motility in dendrites of cultured stage III embryonic cortical neurons of the mouse. Pretreatment of cortical neurons with pharmacological inhibitors of PI3K or genetic ablation of the adapter protein Dab1 abolished the effect of Reelin. Our results suggest a role of the Reelin signaling pathway in modulating the actin cytoskeleton, which results in an increased membrane motility.
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N10

Fibroblast-growth factor 2 (FGF-2) is a growth factor with antidepressive properties

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Depression is a major mood disorder and several lines of ecidence suggest an involvement of fibroblast growth factors (FGFs) in this disorder. Since treatment with antidepressants has been reported to increase mRNA levels of FGF-2 in the forebrain, we hypothesized that FGF-2 may play an important role in depression and/or in the action of antidepressants. We have used olfactory bulbectomy (OBX), an established animal model of depression, and FGF-2 deficient mice to investigate putative antidepressant properties of FGF-2 and the involvement of FGF-2 in the actions of antidepressive drugs. Intraventricularly applied FGF-2 has antidepressant-like effects in bulbectomized mice as well as in the tail suspension test. Like the antidepressants amitriptyline and citalopram, FGF-2 attenuated OBX-mediated neurodegeneration in the piriform cortex (Pir) and posterolateral cortical nucleus of the amygdala (PLCo) as well as depressive-like behaviour. Following OBX, FGF-2 knockout mice, similar to wildtype littermates, developed behavioural alterations, as well as neurodegeneration in the Pir and PLCo. In contrast to bulbectomized wildtype mice, treatment with amitriptyline, however, failed to reverse this behaviour in FGF-2 deficient mice. Our data suggest that FGF-2 has antidepressive properties, since FGF-2 administered to bulbectomized mice can (1) exert antidepressant effects and (2) partially reverse behavioural and cellular changes induced by OBX. Moreover, our data indicate that FGF-2 seems to be an essential mediator in the action of antidepressant drugs, raising the possibility that drugs that selectively stimulate the production of FGF-2 could represent a novel class of antidepressants.
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N11

Detection of steroid receptors in the embryonal tissue and relationship with Huntington Desease

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Background and purpose : Steroid receptors are a special group of receptors with wide efficiency. Androgen and oestrogen receptors and their expression are linked e.g. with reproduction control and sexual behaviour, but their relation with behavioural models, perception, memory and stress are unclear so far. In this project we would like to focus on monitoring SR expression in embryonic tissues and subsequently in adult tissues, such as CNS, monitoring development aspects and relations with neurodegenerative diseases, such as Huntington's disease. HD is autosomally a dominant hereditary disease with full penetration. HD occurrence in the countries of Europe and America is currently estimated at about 1:15,000. HD is in a typical case manifested by a clinical trias: motive, cognitive and psychiatric affliction. Material and Methods : We used imunnohistochemistry, imunnofluorescence and RT-PCR methods for detection SR in the embryonal and adult tissue. Conclusion : Surveying the expression of steroid receptors during development is an essential step for further procedure and monitoring of expression in adult tissues. Supported by Faculty of Medicine and Dentistry Palacky University guideline number 91110071-39 and scholarship cooperation Ministry of Education Czech Republic and Ministry of Education P.R. of China. Key words : steroid hormone receptors(SR); embryonal tissue; Huntington disease(HD).

N12

Focal cerebral ischemia induces up-regulation of Beclin 1 and autophagy

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Autophagy is a highly regulated cellular mechanism for the bulk degradation of cytoplasmic contents which seems to be implicated in a variety of physiological and pathological conditions relevant to neurological diseases. We examined whether autophagy is involved in mechanisms of cell death after focal cerebral ischemia. Protein level and distribution of Beclin 1 (Bcl2 interacting protein) and microtubule-associated protein 1 light chain 3 (LC3) were investigated, both of which were previously found to promote autophagy. We found a dramatic elevation in Beclin 1 levels in the penumbra of rats challenged by cerebral ischemia. Interestly, Beclin 1 upregulation starts at early postischemic stages (6 h) and lasts for at least 48 h. A subpopulation of cells with high Beclin 1-levels is also expressing the active form of caspase-3. In addition, not all cells with high levels of Beclin1 display dense staining of LC3. Some neuronal cells that overexpress Beclin 1 exhibited DNA damage and others not, which indicates that not all the Beclin 1-upregulating cells are predestined to die. Moreover, many blood vessels in the penumbra showed punctuate dots of Beclin 1 expression in the endothelial cells at 24 and 48 hours postischemia. The upregulation of Beclin 1 and related changes of LC3 in the ischemic penumbra may represent an enhanced autophagy either as a mechanism to rescue injured cells or a process leading to cell demise.

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N13

Innate immunity responses at the supraspinal level determine central neuropathic pain

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Microglial activation may be an important factor in the development and chronicity of neuropathic pain. Here we explore whether rats subjected to spinal ischemia that develop allodynic pain and rats that do not show signs of allodynic behavior differ in the degree of presumed microglial activation along the pain neuraxis with particular emphasis on the primary afferent relay center of the brain stem gracile ncl.

Three groups of rats (n=6 in each group) were investigated; rats subjected to spinal ischemic injury with allodynia, without allodynia and sham treated rats. After assessing allodynic behavior with von Frey hairs 2 weeks after photic ischemic lesion of the lower thoracic spinal cord rats were fixed by perfusion with Bouin-Hollande fixative. Brain and spinal cord were postfixed in the same fixative for 24-48 hours and dehydrated in 2-propanol. Deparaffinized serial sections of the lower brain stem bearing the gracile ncl. or spinal cord were immuno-stained for the microglial marker IBA-1, the microglial complement activation marker C1q and the microglial response gene cyclooxygenase-1 (COX-1).

Both allodynic and non-allodynic rats exhibited microglial activation and increased numbers of microglial cells in the gracile ncl. as compared to sham. The increase in the number of IBA-1, C1q and COX-1 positive microglial cells in the gracile nuclei in allodynic rats was significantly higher than that in non-allodynic rats.

Our data indicate that the gracile ncl. in addition to the spinal cord is an important pain relay center in which the degree of microglial activation and production of inflammatory mediators such as complement and prostaglandins but not microglial activation per se determine whether neuropathic pain develops or not. Selective suppression of excess microglial activation at the supraspinal level is proposed as a new strategy for the treatment of central allodynic pain.

C1

Steric hindrance of desmoglein 3 transinteraction is involved in pemphigus vulgaris but not in pemphigus foliaceus

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The severe skin disease pemphigus is clinically defined as pemphigus foliaceus (PF) and pemphigus vulgaris (PV) and caused by autoantibodies against desmosomal cadherins. Whereas PF patients show autoantibodies against desmoglein 1 (Dsg 1), sera of PV patients contain desmoglein 3 (Dsg 3) antibodies alone or in addition to antibodies against Dsg 1. It is still controversially discussed whether pemphigus autoantibodies directly interfere with desmoglein binding by steric hindrance. Using atomic force microscopy, we provide evidence that PV-IgG directly interfere with Dsg 3 but not with Dsg 1 transinteraction. IgG purified from different PV sera either containing Dsg 3 antibodies only or antibodies against both Dsg 1 and Dsg 3 reduced Dsg 3 binding activity in single molecule force distance cycles by 45 and 55 %, respectively, i.e. to a similar extent as Ca2+-depletion by EGTA. AK 23, a monoclonal mouse PV antibody directed against the aminoterminal domain of Dsg 3 also reduced Dsg 3 binding by steric hindrance. However, PV-IgG containing antibodies against both Dsg 1 and Dsg 3 as well as Dsg 1 antibody containing PF-IgG did not block Dsg 1 transinteraction in this cell-free system. Nevertheless, all PV- and PF-IgG used in this study caused keratinocyte dissociation in HaCat monolayers, accompanied by a generalized loss of desmosomes under PV-IgG treatment. Taken together, these data indicate for the first time that PV and PF profoundly differ with respect to the molecular mechanisms involved. Our results show that PV-IgG inhibit Dsg 3 binding, indicating a contribution to pemphigus pathogenesis. On the other hand, because epidermal blistering in PV only occurs when Dsg 1 antibodies are present which do not cause steric hindrance, additional autoantibody-triggered cellular signalling pathways must also be involved.

C2

Modulation of TNF-α induced intrinsic apoptotic pathways in chondrocytes by IL-10

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TNF-α is well known to promote chondrocyte apoptosis which contributes to the pathogenesis of osteoarthritis. The aim of the study was to define the interrelation between TNF-α and the immunomodulatory cytokine interleukin (IL)-10 on chondrocyte survival *in vitro*.
Serumstarved chondrocytes were either stimulated with 10 ng/mL recombinant TNF-α or IL-10 alone or co-treated with 10 ng/ml IL-10 and TNF-α for 48 hours. To evaluate whether effects of these cytokines might be due to modulation of the IL-10 or TNF-α expression in chondrocytes, mRNA analyses were performed using RT-PCR. Activities of the initiator caspase-8, which is associated with TNF-receptor-1 induced extrinsic apoptotic pathways, activated caspase-9 as an indicator of intrinsic pathways and the downstream effector caspases-3/-7 were measured by caspase activity assays. The mitochondrial apoptotic inducer Bax and the suppressor Bcl-2 were assessed using western blot analysis.
TNF-α amplificated the IL-10 mRNA expression in chondrocytes. Compared to the controls, TNF-α stimulation and co-treatment of chondrocytes with TNF-α and IL-10 stimulated significantly the TNF-α mRNA expression, whereas IL-10 alone had no effect. TNF-α induced an increase in all investigated caspase activities in chondrocytes. Despite stimulation with IL-10 alone had no effect on caspase activities in chondrocytes compared to the controls, co-treatment with IL-10 and TNF-α for 48 hours had an inhibitory effect on the caspase activities in comparison to the cultures stimulated with TNF-α alone. TNF-α lead to a significant elevated Bax expression and also the Bax/Bcl-2 protein ratio increased significantly compared to the controls and the co-treated cultures. Additionally, the cotreatment stimulated significantly the Bcl-2 expression in chondrocytes.
IL-10 seems to modulate the pro-apoptotic capacity of TNF-α in human articular chondrocytes as shown by the decrease in caspase activities and significant decrease in Bax/Bcl-2 ratio suggesting an interplay between IL-10 and TNF-α on mitochondrial proapoptotic
pathways.

C3

IGF-I and TGF-β exert common anabolic effects on articular chondrocytes in vitro

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Autologous chondrocyte transplantation (ACT) is used repairing articular cartilage lesions but there are problems associated with the integration of the newly formed tissue within the articular defect. During the expansion phase of chondrocytes in monolayer culture cells gradually loose their phenotype and dedifferentiate into fibroblast like cells, producing nontypical matrix proteins; i.e. collagen type I instead of collagen type II. This dedifferentiation process is a challenging problem for ACT since dedifferentiated chondrocytes are incapable of initiating cartilage defect repair. Growth factors exert anabolic effects on chondrocytes *in vivo* and *in vitro* by influencing chondrocyte differentiation, growth and survival. Here we examined the effects of IGF-I and TGF-β on the chondrogenic potential of dedifferentiated human chondrocytes. Human articular chondrocytes were cultured with interleukin-1β (IL-1β) to induce dedifferentiation, following the co-treatment with either IGF-I, TGF-β or a combination of both at various concentrations (1, 10, 100 ng/ml). The effects of growth factors on monolayer and high-density cultures were then investigated. IL-1β-treated cells rapidly dedifferentiated and lost their phenotype. Expression of collagen type II, β_1-integrin, extracellular regulated kinase (Erk) and the chondrogenic transcription factor Sox9 were down regulated. Cells treated with IGF-I and TGF-β redifferentiated, increasing the expression of collagen type II, β_1-integrin, Erk and Sox9. Immunoprecipitation demonstrated a direct relationship between Sox9 and Erk in IGF-I-treated cells. The results of this study lead to the conclusion that growth factors stabilize chondrogenic potential via activation of the integrin/-MAPK signalling pathway. Furthermore, IGF-I and TGF-β exert similar anabolic effects and positively influence chondrogenic potential, suggesting that they may have practical applications in the fields of tissue engineering and ACT.

C4

17beta-estradiol reduces expression of MMP-1, -3 and –13 in human primary articular chondrocytes cultured in a three dimensional alginate system

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Osteoarthritis (OA) is the most common arthropathy leading to immobility in old people. OA occurs more commonly among women after the age of 50, and its incidence rises around the timepoint of menopause due to a changed estrogen metabolism. Osteoarthritic cartilage is characterized by an imbalance between matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). Here we evaluate the influence of 17beta-estradiol on the expression of MMP-1, -3 and –13 or TIMP-1 and –2 in human primary articular chondrocytes. Primary articular chondrocytes obtained from knee joint surgery in female patients were cultured in alginate beads for 7 days in medium containing steroid hormone free serum at 5% O_2. Chondrocytes were stimulated with 10^{-5}, 10^{-7} and 10^{-9}M 17beta-estradiol during the whole culture period. Expression of MMP-1, -3 and –13 or TIMP-1 and-2 mRNAs was evaluated by real time RT-PCR. Articular cartilage from patients was characterized in vivo and in vitro by immunhistochemical staining for type I- and II-collagens, estrogen receptor alpha and MMP-1. Immunohistochemistry in articular cartilage biopsies from patients revealed positive immunostaining for type II-collagen while type I-collagen immunostaining was negative in all zones of articular cartilage. Antibodies against estrogen receptor alpha reacted only with a few chondrocytes in all articular cartilage zones. Immunostaining for MMP-1 was only detected intracellularly but not in the extracellular matrix. Extracellular matrix of articular chondrocytes cultured in alginate beads showed positive immunostaining for type II-collagen while type I-collagen immunostaining was absent. Some cells stained positive with antibodies against estrogen recpeptor alpha and MMP-1. Compared to control, physiological doses of 17beta-estradiol significantly decreased mRNA levels of MMP-1, -3 and –13. The mRNA levels of TIMP-1 and –2 were not influenced significantly by incubation with 17beta-estradiol. More recent studies about the influence of estrogens on the expression of MMPs and TIMPs were performed in monolayer culture. By contrast, we payed attention that articular chondrocytes did not dedifferentiate to fibroblast-like cells by the use of our well defined culture model in a three-dimensional alginate matrix. Therefore, it can be assured that articular chondrocytes had maintained their phenotype during the whole culture period. Our results show that 17beta-estradiol reduces the mRNA-levels of MMP-1, -3 and –13, enzymes that are involved in articular cartilage degradation. In the present experimental design 17beta-estradiol can be regarded as cartilage protective.

C5

Estrogen and progesterone are implicated in perinatal lung development

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Exposure to estrogen and progesterone derived from the feto-placental unit during the last trimester of pregnancy in humans and rodents appears to be necessary for morphological and functional lung development. We have shown in the past that hormone receptors for both steroids are widely expressed in the perinatal mouse lung (Biol Neonate 2003, 84:59-63). In this study, we have established cell cultures of mouse embryonic alveolar type II cells and lung fibroblast to investigate the physiological response of these cell types to both steroids. We observed by quantitative RT-PCR and ELISA that the expression of a number of developmentally-relevant genes such as vascular endothelial growth factor, glucose transporters, and surfactant proteins are under control of these steroids. These effects required the simultaneous presence of both steroid hormones and functionality of classical estrogen and progesterone receptors. It is noteworthy, that both steroids mimicked in part dexamethasone effects but, beyond it, revealed additional unique cellular responses.

Previously, we demonstrated a lower risk of the acquisition of acute and chronic lung diseases in preterm humans (Pediatr Drugs 2001, 3:629-637) and impaired alveolar formation and fluid clearance in newborn piglets (Ped Res 2006, 60:1-5) after estrogen and progesterone supplementation. The presented data conform to our previous clinical human and experimental studies and extend these observations to the cellular level. Herein, we describe for the first time that both steroids can affect early lung vascularization, surfactant expression, and glucose utilization. Supported by a grant from B. Braun Melsungen AG.

C6

Coexpression of the endogenous modulator SLURP1 with nicotinic acetylcholine receptor subunit alpha10 in mouse airway epithelium

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SLURP1 (secreted mammalian Ly-6/urokinasetype plasminogen activator receptor (uPAR)-related protein) is an endogenous allosteric regulator of nicotinic acetylcholine receptors (nAChR) in human keratinocytes. A mutation of the SLURP1 gene was detected in patients with the skin disease Mal de Meleda that is characterized by inflammation and hyperproliferation of keratinocytes. nAChR are expressed in keratinocytes as well as in the respiratory epithelium, where they also may regulate proliferation and differentiation. Here, we asked whether SLURP1 and nAChR are coexpressed and colocalized and whether they interact with each other in the respiratory epithelium of mice. With RT-PCR we detected SLURP1-mRNA and nAChR subunit alpha3-, alpha4-, alpha5-, alpha9- and alpha10-mRNA in the airway epithelium. SLURP1-immunolabeling and nAChR alpha9- and alpha10-immunolabeling were localized in the apical part of the ciliated cells. Using double-labeling immunofluorescence with subsequent fluorescence resonance energy transfer analysis with the confocal laser scanning microscope, we detected a significant FRET-signal for the combination SLURP1/alpha10-subunit, thereby indicating that SLURP1 is localized in such close spatial association to the nAChR alpha10-subunit that these proteins may interact. Thus, SLURP1 might allosterically regulate the alpha9/alpha10-nAChR that might have an anti-proliferative effect in the airway epithelium.

C7

The extracellular procathepsin D level increases in EA.hy 926 endothelial cells under cytokine treatment: a possible cause for endothelial cell dysfunction?

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Secreted procathepsin D (ProCathD) acts as an autocrine mitogen. Extracellular cathepsin D (Cath D) is able to generate angiogenesis inhibitors such as 16 kDa prolactin by cleavage of 23 kDa prolactin, whereas cytosolic Cath D is crucial in the apoptotic cascade upstream of caspases' activation. We hypothesized that leukocyte-derived cytokines affect the vascular bed during tissue remodelling, e.g. wound healing, by changing the intra- and extracellular ProCath D/ Cath D ratio. We here exposed the endothelial cell line EA.hy 926 to interferon-gamma (IFN-gamma, 200U/ml) and tumor necrosis factor-alpha (TNF-alpha, 1000U/ml) alone or in combination under serum free conditions for 72 hours. The EA.hy 926 cells, which stained positive for vascular endothelial markers (factor VIII-related antigen, CD31/PECAM-1), remained viable (cell counting, DAPI staining and cleaved caspase 3 detection) under single or combined cytokine treatment. The treatment did neither change the total intracellular Cath D amount (cell lysates, Westernblotting) nor its subcellular distribution, i.e. lysosomal bound vs. free protein (lysosomal fraction, postlysosomal supernatant). Cath D was solely present in its active bi-chain form in cell homogenates. In cell supernatants concentrated by Centricon 20 filter devices, however, ProCath D was detected beside the active form. IFN-gamma and TNF-alpha alone and particularly in combination increased the presence of the proenzyme in the supernatants. This increase contributed to a significantly enhanced proteolytic cleavage of the fluorogenic substrate MOCAc-GKPILF~FRLK(Dnp)-D-R-NH2 under acidic conditions. In summary: Our data in EA.hy 926 cells show that IFN-gamma and TNF-alpha alone or in combination, elevate the level of extracellular ProCath D, which is activated under acidic conditions, and contributes to proteolysis. The physiological significance may be related to dysfunctional endothelial cells arising under cytokine stimulation.

C8

Lymphangiogenic properties of the CEA-related cell adhesion molecule 1 (CEACAM1

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Lymphangiogenesis describes sprouting of lymphatics from the pre-existing lymphatic vessels and is essential for tumor growth and metastasis. Vascular endothelial growth factor-C and –D (VEGF-C and –D) and their receptor VEGFR-3 (Flt-4) are the main factors regulating lymphangiogenesis. However, the role of cell adhesion molecules in lymphangiogenesis is not sufficiently studied. Thus we focused on the potential function of CEACAM1 which we previously charaterized as an essential pro-angiogenic factor potentiating the effects of VEGF-A. CEACAM1 is a homophilic, signal transducing adhesion receptor, which is expressed in leukocytes, epithelial and endothelial cells. The CEACAM1-mediated homophilic interaction influences different cellular functions like proliferation, differentiation, apoptosis, immune responses and angiogenesis.
Using immunohistochemical studies for CEACAM1, VEGFR-3 and the lymphendothelial marker podoplanin on different human tumor tissues, overexpression versus gene silencing via siRNA for CEACAM1 and Prox1 in human vascular endothelial cells with subsequent expression studies for lymphendothelial markers VEGFR-3 and podoplanin by immunohistochemistry and FACScan-analyses as well as for Prox1 and LYVE-1 by immunobloting and immunocytochemistry we analysed the role of CEACAM1 in lymphangiogenesis. Here we will present data showing that the CEACAM1-expression in lymphatics is significantly higher compared to small vessel endothelial cells (HDMECs). No further member of the CEACAM-family was expressed in vessel endothelial and lymphendothelial cells. Over-expression of CEACAM1 in HDMECs results in an up-regulation of VEGF-C, -D and their VEGFR-3 at mRNA and protein levels. Furthermore, CEACAM1-transfected HDMECs show enhanced expression of lymphatic markers such as podoplanin, Prox1 and LYVE-1. In contrast, HDMECs transfected with a vector containing the CEACAM1Y-/- mutant missing the tyrosine residues in its cytoplasmic domain had no effect. These data suggest that the expression and signaling of CEACAM1 plays a central role in the lymphangiogenesis and probably in the reprogramming of vascular endothelial cells to lymphatic phenotype.

C9

Urinary proteomics and the role of orosomucoid (ORM) in vascular endothelial cells and angiogenesis

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The determination of protein patterns of body fluids via proteomics opens new opportunities for the development of novel, highly sensitive diagnostic tools for early detection of cancer. Because urine can be collected non-invasively in large amounts, it serves an attractive alternative to blood plasma for disease related proteomics.
We have performed 2-dimansional gel electrophoresis (2-DE) with subsequent mass spectrometric analyses and/or in combination with conventional immunoblotting and immunohistochemical methods to identify new biomarkers for urinary bladder cancer.
Orosomucoid (ORM) and human zinc-alpha2-glycoprotein (ZAG) have been identified in 2-DE to be increased in the urine samples of patients with bladder cancer in comparison to the urine samples of healthy volunteers. Immunohistochemical results let assume that in addition to cancer cells also a part of the tissue resident inflammatory cells and endothelial cells of tumor associated blood vessels may serve as source for this increase of ORM in the urine. Recent studies show that ORM forms a complex with the active form of plasminogen activator inhibitor-1 (PAI-1) in thymosin 4 (T4)-activated but not in quiescent HUVECs. Our recent findings and results presented by Sorensson showed, that, in addition to the cancer cells, human vascular endothelial cells produce ORM endogenously. For functional characterization of ORM in vascular endothelial cells, we have performed ORM-gene overexpression and ORM-gene silencing. Employing the supernatants HDMECs-ORM and HDMECs-ORM-siRNA in in vitro angiogenesis assays, we could show that ORM supports the VEGF-induced endothelial tube formation. This supportive effect of ORM was potentiated by co-treatment of HDMECs with VEGF, ORM and anti-PAI-1 antibody.
Our results demonstrate for the first time that ORM acts pro-angiogenic and support the tube forming effects of VEGF which significantly increased by an additive blockage of PAI-1. Further studies are needed to clarify the interaction between ORM and PAI-1 regarding angiogenesis.

C10

Extravasation of mesenchymal stem cells: Role of endothelial phenotype, VLA-4/VCAM-1 and cytokines

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Human adult bone marrow derived mesenchymal stem cells (MSCs) are in the focus of scientific interest because they differentiate into various mesenchymal tissues. MSCs are not only used in the field of tissue engineering but also as a potential therapy for the regeneration of infarcted myocardial tissue. For a successful therapy, mesenchymal stem cells (MSCs) must contact the endothelial barrier and transmigrate across the endothelium to exit the blood circulation and to finally enter their target tissue. Thus far, most of the underlying morphological and molecular mechanisms of MSC extravasation remain unelucidated.

Therefore, we conducted co-cultivation experiments with endothelial monolayer and MSCs as an in vitro model system to understand transmigration phenomenon and to further characterize this core process for MSC homing. To summarize, our results infere that the time course of MSC integration and the formation of plasmic podia depends on the endothelial phenotype thus indicating a varaiable capacity for the extravasation of MSCs within the vasculature.

Second, to identify cell adherence molecules involved in the interaction, blockade experiments with anti-VLA-4 (integrin alpha4/beta1) and anti-VCAM-1 (CD106) were carried out and resulted in a significant slow down of integration of MSCs into the endothelial monolayer. Thus, we assume that the VLA-4/VCAM-1 interaction plays a key role in the transmigration of stem cells across the endothelial barrier.

Finally, co-cultivation experiments in growth media containing the cytokines bFGF, VEGF, EPO, IL-6, or specific inhibitors enabled to identify factors and signalling pathways which accelerate or decrease the transmigratory capacity of MSCs.

In conclusion, the endothelial phenotype, VCAM-1/VLA-4 and cytokines are the functional key players involved in the transendothelial migration of MSCs.

C11

Shock waves as the first non invasive way for inducing migration in mesenchymal stem cells

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Stem cells are discussed for a very long time as a useful tool for treating various dysfunctions. Different ways are described for placing stem cells into the area of need. Stem cells themselves or factors which are able to attract the stem cells became injected into the target area. All methods described were done in surgery or other ways of smaller invasive methods. Unfortunately, up to now there is no method which is able to attract stem cells to a targeted place in a non invasive way. Here we demonstrate for the first time that it is possible to direct stem cells without any way of invasion.
For this purpose mesenchymal stem cells (MSC) of human origin were obtained from bone marrow of patients undergoing hip joint surgery, isolated and cultured as described before (Schmidt A. et al.; Stem Cells 2006). The isolated MSC were treated with shock waves using the Piezoson 100 (Wolf Inc.). After treating the MSC the migratory activity was analyzed using Boyden chambers. After shock wave treatment the migratory activity was increased significantly up to the three fold compared to the control. In vivo studies in rabbits already proved that extracorporal shock wave treatment increased new bone formation.
Taken together shock waves might be the first approach to mobilize stem cells without invasion. The strong effects onto the behaviour of mesenchymal stem cells indicate that these cells also be sensitized against mechanic influences like shock waves. These mechanic sensitizers can therefore be used to induce a directed migration in mesenchymal stem cells and might be a powerful tool for goal-orientated placement of stem cells.

C12

Noggin ablates tumor formation and invasiveness of B16-F1 mouse melanoma cells transplanted into the eye cup of the chick embryo

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Melanoma cells originate from precursor cells derived from the neural crest and are character-ized by a high potential for migration and invasive growth. In our previous study, we demon-strated that mouse B16-F1 melanoma cells show traits of neural crest cells after transplanta-tion into the chick embryo neural tube. They spontaneously emigrate along the neural crest pathways without tumour formation or malignant growth, respectively. In contrast, B16-F1 cells that were transplanted into the eye cup of the chick embryo formed tumours and revealed signs of invasive growth. As bone morphogenetic proteins (BMPs) induce and the BMP-antagonist noggin blocks emigration of neural crest cells, we asked if the invasive growth of melanoma cells outside the neural crest could be influenced by BMP-2 or noggin.
B16-F1 cells were grown as aggregates, treated with BMP-2 or noggin during aggregation and injected into the eye cup of three day chick embryos. After three days of subsequent incu-bation, embryos were processed for histology.
Immunohistochemical examination with melanoma-specific marker HMB-45 and TUNEL reaction for the detection of apoptosis proved that noggin treated B16-F1 cells survived in the interior of the eye cup without malignant growth. A significant portion of embryos that had been transplanted with untreated melanoma cell aggregates had undergone invasive growth. In the BMP-2 treated group of aggregates, invasive growth was enhanced.
We conclude that BMP-2 enhances and noggin ablates melanoma cells invasiveness after transplantation into the eye cup. Moreover, we demonstrate that the eye cup is an easily ac-cessible and highly reproducible in vivo model for melanoma cell invasiveness.

C13

Noggin inhibits BMP-2 dependent migration of B16-F1 mouse melanoma cells along neural crest pathways after transplantation into the chick embryo

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Melanoma cells are derived from the neural crest and characterized by high migratory potential and invasive growth. After transplantation into the neural tube of the chick embryo, melanoma cells spontaneously migrate along the neural crest pathways. In the same experiment, neural stem cells from the subventricular zone emigrate only after treatment with bone morphogenetic protein-2 (BMP 2). BMPs are constitutively over-expressed in human melanomas. We presumed that emigration of melanoma cells is also BMP 2 dependent. Mouse B16-F1 melanoma cells transfected with GFP-VASP were cultured as aggregates, treated with BMP-2 or the BMP-2 antagonist noggin, and transplanted into the E2 chick embryo. Non-treated and BMP-2 treated melanoma cells emigrated from the neural crest. Noggin treated aggregates showed no emigration. We conclude that migration of melanoma cells depends on the constitutive over-expression of BMP, and that noggin efficiently suppresses migration, thus rendering noggin a promising agent for the inhibition of epithelial mesenchymal transformation of melanoma cells, which is a central process not only in the neural crest, but also in cancer progression and metastasis.