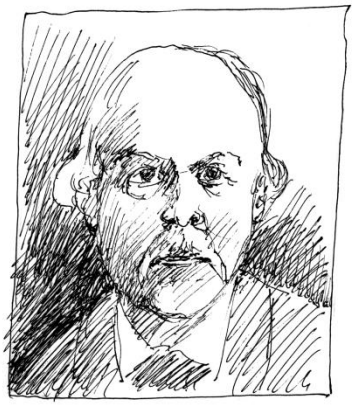
ANATOMISCHE GESELLSCHAFT
1886

In memory of Koelliker's 200th birthday



Rudolf Albert von Koelliker

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Institut für Anatomie und Zellbiologie

der Universität Würzburg

1886

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an abstract of interest
please use the alphabetical list of
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Poster 1:

Titel: SDS-based tissue clearing in histology: technical approaches and new developments

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Abstract:
Evaluating intact organs down to cellular and subcellular resolution is an ambitious goal to put molecular and cell-biological processes in the wider context of gross morphology. Novel techniques allow for the optical clearing of larger tissue samples and entire organs and lay the basis for microscopic analysis.

We employed light sheet, structured-illumination, and confocal scanning microscopy to image cleared human, murine, and piscine tissue samples stained by immunohistochemistry. Moreover, we performed classical histological stainings, and transmission electron microscopy to evaluate cell and tissue architecture in translucent murine specimen.

We were able to clear specimens of the intestine and brain, and stained them using immunostainings for neuronal, epithelial, and vascular markers. This enabled us to follow complex three-dimensional structures, like nerve fibers, vessels, and epithelial barriers throughout large specimens (e.g. brain slices of >2 mm thickness) or even entire intestinal segments. We report the possibility to stain cleared tissues with classical histological methods suggesting potential use in pathohistology. We systematically evaluated the ultrastructure of cleared specimens and were able to confirm good tissue-retention at neutral pH. Moreover, we present novel approaches to combine tissue clearing with bleaching of pigment in order to apply this in entire murine and piscine eyes.

Our data shows the high potential of modern tissue clearing. It supports the use of tissue clearing in research and diagnosis and contributes to the technical discussion of ultrastructural tissue-retention during the clearing process. We also advance this technology by combining clearing and pigment-bleaching for ophthalmologic research.

Poster 2:

Titel: Step-by-step protocol to perfuse and dissect the mouse parotid gland and optimized procedures to isolate high quality RNA from murine and human parotid tissue

Autoren/Adressen: Christoph Watermann (Justus Liebig University), Klaus-Peter Valerius (Justus Liebig University), Steffen Wagner (Justus Liebig University), Claus Wittekindt (Justus Liebig University), Jens Peter Klussmann (Justus Liebig University), Eveline Baumgart-Vogt (Justus Liebig University); Srikanth Karnati (Justus Liebig University), Christoph.Watermann@anatomie.med.uni-giessen.de

Abstract:
Macroscopic identification and surgical removal of the mouse parotid gland is demanding because of its anatomic location and size. Moreover, the mouse parotid gland contains high concentrations of intrinsic RNases, making it difficult to isolate high quality RNA. So far, appropriate methods for optimal perfusion-fixation and dissection of mouse parotid glands, as well as isolation of high quality RNA from this tissue are not available:

Therefore, we have established a simple, optimized step-by-step surgical method to perfuse and isolate murine parotid glands. Further, we compared different protocols of the well-known RNA extraction methods (RNeasy Mini Kit® vs. TRIzol®) to yield high quality of intact RNA from human and murine parotid gland tissues that were either snap-frozen or immersed in RNAlater®.

Mouse parotid tissue that was perfused and immersed in RNAlater®, and human samples immersed in RNAlater® exhibited the best RNA quality independent of the isolation method.

In conclusion, we recommend to perfuse mice via left ventricle or aortic arch, dissect the parotis quickly out and immediately immerse in RNAlater® for optimal high quality RNA preparation from mouse parotid tissue. For human samples, immediate immersion into RNAlater® of the material obtained in the operation room yields good RNA quality.

Poster 3:

Titel: The ultimate near-peer teaching approach

Autoren/Adressen: Luis Filgueira (University of Fribourg), Elisabeth Eppler (University of Basel); luis.filgueira@unifr.ch

Abstract:
Anatomy education often embraces near-peer teaching where more advanced students teach their younger peers in anatomy. However, students have been seldom included in the development of new courses. In the context of this educational project, a student initiative to introduce a new course by 3rd year students for their 2nd year peers, has been followed and evaluated.

A new abdominal ultrasound course of the normal anatomy was designed as an optional (elective) course for 20 2nd year participants taught by 8 3rd year peer-teachers. The course was approved by the Curriculum Committee. The peer-teachers were prepared and coached for their role by expert ultrasound teachers. The course was delivered in 6 x 3 hours blocks for the following topics: (1)Abdominal blood vessels, (2) biliary ways and pancreas, (3) liver, (4) spleen and abdominal urogenital organs, (5) FAST and (6) practical exam. Each group and topic was taught in small groups of 5 students. The course was evaluated anonymously through a standard questionnaire for student, as well as an online questionnaire for the students and another one for the peer-teachers. In addition, written feedback was evaluated.

The course got excellent feedback for content and delivery from the students, as well as from the near-peer teachers. At the end, all students were able to handle various models and brands of ultrasound machines and knew well how to do the basic ultrasound investigation of the abdomen and recognized all relevant anatomical structures.

We conclude from our very positive experience that student participation in the design and delivery of courses should be more often considered in the future.

Poster 4:

Titel: Is there a place for tricks in anatomy teaching?

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Abstract:
There are many methods available to support anatomy teaching, among them 3-D views, CT-imaging and MRI-imaging. For many students the process of learning and remembering the anatomical features is still difficult and overhelming. During the cadaveric examination some anatomical characteristics could not be disclosed and shown to the medical students. After the initial process of anatomical presentation and presenting connections with pathophysiology and clinics, that is a time for remembering and keeping in mind.

Presentation of „typical" tricks (mnemonic devices) used in anatomy teaching.
We present some examples of the different devices to simplify the remembering - the features of the clavicle, muscles of the anterior abdominal wall, maxillary artery, peripheral and central nervous system.

Muscles of the anterior abdominal wall - the most difficult to remember is the attachments on the ribs
External oblique 5th - 12th ribs, internal oblique 10th - 12th ribs, transverse abdominal 7th - 12th costal cartilages.
The muscles are attached on the last rib.
The beginning: Letter E (5th in the alphabet). The letters IO (internal oblique muscle) are similar to number 10. Transvere is described as third -so you van add 2 to the first muscle (EO) = 5 + 2 = 7
Rectus abdominis 5th - 7th costal cartilages. The numbers described above are used to describe the attachment of rectus muscle.

The mnemonic devices or tricks are very useful in anatomy teaching.

Poster 5:

Titel: Early internationalization of German medical students as part of a collaboration project between the Departments of anatomy from the Columbia University NYC and the Martin Luther University Halle-Wittenberg

Autoren/Adressen: Camilla Gölkel (Medical Faculty, Martin Luther University Halle-Wittenberg), Heike Kielstein (Medical Faculty, Martin Luther University Halle-Wittenberg), Anette Wu (Columbia University New York), Paulette Bernd (Columbia University New York); camilla.goelkel@gmx.de

Abstract:
Internationalization and international exchange are important topics for the academic formation in times of globalization and international mobility. Nowadays the importance of the dissection course is highly controversial and diverges from Medical Faculty to Medical Faculty. The aim of this project was to combine the traditional concept of the dissection course with the modern idea of internationalizing medical education.

As part of the dissection course a voluntary group of 140 medical students from Halle dissected certain topics with the help of an English, picture-run dissection script. Furthermore, these students were matched with American students, with whom they discussed subjects via voice over IP (Skype), including health care systems, structure and content of the anatomy course and the organization of their studies. The students organized the dates of their Skype meetings autonomously. It was obligatory to implement at least one meeting.

The project was evaluated by questionnaires at the end of the term. There was no preference between the two different scripts. However, the students enjoyed making international medical experiences as part of their anatomical formation. A few students kept in touch with their peers after the end of the study.

The realization of this project requires a broad financial scope, additional manpower and high motivation and preparation of the students. In the future we won’t take over the American script completely, but the cooperation between the two faculties will persist and the exchange between the students will be expanded.

Poster 6:

Titel: Virtual microscopy versus optical microscopy: evaluation of search strategies of histological structures by undergraduate medical students

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Abstract:
In the last ten years e-learning in the form of virtual microscopy in the histological education and training of undergraduate students expanded dramatically. Therefore several articles examined the strengths and weaknesses of the virtual microscopy (VM) compared to the conventional optical microscopy (OM). Lots of these publications focused on the acceptance of VM, the limits of its use and its influence on collaborative learning and performance in histology exams. To our knowledge, there have been no well controlled studies comparing visual search strategies of medical students using VM and OM. Thus leads us to plan this study with questionnaires, formative post-tests, cross-over study design and summative exams.

In the first phase, the first semester students were divided into two groups. Group 1 performed the practical sessions with OM, Group 2 performed the same sessions with VM. In the second phase, the research subjects switched conditions. After each phase learn and search strategies of histological structures and acceptance of the microscopy form were measured with a practical formative post-test and a questionnaire.
The search strategies were tracked by slide movement observations. Additionally performances in written and practical histology exams were collected.

The acquisition of the histology knowledge is independent of the microscopy representation mode. Virtual microscopy (VM)is equivalent to optical microscopy (OM).

Poster 7:

Titel: Topographic anatomy of the extrinsic autonomic nerve supply of the internal anal sphincter - a prerequisite for nerve-sparing rectal surgery

Autoren/Adressen: Thilo Wedel (Christian-Albrechts-University Kiel), Martina Böttner (Christian-Albrechts-University Kiel), Werner Kneist (University Medical Center of the Johannes Gutenberg-University Mainz), Philip Quirke (Leeds Institute of Cancer and Pathology, University of Leeds), Nicolas West (Leeds Institute of Cancer and Pathology, University of Leeds), Juliane Kupsch (Dresden-Friedrichstadt General Hospital), Helmut Witzigmann (Dresden-Friedrichstadt General Hospital), Sigmar Stelzner (Dresden-Friedrichstadt General Hospital); t.wedel@anat.uni-kiel.de

Abstract:
Extrinsic nerve fibers supplying the internal anal sphincter are endangered during surgical resection of rectal carcinoma often leading to postoperative anal incontinence. To prevent damage of these autonomic nerves, a detailed macroscopic topographic description of their exact course is mandatory.

Seven (5 males, 2 females) hemipelvises obtained from body donors (67-92 years) were subjected to a macroscopic dissection of pelvic autonomic nerves using magnification glasses. Dissection involved the superior hypogastric plexus, hypogastric nerves, pelvic splanchnic nerves and pelvic plexus. Special emphasis was given to connections between the pelvic plexus and the internal anal sphincter. The presence of nerve fibers running towards the internal anal sphincter was confirmed by histologic and immunohistochemical stainings.

Nerve fibers could be traced from the anteroinferior edge of the pelvic plexus to the anorectal junction running along the urogenital neurovascular bundles ("Walsh bundles") anterolaterally to the rectum and posterolaterally to the prostate/vagina. Nerve fibers penetrated the longitudinal rectal muscle layer just above the fusion with the levator ani muscle (conjoint longitudinal muscle) and entered the intersphincteric space to reach the internal anal sphincter. Histologic and immunohistochemical findings confirmed the presence of nerve tissue.

Autonomic nerve fibers supplying the internal anal sphincter emerge from the pelvic plexus and are distinct to nerves entering the rectum via the lateral rectal pedicles. Thus, they should be classified as internal anal sphincter nerves. The macroscopic topographic description provides a basis for nerve-sparing rectal resection procedures and may help to prevent postoperative functional anorectal disorders.

Poster 8:

Titel: Michaelis – more than a rhombus

Autoren/Adressen: Ibrahim Alkatout (), Ulrich Mechler (), Eva Fuhry (), Michael Illert (), Nicolai Maass (), Bernhard Tillmann ();bntill@t-online.de

Abstract:
The Rhombus of Michaelis, also known as the Michaelis-Raute is named after the former Professor of Obstetrics in Kiel, Gustav Adolf Michaelis (1798 – 1848). Today, it is known only to older anatomists and obstetricians. Its description is missing in many of the textbooks for anatomy and obstetrics.

The aim of this medical-historical investigation is to recall the anatomical structures of the pelvis analyzed by Michaelis as they have not lost their relevance in the contemporary teaching of obstetrics.

For this study, use is made of the Michaelis monography, "The narrow pelvis" (1890), published posthumously with the analysis of 5 pelvic measurements, the Michaelis-Raute and the documentation of 1000 pregnant women with their birth histories in the register of the "Royal lying-in hospital". Furthermore, the "Kiel pelvis cabinet" contains 31 pelvises, mostly deformed by severe rickets from women who died postpartum.
Abnormalities of the normal pelvis, e.g. transverse constricted pelvis and oblique constricted pelvis can be readily diagnosed by an analysis of the changes in shape of the Michaelis-Raute.

By comparing the measurements taken before the delivery and afterwards, the validity of the estimated pelvic measurements can be analyzed. According to Michaelis the narrow pelvis is more common than believed. He also concluded that a narrow pelvis is not inevitably associated with an obstetric obstacle. Regarding the pathological course of parturition, Michaelis derived the necessary steps of intervention from his examinations.
Despite modern diagnostic tools in obstetrics, pelvic measurements and the Michaelis-Raute should not fall into oblivion.

Poster 9:

Titel: Frequency of branching pattern of the deep femoral artery and the circumflex femoral arteries with respect to the branches of the femoral nerve

Autoren/Adressen: Horst Claassen (Martin-Luther-University Halle-Wittenberg), Oliver Schmitt (Rostock University Medical Center), Marko Schulze (Rostock University Medical Center), Andreas Wree (Rostock University Medical Center); horst.claassen@medizin.uni-halle.de

Abstract:
The branching pattern of the femoral artery (FA), especially of the deep femoral artery (DFA), is highly variant.

In 111 enbalmed lower extremities, the frequency of the origin of DFA in relation to the inguinal ligament (IL) and the respective origins for the medial (MCFA) and lateral (LCFA) circumflex femoral arteries with respect to the position of the branches of the femoral nerve (FN) were investigated. Gender and side differences were analyzed statistically using the Chi-Square and the Mann-Whitney-U tests. In addition, we present two seldom cases.

High origin of DFA (1-2 cm below IL) was observed in 37.8%, middle origin (3-5 cm below IL) in 39.6% and deep origin (6-10 cm below IL) in 18.0 %. A weak tendency for a male expression of the deep origin of DFA was observed. An isolated origin of LCFA from FA was seen in 19.8%, a respective one of MCFA in 14.4%. Branches of FN passed mostly before and behind LCFA (47.8%) or only before LCFA (46.4%), but very seldom (5.8%) only behind it. A strong tendency to left side expression was seen in the constellation that all FN branches passed behind LCFA. Case 1: Isolated origins of LCFA and MCFA from FA were observed in the right leg of a 100-year-old woman. Case 2: A seldom (9.9%) trifurcation into DFA, MCFA and LCFA was detected in the right leg of a 81-year-old man.

Our results are of clinical significance during vascular reconstructive surgery, catherization procedures and surgical intervention for embolism.

Poster 10:

Titel: Aminolipin: a novel alternative for formalin in human cadaver embalming

Autoren/Adressen: Peter Neckel (University of Tübingen), Corinna Gleiser (University of Tübingen), Andreas Kramer (University of Tübingen), Claus Zeyher (University of Tübingen), Hubert Kalbacher (University of Tübingen), Gerhard Feil (University of Tübingen), Lothar Just (University of Tübingen), Bernhard Hirt (University of Tübingen); peter.neckel@uni.tuebingne.de

Abstract:
The anatomical dissection courses are in danger, as new guidelines for occupational safety concerning the currently widely-used formalin-based embalming procedure are being enforced by the authorities worldwide. Therefore, anatomical teaching faces a compelling urge to invent and evaluate novel, non-hazardous fixation methods to keep up the high quality of medical training.

We used mass-spectrometry, gas-chromatography, and 1H-NMR-spectrometry to analyze the chemical properties of aminolipin. We characterized its fixative activity by circular-dichroism-spectroscopy, 1H-15N-NMR-spectrometry, and FRET-based endopeptidase assays. Moreover, we evaluated the histology and gross tissue retention of aminolipin-embalmed human cadavers and quantified haptics and joint-mobility. Biological and occupational safety was evaluated by microbiological assays and ambient air analysis by HPLC.

We found that aminolipin exerts a potent denaturating effect on proteins, thereby unfolding tertiary and secondary structure and arresting enzymatic activity. Further, we present strong histological and gross-anatomical evidence that aminolipin is a useful fixative for the embalming of human cadavers. Due to the in vivo-like haptics and joint-flexibility, aminolipin even is superior to the currently used formalin-based embalming procedures for specific applications such as surgical training. Additionally, we were not able to detect any volatile hydrocarbons as derivatives of aminolipin by HPLC evaporating form the fixed specimens. Moreover, we found that aminolipin has a potent anti-microbial effect covering a wide spectrum of anaerob-aerob, gram-positive and gram-negative bacteria and, in contrast to formalin, even acts anti-mycotic.

Aminolipin is an alternative useful for the preservation of human cadavers having favorable properties in terms of biological and occupational safety.

Poster: 11

Titel: Longitudinal changes in specific muscle strength prior and concurrent to changes in knee function - data from the osteoarthritis initiative (oai)

Autoren/Adressen:
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Abstract:
A positive, concurrent association between longitudinal change in isometric muscle strength vs. change in knee function ([KF], worsening/improvement ≥6 [minimal clinically important difference (MCID)] on the WOMAC function score) was investigated. Within a sub-cohort, no relationship between KF-change and muscle morphology (thigh anatomical cross sectional areas [ACSAs]) was observed. Hence, we investigate whether changes in KF are then associated with changes in specific muscle strength (SMS [N/cm²]).

2675 OAI participants with baseline (BL), year-2 (Y2) and year-4 (Y4) follow-up data were divided into a) those with worsening, b) with improvement, or c) without relevant change in KF. From this sample, the first 25 men and women in each group were investigated. Changes in SMS, preceding (BL→Y2) and concurrent (Y2→Y4) to KF-change were compared between groups (ANCOVA) and over time (ANOVA).

A significant increase in extensor SMS was observed in knees with functional improvement. SMS changes in those with worsening did not significantly differ from those without change. During the preceding change in KF, knees with subsequent improvement, but not with subsequent worsening, surprisingly showed significant reduction in extensor (but not in flexor) SMS when compared to the no-change group.

Whereas longitudinal change in muscle morphology does not appear to be related to change in KF, change in extensor SMS (force per unit muscle area) is observed to be associated with concurrent improvement in KF. However, there is no rational explanation why a reduction in SMS is observed in those knees prior to the period of functional improvement.

Poster 12:

Titel: Renal vessels variability highlighted via dissection and biodur S10 plastination technique

Autoren/Adressen: Sorin Lucian Bolintineanu (Victor Babes University of Medicine and Pharmacy), Elena Pop (Victor Babes University of Medicine and Pharmacy), Monica Adriana Vaida (Victor Babes University of Medicine and Pharmacy), Alina Maria Sisu (Victor Babes University of Medicine and Pharmacy); alinasisu@gmail.com

Abstract:
Renal arteries variability ranges between 28%-30% in anatomic and cadaver studies. Renal artery variations are important due to the gradual increase in interventional radiological procedures, urological and vascular operations, and renal transplantation. The aim of this study was to determine the location of origins of renal arteries and the variation rates of renal arteries in patients suffering from renal diseases. Literature reports great variability in renal blood supply, the number of renal arteries and the arrangement of hilum structures on the left side.

Macroscopic dissection and S10 Biodur plastination technique was performed on all the variable specimens.

In a study of 12 bilateral kidneys we discovered in 4 specimens on the left side double renal artery and double renal vein having the same calibre originated from a common trunk coming out of the lateral aspect of abdominal aorta. The renal arteries were in 4 cases (30%) double and in the rest (8=60%) single. The renal artery was situated posterior to the vein and anterior to the left ureter in all 4 cases. Such variation has great implications when surgery is indicated, as in renal transplants, urological and radiological procedures, renovascular hypertension, renal trauma and hydronephrosis.

In order to avoid any vascular complication, angiography and arteriography should be performed before any surgical procedure.
Present study was supported by funds kindly provided by Victor Babes University of Medicine and Pharmacy Timisoara, Romania, through P-III-C5-PCFI-2017/2018-03 Internal grant-acronym UROVESSELS.
Keywords: renal artery, variation, plastination.

Poster 13:

Titel: Provenance research in colonial collections – the case of a skull from Tasmania

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Abstract:
Following earlier inquiries by the Australian government, human remains of the then Charité collections were scanned for items of Australian origin. Only one of more than 5000 skulls was labeled as stemming from "Van Diemensland", i.e. Tasmania. Provenance research was initiated to confirm the origin of this skull and to elucidate the historical context.

Combination of (non-invasive) physical-anthropological investigations and historical research.

An inscription on the frontal bone 'Durch Schayer aus Van Diemensland … "Nanny" native of Kangaroo Island' led to collector Adolph Schayer (*1793), who worked as a sheepbreeder in Tasmania 1831-1843 and then returned to Berlin, likely giving the skull to anatomist Johannes Müller. In a Tasmanian archive, a burial register could be identified stating that in 1836 a "Nanny Allan, Native of Kangaroo Island" was buried in Launceston, aged "about 14 years". Anthropological inspection revealed an age at death of about 15-16 years and a large intravital defect of the left petrous bone, making a massive mastoiditis/petrositis with subsequent meningitis the most likely cause of death.

The anthropological and historical findings match very well and leave no doubt that these remains originated from Tasmania. The remains have since been handed over to the Tasmanian Aboriginal Centre. This is one of the rare cases in which a personal identity could be traced and the cause of death determined. How the skull came into the hands of the collector remains unclear, but either dissection immediately after death or grave robbing immediately after burial are likely.

Poster 14:

Titel: Anatomical variants of the circle of Willis

Autoren/Adressen: Cristinel Ionel Stan ("Gr. T. Popa" University of Medicine and Pharmacy Iași), Daniela Cătălina Stan ("Gr. T. Popa" University of Medicine and Pharmacy Iași), Ana Maria Dumitrescu ("Gr. T. Popa" University of Medicine and Pharmacy Iași), Lucia Indrei ("Carol Davila" University of Medicine and Pharmacy Bucharest), Gabriela Florența Dumitrescu (“Prof. Dr. Nicolae Oblu” Emergency Clinic Hospital Iași), Marius Valeriu Hînganu ("Gr. T. Popa" University of Medicine and Pharmacy Iași), Anca Sava ("Gr. T. Popa" University of Medicine and Pharmacy Iași);
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Abstract:
The aim of the study was to detect the variations of the arterial circle of Willis.

We studied the variants of this circulus arteriosus on 102 bodies which were necropsiated during two years (2014 and 2015) in the Laboratory of Pathology of the Emergency Hospital ,,Prof. Dr. Nicolae Oblu’’ Iași.

In the wide majority of cases, the circle of Willis has a shape of a heptagon around the interpeduncular fossa, with the anterior border represented by the anterior communicating artery, the antero-lateral borders represented by the anterior cerebral arteries, the postero-lateral borders formed by the posterior communicating arteries and the posterior borders represented by the posterior cerebral arteries.

However, we could find many variations of this circle, from double anterior communicating arteries to the absences of the posterior communicating arteries, or many asymmetries and we tried to find a correlation between some variants of the arterial circle of Willis and the arterial aneurysms, the age and the sex of these pacients.

We can conclude that this study reaffirms the great variability of the arterial circle of Willis, thus bringing its contribution to neurosurgery, where these variants are of a great importance

Poster 15:

Titel: Markers of locoregional extension in colorectal cancers

Autoren/Adressen: Delia Hinganu (University of Medicine and Pharmacy "Gr. T. Popa" Iasi), Anca Sava (University of Medicine and Pharmacy "Gr. T. Popa" Iasi), Cristinel Ionel Stan (University of Medicine and Pharmacy "Gr. T. Popa" Iasi), Marius Valeriu Hinganu (University of Medicine and Pharmacy "Gr. T. Popa" Iasi); delia_f24@yahoo.com

Abstract:
Neoplastic growth and locoregional extension determination technique to be used in the positive diagnosis and prognosis of colorectal neoplastic lesions

We followed the histopathological and immunohistochemical evaluation of patients in the study group - 28 patients diagnosed with rectal cancer. The evaluation was performed on the sections stained with hematoxylin-eosin, and for the mucinous forms, on the sections stained with Alcian blue. The examination of immunohistochemically stained sections for Ki-67 demonstrated that the topography of the reaction is strictly nuclear.

The lack of Ki-67 expression in nearby neoplastic tissues suggests that cancerous tissue proliferates in a pathway that is not correlated with adjacent tissues. In cases with a strong positive response to Ki-67, prognosis is worse, with invasion of the perirect tissues, regional lymph nodes and remote metastases. In these situations there is a direct correlation between the expression and accumulation of the Ki-67 protein and the local, regional or remote extension.

Ki-67 immunohistochemical marker should be used to determine the local extension of a colorectal cancer, especially associated with radiological investigations and, in particular, with the histopathological examination of the biopsy piece

Poster 16:

Titel: Contrast-enhanced cadaver-specific post mortem computer tomography yields improved teaching of vascular and topographic anatomy in human gross anatomy

Autoren/Adressen: Kerstin Klopries (University Heidelberg), Daniel Paech (German Cancer Research Center); Kerstin.Klopries@gmx.de

Abstract:
The purpose of this study was to analyze the benefit of contrast-enhanced post mortem computer tomography (CEPMCT) in comparison to non-enhanced post mortem computer tomography (NEPMCT) in teaching human gross anatomy with the focus on vascular and topographic anatomy.

Since October 2012, teaching of gross anatomy supported by NEPMCT is an integral component of first-year medical curriculum at Heidelberg University.

In 2015, contrast agent was used to acquire NE- and CEPMCT data sets in three body donors. During the winter semester 2016/17, sixty out of five hundred first-year medical students were provided with these NE- and CEPMCT data sets. The students were randomly selected in this IRB approved study. After having completed the gross anatomy course, the attendees were invited to fill out a 20 point questionnaire based on a five-point Lickert scale.

In total, 53 first-year (53/60) medical students answered the questionnaire. According to the evaluation of the questionnaire, 94.3% of the students agreed that CEPMCT improved their understanding of vascular anatomy.

98.1% students decided that CEPMCT was supportive for anatomic teaching units of the abdomen, 96.1% of the thorax, 73.1 of the extremities and 69.8% of the head/neck. 100% believed that getting familiar with cross sectional imaging at an early point of medical education is important and 90.6% decided that getting to know the advantages and disadvantages of both, CE and NE, is crucial for the time after postgraduate studies.

CEPMCT improved especially the understanding of the complex vascular and topographic anatomy and should therefore be considered to be part of the standard curriculum of gross anatomy.

Poster 17:

Titel: The gleno-humeral joint revisited in light of anatomical and reverse total shoulder arthroplasty: a cadaver dissection- and 3D-CT-based comparative study

Autoren/Adressen: Elisabeth Eppler (University of Basel), Sandra Mathews (University of Zürich), Nabil Serrano (University of Zürich), Karl Link (University of Zürich), Marco Burkhard (University of Zürich), Hannah Krafft (University of Zürich), Magdalena Vich (University of Zürich), Oliver Ullrich (University of Zürich), Dominic Gascho (University of Zürich), Michael Thali (University of Zürich), Magdalena Müller-Gerbl (University of Basel), Frank-Jakobus Rühli (University of Zürich), Martin Häusler (University of Zürich); elisabeth.eppler@unibas.ch

Abstract:
Optimal placement of the implant components is crucial for the outcome of anatomical and reverse total shoulder arthroplasty, and preoperative treatment planning is gaining increasingly in importance. However, the database with regard to true glenoid and scapular body size is weak. In this multimodality cadaver study, we systematically examine the glenohumeral joint using morphological and CT measurements and compare the data set with the donor body size.

CT scans including the shoulder girdle and arms were performed from 11 female and 7 male body donors (average age of 84 years, range: 60-98) prior to dissection. Glenoid size was determined on subsequently isolated scapulae and on 3D-CT reconstructions of the glenohumeral joint according to the method of Friedman as described (Mathews et al., BMC Musculoskelet Disord. 2017; 18:9). Body length is measured by CT and humerus length and compared to correlate to the glenoid size.

Mean glenoid height was 36.6 mm ± 3.6, and width 27.8 mm ± 3.1 with a significant sex dimorphism ($p \leq 0.001$): in males, glenoid height 39.5 mm ± 3.5, and width 30.3 mm ± 3.3, and in females, glenoid height 34.8 mm ± 2.2, and width 26.2 mm ± 1.6. Comparison of body size measurements using CT scans and humerus length, respectively, reflects the necessity to establish a data set to extrapolate body size in the elderly.

This study is one of the first to combine dissection with anatomical measurements and radiological CT data to systematically correlate the glenoid size with the body size.

Poster 18:

Titel: Sonographic diagnosis of carpal tunnel syndrome by calculating ratios of cross sectional areas of the median nerve

Autoren/Adressen: Ulrike M. Hamscha (Medical University of Vienna), Stefan Meng (KFJ Hospital), Lukas F. Reissig (Medical University of Vienna), Johannes Steinbacher (Medical University of Vienna), Mahmoud Moussa (Medical University of Vienna), Wolfgang Grisold (KFJ Hospital), Wolfgang J. Weninger (Medical University of Vienna); ulrike.hamscha@meduniwien.ac.at

Abstract:
Ultrasound is a widely used tool for diagnosing carpal tunnel syndrome (CTS). Usually the ratio of cross sectional areas (CSA) are measured at various sites along the nerve in order to diagnose nerve swelling proximal to the carpal tunnel. Yet, no valid data exist regarding the prevalence of CTS detected with the described method.

In 332 arms of fresh, non-embalmed whole cadavers (mean age: 80.52 years, 10.04 years SD) median nerves were identified using a z.one US-system (ZONARE Medical Systems GmbH) with a 14 Mhz transducer. Along each nerve, its CSA was measured at two sites: 1.) halfway between elbow joint and wrist, 2.) directly proximal to the carpal tunnel. Using the software package Excel, the CSA ratio was calculated for each specimen.

In 282 (84.93%) arms the CSA ratio was < 1.5. In 40 (12.07%) the CSA ratio was ≥ 1.5 and in 10 (3%) the CSA ratio was ≥ 2. 17.58% of females and 12% of males had a CSA ratio ≥ 1.5. A significant correlation between age and CSA ratio was found in females (Spearman-

Based on a CSA ratio ≥ 1.5 as a criterion for CTS, the present results are consistent with the CTS prevalence reported in previous in vivo studies. It can therefore be assumed that cadaver specimens are valid models for researching median nerve pathologies.

Poster 19:

Titel: Potentials and limitations of Thiel embalming and ethanol-glycerol fixation of the knee

Autoren/Adressen: Cindy Richter (University Leipzig), Patrick Stumpp (University Hospital Leipzig), Nina Barth (University Leipzig), Ingo Bechmann (University Leipzig), Pierre Hepp (University Hospital Leipzig); cindy.richter@medizin.uni-leipzig.de

Abstract:
The knee is the most complex joint in the human body. A complex ligament arrangement, vulnerable to injury, has evolved to counteract its tendency to instability. In a great measure driven by the improvement of new reconstructive surgery our knowledge of the anatomy of the knee has improved considerably.

In cooperative work with a radiologist and a trauma surgeon important structures were first dissected in knees with Thiel embalming in addition with MRI imaging of the same knee. Afterwards ethanol-glycerin fixation was tested for suitability in student dissecting courses.

Against all expectations ethanol-glycerin fixation works even better for identification of ligamentous structures such as posterolateral corner, arcuate complex, anterolateral ligament, medial patellofemoral ligament and medial collateral complex. Nerves and vessels even keep a resistant surface and distinct color. Structures as the anterolateral ligament and fibre bundles of the cruciate ligaments which need a sufficient mobilization to be identified require Thiel embalming. For demonstration of structures in functional context Thiel embalming is still unrivalled.

Even though ethanol-glycerol fixation allows limited mobilization of a joint, it is possible to demonstrate the complex ligament arrangement of the knee even in student dissecting courses in alcohol-fixed specimens.

Poster 20:

Titel: Dynamic visualization of skin angiosomes

Autoren/Adressen: Ines Tinhofer (Medical University of Vienna), Lena Hirtler (Medical University of Vienna), Lukas Reissig (Medical University of Vienna), Max Zaussinger (Medical University of Vienna), Stefan Meng (Medical University of Vienna), Chieh-Han John Tzou (Medical University of Vienna), Lars-Peter Kamolz (Medical University of Graz), Wolfgang Weninger (Medical University of Vienna); ines.tinhofer@meduniwien.ac.at

Abstract:
Defining angiosomes of the skin is the basis for designing skin flaps. Usually the area a skin angiosome covers is identified by injecting dyed solutions into perforator arteries of body donors. This however neglects the blood pressure in neighbouring skin areas and therefore does not provide the extent of an angiosome under physiologic conditions. We aimed at developing a technique that closely mimics physiologic conditions and applied it for researching the extent of the angiosome of the descending genicular artery.

We used 20 fresh frozen anatomical lower limb specimens and injected the descending genicular artery with a pressure of 100mmHg (flow 30%). In addition we cannulated the femoral artery at the level of the adductor hiatus and perfused this artery and both vessels simultaneously.

An angiosome of the descending genicular artery could be identified in 19 specimens. Its dimension during parallel perfusion via both arteries was on average 312,61 cm2. Its dimension during sole perfusion via the descending genicular artery was 415,63 cm2. Its dimension during perfusion via sole perfusion of the femoral artery was 210,64 cm2.

We designed a dynamic method for defining the minimal and maximal extent of angiosomes. Applying it for measuring the dimension of the angiosome of the descending genicular artery revealed that, in a post mortem setting, the dimension of an angiosome significantly varies if only the angiosome is perfused or neighbouring angiosomes are perfused as well. This must be considered when researching angiosomes in human cadaver studies.

Poster 21:

Titel: Vessel and valve morphology of the human femoral vein – complex templates for artificial valve implants

Autoren/Adressen: Jonas Keiler (Rostock University Medical Center), Marko Schulze (Rostock University Medical Center), Ronald Seidel (Helios Medical Center), Armin Springer (Rostock University Medical Center), Horst Claassen (Martin Luther University Halle-Wittenberg), Heike Kielstein (Martin Luther University Halle-Wittenberg), Andreas Wree (Rostock University Medical Center); jonas.keiler@med.uni-rostock.de

Abstract:
Valve reflux in the femoral vein (FV) is suggested as the principal cause for chronic venous insufficiency either by valve damage or venous wall dilatation. Surgical repair of the insufficient valves is not always feasible especially in elderly, multimorbid patients. Therefore, the minimally invasive application of an artificial valve implant is a promising therapeutic approach. Previous prototypes, however, have failed to meet the complex anatomical and physiological requirements. We therefore re-investigated various anatomical aspects of the human FV and its valves.

In vivo: FV diameters were sonographically measured in 82 probands. Post mortem: Donor FVs (n=90) were dissected to analyze valve and tributary topography, and inner circumference. Morphology of fixed valve segments, both untreated and decellularized, was studied with high resolution micro computer tomography and electron microscopy. Paraffin sections of FV segments were stained histochemically (e.g. AZAN) and immunohistochemically (e.g., anti-α-SMA, anti-Col3).

In vivo, the common FV diameter (CVFD) averages 13.6±3.0 mm in supine position and dilates to a mean of 120 % (16.4±2.6 mm) in upright position plus Valsalva maneuver. A slight positive correlation between BMI and diameter is observed.
Post mortem, the CVFD averages 11.6±2.3 mm. FV valve and tributary topography is highly variable between left and right leg and between donors. Approximately 10 nm thin fibrils variably interconnect subendothelial type-I-collagen fibers. Likewise variably present, type-III-collagen-rich longitudinal fibers run in the inner media.

FV morphology is highly variable and complex. Mimicking valve geometry and subendothelial fiber structure might improve both endothelialization and anti-thrombotic properties of future valve prosthetics.

Poster 22:

Titel: Anatomy of Canto

Autoren/Adressen: Marius Valeriu Hinganu (University of Medicine and Pharmacy "Gr. T. Popa" Iasi), Anxa Sava (University of Medicine and Pharmacy "Gr. T. Popa" Iasi), Cristinel Ionel Stan (University of Medicine and Pharmacy "Gr. T. Popa" Iasi), Delia Hinganu (University of Medicine and Pharmacy "Gr. T. Popa" Iasi);
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Abstract:
The aim of the paper is to appreciate the presence of palatine reflexive areas and their functional importance in the voice of the singer

We studied these phenomena on a group of 15 students from the canto faculty in Iassy, using clinical examination of the oro-pharyngian pavilion and glotic flor of the larynx during singing and passing from a register of voice to another.

We were able to confirm the existence of reflexive areas at the palatine level which, when properly stimulated, cause vocal cords tensioning and larynx to descent, with a feeling of comfort and even pleasure at the laryngeal level.

The projections of oropharyngeal subjectivities at Palatinate level are the basis of the learning of canto techniques. These should be included in the anatomy textbooks, and especially in phoniatrics. The anatomical changes underlying the formation of a voice are not fully known.

Poster 23:

Titel: Unravelling the role of muscle in knee osteoarthritis: quadriceps specific-strength, but not mass is associated with subsequent symptomatic knee osteoarthritis progression in women

Autoren/Adressen: Jana Kemnitz (Paracelsus Medical University(PMU)), Wolfgang Wirth (Paracelsus Medical University(PMU)), Felix Eckstein (Paracelsus Medical University(PMU)), Adam Culvenor (Paracelsus Medical University(PMU); Jana.Kemnitz@pmu.ac.at

Abstract:
Quadriceps muscle weakness has been reported to be a modifiable risk factor for symptomatic and radiographic knee osteoarthritis (KOA) incidence and progression in women. To explore the mechanisms behind this relationship, we investigated whether quadriceps mass and/or specific-strength are associated with KOA progression.

Female Osteoarthritis Initiative participants with Kellgren-Lawrence grade ≤3 at baseline, isometric quadriceps strength measurements, and thigh magnetic resonance images (MRIs) at 2- (Y2) and 4-year (Y4) follow-up (n=747, age 62±9 years; BMI 28.7±4.9 kg/m²) were included. Cross sectional (Y2) and longitudinal (Y2→Y4) comparisons of (change in) quadriceps mass and specific-strength (=isometric strength÷mass) were performed between controls without progression Y2→Y4, women with: isolated symptomatic progression (i.e. increase ≥9% in WOMAC pain); isolated radiographic progression (i.e. decrease in minimum joint space width ≥0.7 mm); and combined symptomatic and radiographic progression, using ANOVA.

Women with symptomatic progression between Y2→Y4 displayed a deficit in specific-strength at Y2 relative to controls ($p<0.001$) but no differences in muscle mass, whereas radiographic progression was not predicted by specific-strength or mass. A longitudinal loss of quadriceps mass was observed concurrent with symptomatic progression ($p=0.005$) (but no loss in specific-strength), whereas those with radiographic progression displayed concurrent improvement in quadriceps specific-strength ($p=0.040$).

These findings suggest that, in women, quadriceps specific-strength (but not mass) predicts subsequent symptomatic progression and that quadriceps weakness is not associated with radiographic progression. Future studies should explore whether low quadriceps specific-strength is explained by a lower muscle quality (contractile elements per mass) or a limited ability of muscle-fiber activation.

Poster 24:

Titel: Safe reduction of formaldehyde vapors in breathing air during dissection courses

Autoren/Adressen: Sonja Pfeil (Justus-Liebig-Universität Gießen), Hans Hieke (Justus-Liebig-Universität Gießen), Monika Wimmer (Justus-Liebig-Universität Gießen); sonja-pfeil@posteo.de

Abstract:
Formaldehyde is widely used as an ingredient in anatomical perfusion solutions. Classified as a cancer hazard in high concentrations in vapors from cadavers embalmed with formaldehyde it can cause severe health problems. The new German permissible exposure limit (PEL) for formaldehyde in the workplace of 0.37 mg/m³ urged new methods for the reduction in vapors during dissection courses.

Preliminary investigations were made with preservative solutions of lower formaldehyde content, different kinds of ventilation systems, and post-embalming treatment of cadavers with a formaldehyde destroying agent. In ongoing dissection courses the concentration of formaldehyde in the breathing air of students and in the ambient air was measured while skin, subcutaneous adipose tissue, muscles, the thorax and the abdominal wall were opened.

Best results were achieved with the combination of a formaldehyde destroying agent applied to commonly fixed cadavers (3% formaldehyde) and a modified ventilation system using a long throw nozzle with air coming directly down to each dissection table. Concentrations of formaldehyde in the breathing air did not exceed 0.06 mg/m³ and in the ambient air it did not exceed 0.023 mg/m³.

The data clearly revealed the necessity for a combination of different methods – chemical destruction and ventilation system - to reduce formaldehyde loads in a dissection course using conventionally formaldehyde fixed cadavers. Thus, we could reach sufficient low values below the PEL and even lower than 0.1 mg/m³ as requested by the indoor-air-guideline for non-working-places. According to TRGS 402 “protective measures adequate” can be classified. This ensures safe working not only for students, but also for teachers.

Poster 25:

Titel: Anatomical evaluation of the risk of common peroneal nerve injury in intramedullary tibial nailing

Autoren/Adressen: Lukas Reissig (Medical University of Vienna), Matthias Stockinger (Medical University of Vienna), Barbara Maurer-Gesek (Medical University of Vienna), Wolfgang Weninger (Medical University of Vienna), Michael Humenberger (Medical University of Vienna); lukas.reissig@meduniwien.ac.at

Abstract:
Injuring the common peroneal nerve (CPN) while drilling the bore for the locking screws of tibial nail systems is a rare but feared surgical complication in elderly patients with decreased refractory bone-cortices. This study aimed at examining, if the insertion depth of a tibial nail correlates with the risk of injuring the CPN while using the proximal oblique locking option.

This investigation included 11 right lower limbs of embalmed cadavers. In every limb, a 300 millimeters T2 nail (Stryker) was inserted in 2 different insertion depths and the proximal oblique and mediolateral locking possibilities were drilled. The limbs were then dissected and the distance between CPN and the boreholes was measured. Following this, the soft tissue was removed and the lengths of the bore channels were measured.

In the group of specimens with lower insertion depth of the tibial nail the distance between the proximal, oblique bore channel and CPN measured 0 to 15 millimeters. In 36.4 % the nerve was injured by drilling. In the group of specimens with deeper insertion depths the distance ranged from 0 to 8 millimeters. The nerve injury rate was 27.3 %. Using the mediolateral locking option in neither of the two groups a nerve injury occurred.

We recommend using the oblique locking option only if high stability of the tibial nail is necessary for the optimal healing procedure. In patients with reduced compact bone we recommend using an x-ray image intensifier for monitoring the drilling procedure.

Poster 26:

Titel: Surfactant replacement therapy attenuates abnormal alveolar micromechanics in bleomycin induced lung injury

Autoren/Adressen: Lars Knudsen (Hannover Medical School), Elena Lopez-Rodriguez (Hannover Medical School), Lennart Berndt (Hannover Medical School), Lilian Steffen (Hannover Medical School), Clemens Ruppert (Justus-Liebig-University), Jason Bates (University of Vermont College of Medicine), Heinz-Gerd Hoymann (Fraunhofer Institute for Toxicology and Experimental Medicine), Matthias Ochs (Hannover Medical School), Bradford Smith (University of Colorado Denver, Anschutz Medical Campus); knudsen.lars@mh-hannover.de

Abstract:
Alterations of alveolar micromechanics including intra-tidal alveolar recruitment and derecruitment (R/D) and dynamic heterogeneous ventilation might contribute to acute lung injury by increasing dynamic stresses. We tested the hypothesis that abnormalities in alveolar dynamics are a direct consequence of surfactant dysfunction in bleomycin-induced lung injury.

After bleomycin instillation the Surf group recieved surfactant replacement therapy (SRT) while the Bleo group did not. At day 3 after bleomycin instillation invasive pulmonary function tests were performed to determine lung elastance (H) at positive end-expiratory pressures 1≤(PEEP)≤10cmH2O. Lungs were then perfusion-fixed at end-expiratory airway opening pressures (Pao) of 1-20cmH2O and assessed by design-based stereology to determine the surface area of air-filled alveoli, total volumes of alveoli, alveolar edema as well as number, size and size distribution of alveoli.

Reducing Pao from 10cmH2O to 1cmH2O in the Bleo group resulted in a loss of air-covered alveolar surface area, volume of alveolar airspaces and alveolar number. These changes as well as the increased alveolar size heterogeneity at Pao=10cmH2O could significantly be attenuated by SRT. The SRT-related stabilization of alveoli at lower lung volumes as well as the decrease in alveolar size heterogeneity correlated with improvement in H following SRT. Also, alveolar edema was reduced.

SRT reduces alveolar R/D and alveolar size heterogeneity which correlate with a significant reduction in H at PEEP=1 and 10cmH2O in bleomycin-injured lungs. Hence, SRT might reduce dynamic mechanical stress during the respiratory cycle, representing a potential preventive therapeutic strategy in conditions characterized by high surface tension.

Poster 27:

Titel: Experimental model upper-airway epithelium regeneration.

Autoren/Adressen: Elisabeth Safronova (Sechenov First Moscow State Medical University), Sergey Dydykin (Sechenov First Moscow State Medical University), Andrey Panteleev (Kurchatov Institute), Olga Romanova (Kurchatov Institute), Anna Denisova (Sechenov First Moscow State Medical University), Evgeny Grigoryevsky (Sechenov First Moscow State Medical University), Stepan Kolchenko (Sechenov First Moscow State Medical University), Nataliia Piskunova (Sechenov First Moscow State Medical University); elisabeth-snu@yandex.ru

Abstract:
In this research we developed a rabbit model of mucosal layer injury in trachea and a new method of fixing a tissue-engineering graft inside the trachea to cover the injury and improve a tracheal mucosa reparation.

Fifteen Chinchilla rabbits weighing 4.5 kg were divided into five groups.

In our experiment, we have probed a new method to fix the tissue-engineering scaffold in lumen of any hollow tubular organ to cover the injury of mucosal layer. We have developed an animal model that is suitable for modelling different injuries of upper airways and appears to be less traumatic for an animal.

The experimental model that was described in our study is suitable to perform any injury and any method to influence the regeneration process in upper airways.
Fixing the scaffold with a stent is rather simple and less traumatic method that can be performed by using bronchoscope assisted technique. The scaffold may be fixed this way in lumen of any hollow tubular organ to cover the inner injury or even to replace minor full-thickness defects. However, the scaffold and the stent have to be selected carefully, considering features of the materials and properties of tissues and organs.

Poster 28:

Titel: Effect of prenatal exposure of various doses of synestrol on ovarian morphology in adult life of offspring

Autoren/Adressen: Radik Khayrullin (Ulyanovsk State University), Rimma Sulaymanova (Bashkir State Medical University), Lyaysyan Yusupova (Ulyanovsk State University); prof.khayrullin@gmail.com

Abstract:
Physiological hyperestrogenism during pregnancy in mammals and humans is one of the hormonal mechanisms of normal pregnancy and prevention of miscarriages. This phenomenon has received wide practical use for the prevention of spontaneous abortions in obstetrics. The aim of the study was to determine the morphological changes in the ovaries of adult mature mice, whose mothers underwent various doses of synestrol during pregnancy.

Pregnant mouses was exposed by synestrol during critical dates and the initial stages of morphogenesis of ovaries of offspring.

Depending on the dose of synestrol, which was administered to their mothers, significant macroscopic and microscopic changes in the ovaries were found, the frequency and intensity of manifestation of which were statistically significantly different from those of the control groups. As a result of the study, it was established that macroscopic changes in the ovaries were observed, both unilateral and bilateral, of varying degrees of agenesis and dysgenesis. Microscopically observed a violation of the overall structure of the ovary, the ratio of vascular, stromal and follicular tissue components, of immunohistochemically markers of cell proliferation and apoptosis. A distinctive feature is the presence of numerous atretic follicles and of Call-Exner bodies.

On the basis of the results obtained, conclusions are drawn about the possible risk of a pro-cancerogenic effects of artificial changes in the estrogen level in the prenatal period of development.

Poster 29:

Titel: Experimentally induced intrauterine growth restriction in rabbits leads to differential remodelling of left versus right ventricular myocardial microstructure

Autoren/Adressen: Julia Schipke (Hannover Medical School), Alper Willführ (Hannover Medical School), Christian Mühlfeld (Hannover Medical School); Schipke.Julia@mh-hannover.de

Abstract:
Intrauterine growth restriction (IUGR) is a major cause of perinatal mortality and associated with fetal cardiovascular remodelling accompanied by myocardial dysfunction. Previous studies addressing IUGR effects on cardiomyocyte and microvascularization anatomy are contradictory. Moreover, it was not elucidated yet whether the left and the right ventricle are similarly vulnerable to in utero undersupply.

At 25 days of gestation, IUGR was induced in pregnant rabbits by selective ligation of uteroplacental vessels thus combining restriction of nutrients and oxygen. Design-based stereology was used to quantify the number, volume and maturation status of cardiomyocytes as well as the number, length and supply area of capillaries in the fetal heart, analysing left and right ventricles separately.

Body and heart weight of IUGR offspring were significantly reduced. Cardiomyocyte numbers were decreased in both ventricles in response to IUGR. Only in the left ventricle this was accompanied by significantly higher cardiomyocyte mean volumes. Length and numbers of cardiac capillaries were diminished in left but not in right IUGR ventricles. The cardiomyocyte maturation status and the capillary supply area did not differ between the groups.

While cardiomyocyte numbers are diminished in both ventricles, hypertrophic remodelling of cardiomyocytes and alterations in microvascularization is rather a left ventricular adaptation to IUGR. Thus, fetal left and right ventricles are differently affected by placental insufficiency. These unequal structural changes may be related to developmental differences of the left and the right ventricle.

Poster 30:

Titel: Impact of vasopressin on cell-autonomous expression of membrane transport proteins in rat distal nephron

Autoren/Adressen: Alina Smorodchenko (Institute for Vegetative Anatomy), Yulia Sharkovska (Institute for Vegetative Anatomy), Carsten Dittmayer (Institute for Vegetative Anatomy), Alejandro Cornejo-Müller (Institute for Vegetative Anatomy), Kerim Mutig (Institute for Vegetative Anatomy), Sebastian Bachmann (Institute for Vegetative Anatomy); alina.smorodchenko@charite.de

Abstract:
Reabsorption of NaCl in kidney thick ascending limb (TAL) is controlled by NKCC2 and involves the action of luminal (ROMK) and basolateral (Kir4.1) potassium channels, a basolateral calcium sensing receptor (CaSR), and claudin family of proteins. A morphological cell heterogeneity and mosaic expression of ROMK and Kir4.1 was reported.

We studied TAL EM morphology, zonal and cell-autonomous heterogeneity of the transport proteins at steady state and under stimulation by vasopressin (AVP; V2R agonist dDAVP for 7 or 72 h) using AVP-deficient Brattleboro (BB) and Long Evans (LE) control rats. NKCC2, ROMK, Kir4.1, CaSR and Claudin (Cld)-16 signals were analyzed using immunohistochemistry, in situ hybridization (ISH), EM and Western blot (WB).

TAL morphological cell heterogeneity was obvious among cortex and medullary (m) kidney zones, but not at a cell-to-cell level within a zone. NKCC2 was continuously expressed in all TAL cells, but ROMK and Kir4.1 protein expression showed conspicuous heterogeneity in a mutually exclusive pattern. CaSR and Cld-16 signals were moderate to absent in ROMK-positive cells, but strong in ROMK-negative cells. 72h dDAVP increased the number of ROMK- and Kir4.1-positive cells as well as the overlap of both signals in mTAL. WB showed 2.2-fold increase for ROMK, 1.3-fold for Kir4.1, but down-regulation for CaSR to 60%. ISH revealed no differences in mRNA abundances. No changes were detected after 7h.

These results demonstrate expressional cell heterogeneity in TAL with respect to ROMK, Kir4.1, CaSR, and Cld-16 signals and reflect an adaptive mechanism in renal potassium and calcium handling to maintain electrolyte homeostasis.

Poster 31:

Titel: Loss and recovery of excitatory synapses in the hippocampus after blunt chest trauma

Autoren/Adressen: Silvia Cursano (University of Ulm), Michael Schön (University of Ulm), Stefanie Grabrucker (University of Ulm), Markus Huber Lang (Ulm University Medical Centre), Tobias M. Böckers (University of Ulm); tobias.boeckers@uni-ulm.de

Abstract:
Blunt chest trauma (BCT) is a leading cause of death, morbidity, hospitalization and disability from the age of 1 year to the middle of the fifth decade of life. Up to now, there are only a very limited number of investigations focusing on the effects of a peripheral trauma on the brain.

We employed a mouse model for blunt chest trauma and analyzed selected brain regions after defined time points post BCT with respect to degeneration and regeneration of neurons and synaptic contacts.

Surprisingly, we found that blunt chest trauma induced a massive loss of about half the excitatory synapses in the hippocampus at 5 and 10 days after BCT and observed an almost complete recovery after posttraumatic day 18. In line with this, we observed a partial loss of contextual memory 5 days after the trauma using the trace fear conditioning test.

Preliminary results indicate that the stress-induced local release of hippocampal corticotropin-releasing hormone (CRH) could be the underlying cause for the synapse loss seen in the trauma model. We are currently challenging this hypothesis by either activating or deactivating the stress-related circuitry within the brain to amplify or rescue trauma-induced synaptic and memory loss.

Poster 32:

Titel: Effect of medial longitudinal myelotomy (MLM) plus whole body vibration (WBV) plus riluzole (RLZ)-treatment on motor functions recovery in spinal cord-injured (SCI) rats

Autoren/Adressen: Dilyana Cvetkova (University of Cologne), Stein Gregor (University of Cologne), Carolin Meyer (University of Cologne), Ramona Jansen (University of Cologne), Zeynep Isik (University of Cologne), Hüseyin Erdem (University of Cukurova), Doychin Angelov (University of Cologne); angelov.anatomie@uni-koeln.de

Abstract:
In an attempt to reduce secondary injury after SCI by surgical removal of neural debris (medial longitudinal myelotomy, MLM) we reported that this operation did not improve functional and morphological parameters. At the same time however, MLM did not worsen the values, which rendered its combination with additional treatments possible. In the present study we combined MLM with whole-body vibration (WBV) and early neuroprotection treatment with riluzole (RLZ). This Na-channel blocker impedes the stimulation of NMDA-receptors, reduces the calcium influx into neurons and is thus especially effective for lesioned neurons under ischaemic conditions.

Severe compression SCI at low-thoracic level (Th10) in 14 rats was followed by MLM 48 h later. Seven rats received intra-peritoneal injections with Riluzole (Calbiochem, Cat. # 557324; 8 mg/kg) twice daily over the first week after SCI. The other 7 animals served as controls. All rats were subjected to daily WBV (onset at 14 days after SCI, WBV14) over a 12-week post-injury period. Recovery was analyzed at 1, 3, 6, 9 and 12 weeks after SCI. We determined : (i) BBB-locomotor score, (ii) foot-stepping angle (FSA), (iii) rump-height index (RHI), (iv) number of correct ladder steps (CLS), (v) bladder score (BS), and sensitivity by measuring the withdrawal latency (WL) after thermal stimulus.

Compared to rats treated only with MLM+WBV14, the combination of MLM+WBV14+RLZ did not improve any functional parameter.

RLZ-therapy neither improved recovery, nor reduced the lesion volume nor recovered the amount of synapses in the ventral horn below the lesion.

Poster 33:

Titel: Effect of combined therapy with whole body vibration (WBV) plus erythropoietin (EPO) on functional recovery in spinal cord-injured (SCI) rats

Autoren/Adressen: Julia Frenz (University of Cologne), Aliona Woehler (University of Cologne), Svenja Rink (University of Cologne), Stefanie Richter (University of Cologne), Dominik Arnold (University of Cologne), Zeynep Isik (University of Cologne), Doychin Angelov (University of Cologne); angelov.anatomie@uni-koeln.de

Abstract:
Recently we showed in rats that WBV after SCI improved body weight support, increased the density of synaptic terminals in the lumbar spinal cord and restored bladder function. In the present study we combined WBV with EPO-treatment which should inhibit oxidative stress, suppress glutamate release, decrease lipid peroxidation, reduce local oedema and icrease blood flow and tissue oxygenation.

Following moderate compressive SCI (75 mm/sec) at low-thoracic level (segment Th10) 18 rats were distributed in 3 groups (each of 6 animals). Rats in group 1 (SCI) received no further treatment. Those in group 2 (SCI+WBV) were subjected to daily WBV (onset at 14 days after SCI, WBV14) over a 12-week post-injury period. In addition to WBV, rats in group 3 (SCI+WBV+EPO) received a single i.p injection of rhEPO (Cat. Nr. 11120166001, Roche) 2 hours after SCI. The dosage was 10 µg/kg, i.e. 2,5 µg rhEPO were dissolved in 1 mL phosphate-buffered saline, pH 7.4. Recovery was analyzed at 1, 3, 6, 9 and 12 weeks after SCI. We determined : (i) BBB-locomotor score, (ii) foot-stepping angle (FSA), (iii) rump-height index (RHI), (iv) number of correct ladder steps (CLS), (v) bladder score (BS), and sensitivity by measuring the withdrawal latency (WL) after termal stimulus.

Compared to rats treated with WBV14, the combination of WBV14+EPO did not improve any functional parameter.

Although we cannot exclude beneficial effects of EPO in other SCI models, we conclude that the use of the combination WBV14+EPO requires further investigation before implementing clinical trials.

Poster 34:

Titel: Intrastriatally injected botulinum neurotoxin-a differently effects cholinergic and dopaminergic fibers in c57bl/6 mice

Autoren/Adressen: Alexander Hawlitschka (Rostock University Medical Center), Carsten Holzmann (Rostock University Medical Center), Sarah Witt (Rostock University Medical Center), Juliane Spiewok (Rostock University Medical Center), Anne-Marie Neumann (Rostock University Medical Center), Oliver Schmitt (Rostock University Medical Center), Veronica Antipova (Medical University of Graz), Andreas Wree (Rostock University Medical Center); alexander.hawlitschka@med.uni-rostock.de

Abstract:
Unilateral intrastriatal BoNT-A injection abolished apomoprhine-induced rotational behavior in a rat model of hemiparkinsonism (hemi-PD) up to 6 months. We hypothesized that the beneficial effect of BoNT-A grounded on the reduction of the PD-associated striatal hypercholinism. Intrastriatal injection of BoNT-A was not cytotoxic in rat brain, but neuronal fiber swellings in the BoNT-A infiltrated striata appeared and named BoNT-A-induced varicosities (BiVs). In the rat BiVs were immunoreactive (ir) either for choline acetyltransferase (ChAT) or tyrosine hydroxylase (TH). In the present study the structural effect of unilateral intrastriatal BoNT-A injection in naïve mouse brain was analyzed to extend possible therapeutic BoNT-A applications to genetical Parkinsonian strains.

We investigated the effect of either a single dose of 25 pg BoNT-A injected into the right striatum for up to 9 months, or of increasing doses up to 200 pg on striatal volume, number of ChAT-ir interneurons, and numeric density and volume of the ChAT-ir BiVs in comparison to the uninjected hemisphere.

Intrastriatal BoNT-A injection did not alter the number of ChAT-ir interneurons irrespective of survival time and dosage tested. However, the numeric density of the ChAT-ir BiVs at a dose of 25 pg increased from 1 to 3 months after BoNT-A, followed by a time dependent decrease. In parallel, with increasing BoNT-A survival time, the mean BiV volume increased as the number of small BiVs decreased.

Most interestingly, in contrast to rats we did not find TH-ir BiVs in BoNT-A injected mouse striatum giving further evidence, that mice are not small rats.

Poster 35:

Titel: Differential behavioral effects of botulinum neurotoxin-a injected ipsilaterally or contralaterally into the striatum in hemiparkinsonian rats

Autoren/Adressen: Veronica Antipova (Medical University of Graz, Austria), Carsten Holzmann (University Rostock Medical Center), Oliver Schmitt (University Rostock Medical Center), Alexander Hawlitschka (University Rostock Medical Center), Andreas Wree (University Rostock Medical Center); veronica.antipova@medunigraz.at

Abstract:
In Parkinson's disease loss of dopaminergic neurons in the substantia nigra leads to striatal hypercholinism via disinhibition of cholinergic interneurons. We showed that ipsilateral intrastriatal injections of 1 ng botulinum neurotoxin-A in hemiparkinsonian rats reduced apomorphine-induced rotation behaviour significantly up to six months.

In the present study we extended the behavioral testing of intrastriatal BoNT-A injection ipsilateral to the dopaminergic deprivation, and additionally investigated the impact of contralateral intrastriatal BoNT-A injections on motor and cognitive functions. We hypothized that ipsilateral or contralateral injections of BoNT-A diffentially and temporally influenced behavior due to interhemispheric differences of acetylcholine seen in hemi-PD. Hemi-PD rats were injected with 1 ng BoNT-A or vehicle into either the ipsilateral or contralateral striatum.

In hemi-PD rats intrastriatal ipsilateral BoNT-A injections significantly reduced apomorphine-induced rotations and increased amphetamine-induced rotations, but showed no improvement of forelimb usage, akinesia, lateralized sensorimotor integration and also no effect on spontaneous locomotor activity. However contralateral intrastriatal BoNT-A injections led to a significant increase of the apomorphine-induced turning rate only two weeks after the treatment. Amphetamine-induced rotations were not significantly changed after BoNT-A in comparison to sham-treated animals. Forelimb usage was temporally improved by contralateral BoNT-A injection at two weeks after BoNT-A. Akinesia and lateralized sensorimotor integration were also improved, but contralateral BoNT-A injection had no significant effect on spontaneous locomotor activity.

The different effects suggest that intrastriatally applied BoNT-A acts not only as an inhibitor of acetylcholine release but has possibly also long-lasting impact on transmitter receptors, actually evaluated in our group.

Poster 36:

Titel: Connectomics of the rat hypothalamus

Autoren/Adressen: Oliver Schmitt (University Rostock), Felix Lessmann (), Sebastian Schwanke (), Peter Eipert (), Jennifer Meinhardt (), Julia Beier (), Kanar Kadir (), Adrian Karnitzki (), Linda Sellner (), Ann-Christin Klüncker (), Lena Kuch (), Frauke Ruß (), Jörg-Christian Jenssen (), Andreas Wree ();schmitt@med.uni-rostock.de

Abstract:
The hypothalamus of the laboratory rat needs a complex intrinsic and extrinsic connectivity to realize the control of different aspects of homoeostasis. The connections of the hypothalamus were collated from tract tracing publications in order to characterize its structural connectivity applying graph theoretical investigations.

In a metaanalysis of 7400 publications which describe tract tracing results of normal laboratory rats all connections and experimental data of individual tract tracing experiments (multiple experiments per publication) were extracted and imported into a generic framework (neuroVIISAS) that allows visualization, statistical and graph theoretical analysis as well as dynamic investigations of structural connectomes. Each connection is linked to a according publication. Regions of the connectome are arranged in a hierarchy and are related to each other by neuroontological definitions.

The bilateral hypothalmus is subdivided into 94 regions in latest version of the steretaxic atlas of Paxinos and Watson. These regions are connected by 105 connections in each hemisphere (ipsilateral) and 60 contralateral connections. $5 connections are reciprocal. The average path length is 2.6. The hypothalamic network has a relative large small-worldness coefficient of 8.5 The average cluster coefficient is relative small (0.19). The ventromedial hypothalamic nucleus dorsomedial part (VMdm) has most connections (26) followed by VMvl (23) and VMc (23).

The intrinsic hypothalamic connectome has a specific connectional architecture which has been characterized in terms of stereotaxic atlas regions. We found a typical small-world connectivity with a larger amount of extrinsic connections in comparison with intrinsic interconnections.

Poster 37:

Titel: Dopamine and serotonin receptor densities in the striatum of hemiparkinsonian rats following botulinum neurotoxin-a injection

Autoren/Adressen: Teresa Mann (Institute of Anatomy), Karl Zilles (Institute of Neuroscience and Medicine INM-1/JARA - Translational Brain Medicine, and Department of Psychiatry, Psychotherapy and Psychosomatics), Hanne Dikow (Institute of Anatomy), Markus Cremer (Institute of Neuroscience and Medicine INM-1), Alexander Hawlitschka (Institute of Anatomy), Oliver Schmitt (Institute of Anatomy), Andreas Wree (Institute of Anatomy); teresa.mann@med.uni-rostock.de

Abstract:
Parkinson's disease (PD) is characterized by a massive dopamine deficit in the caudate-putamen (CPu) accompanied by compensatory changes in other neurotransmitter systems. The hemiparkinsonian (hemi-PD) rat is an animal model for PD and used to investigate pathogenic mechanisms and therapeutic strategies. Dopaminergic denervation in hemi-PD rats is induced by unilateral stereotaxic injection of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle. We recently demonstrated that intrastriatal application of Botulinum neurotoxin-A (BoNT-A) abolishes apomorphine-induced rotations in hemi-PD rats up to 6 months. The exact underlying mechanisms have not been fully disclosed yet.
Beside dopaminergic receptors, also serotonergic receptors might be involved in PD's pathology and a close interaction of the serotonergic and dopaminergic system is postulated.

To longitudinally analyze the role of dopamine (D1, D2/D3) and serotonin (5HT2A) receptor densities we performed in vitro quantitative receptor autoradiography in the CPu of unoperated control rats, BoNT-A only injected rats, hemi-PD rats, BoNT-A injected hemi-PD rats, and sham injected hemi-PD rats.

Dopaminergic deafferentation causes a moderate increase in D1 receptor density early post lesion that decreases during longer survival, a significant increase of D2/D3 receptor density, and 50% reduction in 5HT2A receptor density. Application of BoNT-A reduces the interhemispheric imbalance in D2/D3 receptor density in hemi-PD rats.

Our novel data provide insights of the postlesional plasticity of dopaminergic and serotonergic receptors in the hemi-PD rat model. The results further suggest an explanation for the therapeutic effect of BoNT-A on the motor behavior of hemi-PD rats by reducing increased D2/D3 receptor density.

Poster 38:

Titel: Effect of combined therapy with whole body vibration (WBV) plus intracellular sigma peptide (ISP) on functional recovery in spinal cord-injured (SCI) rats

Autoren/Adressen: Dominik Arnold (University of Cologne), Aliona Wöhler (University of Cologne), Svenja Rink (University of Cologne), Julia Frenz (University of Cologne), Zeynep Isik (University of Cologne), Doychin Angelov (University of Cologne); angelov.anatomie@uni-koeln.de

Abstract:
Therapy with WBV after SCI in rats has been shown to improve body weight support, increase the density of synaptic terminals in the lumbar spinal cord and restore bladder function. In the present study we combined WBV with ISP-treatment. By competitive binding with the protein tyrosine phosphatase σ (PTPσ) receptor of the chondroitin-sulphate-proteoglycans (CSPGs) ISP weakens their stable contact with axons, which in turn enables neurite regrowth.

Following severe compressive SCI (100 mm/sec) at Th10-segment 18 rats were distributed in 3 groups (each of 6 animals). Rats in group 1 (SCI) received no further treatment. Those in group 2 (SCI+WBV14) were subjected to daily WBV (onset at 14 days after SCI) over a 12-week post-injury period. In addition to WBV14, rats in group 3 (SCI+WBV+ISP) received 2 h after SCI and then daily i.p injections of ISP (CSBio Co., Menlo Park, CA 94025 USA; Cat. Nr. PEP 200) in dosage 44 microgram in 500 microliter phosphate buffered saline, pH 7.4 over 7 weeks. Recovery was analyzed at 1, 3, 6, 9 and 12 weeks after SCI. We determined: (i) BBB-locomotor score, (ii) foot-stepping angle (FSA), (iii) rump-height index (RHI), (iv) number of correct ladder steps (CLS), (v) bladder score (BS) and sensitivity by measuring the withdrawal latency (WL) after thermal stimulus.

Compared to rats treated with WBV14, the combination of WBV14+ISP did not improve any functional parameter.

We conclude that the use of the combination WBV14+ISP requires further investigation before implementing clinical trials.

Poster 39:

Titel: Adult newborn rat hippocampal granule cells exhibit structural homo- and heterosynaptic plasticity

Autoren/Adressen: Tassilo Jungenitz (Goethe-University Frankfurt), Marcel Beining (Ernst-Strüngmann Institute (ESI)), Tijana Radic (Goethe-University Frankfurt), Hermann Cuntz (Ernst-Strüngmann Institute (ESI)), Thomas Deller (Goethe-University Frankfurt), Peter Jedlicka (Goethe-University Frankfurt), Stephan W. Schwarzacher (Goethe-University Frankfurt); tassilo.j@gmx.de

Abstract:
Adult neurogenesis of dentate gyrus granule cells (GCs) is present in mammals, including humans, and has been implicated in hippocampal forms of learning and memory. Here we study structural maturation, synaptic integration and plasticity of adult newborn GCs (abGCs).

We injected a retrovirus to label abGCs and an adeno-associated virus to label mature GCs. Structural analysis and GC reconstructions were combined with in vivo high-frequency stimulation (HFS) of the medial perforant path known to induce homosynaptic LTP (hom LTP) at tetanized synapses and simultaneous heterosynaptic LTD (het-LTD) at non-tetanized synapses of the lateral perforant path.

Spine numbers in abGCs increased from 21 -77 dpi. HFS of the medial perforant path induced hom-LTP in the middle molecular layer (MML) and conversely het-LTD in the outer molecular layer (OML). Analysis of mushroom spines revealed a homosynaptic spine head enlargement in the stimulated MML and heterosynaptic spine head shrinkage in the adjacent OML. Spine enlargement and shrinkage occurred in parallel on dendritic segments of the same neuron and appeared gradually in abGCs from 21 dpi on, with a sharp increase between 28 dpi and 35 dpi. Application of the non-competitive NMDA receptor antagonist MK801 abolished hom-LTP and het-LTD and completely blocked concurrent structural spine changes.

The gradual appearance of NMDA receptor-dependent structural hom-LTP and het-LTD during the phase of integration may impart a competitive advantage to abGCs helping them to facilitate and stabilize synaptic connectivity.

Poster 40:

Titel: Amount of axons, but not the area of preserved neural tissue bridges (PNTB) determines functional recovery in spinal cord-injured (SCI) rats

Autoren/Adressen: Svenja Rink (University of Cologne), Sina Wennmachers (University of Cologne), Stefanie Richter (University of Cologne), Gregor Stein (University of Cologne), Carolin Meyer (University of Cologne), Kirsten Lütkemeier (University of Cologne), Meysam Dolatnejad Gargari (University of Cologne), Aliona Woehler (University of Cologne), Miriam Wechsler (University of Cologne), Ramona Jansen (University of Cologne), Zeynep Isik (University of Cologne), Doychin Angelov (University of Cologne); angelov.anatomie@uni-koeln.de

Abstract:
It is well known that severity of damage after SCI is proportional to the amount of kinetic energy delivered by the mechanical impact. The subsequent tissue alterations are not well understood, e.g. patients with large post-injury medullar cavities may recover well, regain micturition control and even start to walk. The aim of this study was to correlate the severity degree of SCI with functional recovery.

Spinal cord compression at Th10 was produced in adult rats at impact speed of 50, 75, 100 and 200 mm/s. Recovery was analyzed after 1, 3, 6, 9 and 12 weeks by determining ground locomotion, bladder function and sensitivity. Following fixation spinal cords with the injury site and preserved neural tissue bridges (PNTB) around it were cut in longitudinal sections and stained with cresyl violet and anti-neuronal beta tubulin. Data for functional parameters were correlated with PTB area and amount of axons in them.

Only SCI at speed of 75 mm/sec was suitable for our aim: compression at 100 or 200 mm/s was not followed by functional recovery; SCI at 50 mm/s caused no paraparesis. Interestingly, the lesion length and the PNTB area did not differ among groups. In contrast, linear analysis of the amount of axons in PNTB showed correlation with functional recovery.

We conclude that it is not the PNTB area, that can be determined by e.g. magnetic resonance imaging (MRI), but the amount of axons that enables recovery after SCI. Prognostic statements using MRI-measured area of PNTB should be considered with caution.

Poster 41:

Titel: Does domestication affect predator odor induced innate fear behavior in rats

Autoren/Adressen: Silke Storsberg (Otto-von-Guericke-University), Katharina Gottswinter (Otto-von-Guericke-University), Rafał Stryjek (Polish Academy of Sciences), Andrea Kröber (Otto-von-Guericke-University), Rüdiger Linke (Otto-von-Guericke-University), Thomas Roskoden (Otto-von-Guericke-University); silke.storsberg@med.ovgu.de

Abstract:
Innate defensive fear behavior including avoidance is part of the behavioral system of prey animals such as rats in order to survive and avoid predator threats.
Wild and domesticated rats share a neuronal circuitry which underlies innate fear behavior. Knowledge about this circuitry is important to understand anxiety disorders and may lead to a prospective improvement of these mental conditions such as posttraumatic stress disorder. It is required to compare domesticated and wild animals to extrapolate the results to other species including humans.
We investigated the innate fear behavior of wild Norway rats and compared these behaviors to laboratory rats.

All rat strains are exposed to predator odor or a control stimulus in an open field paradigm. The behavior of the animals is recorded and analyzed. After the test the rats are sacrificed, blood samples are tested for corticosterones and the brains are either immunhistochemically stained or tested via ELISA for c-fos to determine whether there are differences in the quantity of activated neurons.

Our analysis revealed a significant change of corticosterone levels in wild rats but not in domesticated rats which is quite surprising. Both strains showed avoidance behavior and freezing reaction in the test sessions, but only domesticated rats increased the number of freezing events remarkably but not the duration of single events and domesticated rats kept the biggest possible distance to the odor source.

Taken together domesticated rats show a behavior triggered from a stressful event but wild rats do not. The corticosterone levels show something different.

Poster 42:

Titel: Position of nervus abducens in sinus cavernosus: microsurgical preliminary study

Autoren/Adressen: Hasan Orkun IPSALALI (Bahcesehir University), Ahmet Can CIFTCI (Bahcesehir University), Deniz KILIC (Bahcesehir University), Gülfem SENDEMIR (Bahcesehir University), Ilayda KAYA (Bahcesehir University), Sergen SEYHAN (Bahcesehir University), Sule KUTAY (Bahcesehir University), Gursel ORTUG (Bahcesehir University (Supervisor); hoipsalali@outlook.com

Abstract:
The aim of this microsurgical preliminary study is to present the variations of nervous abducens in localization and number as it pierces the clival dura mater.

The calvaria of 15 cadaveric heads fixed in 10% formalin solution were removed by making horizontal cuts from Glabella to Inion in both sides of the head. The dura mater was dissected. Cerebrum and cerebellum were taken out by obtuse dissection. Dissections of sinus cavernosus were made using Leica S6D stereomicroscope and the findings were photographed.

Out of 15 specimens, one of them was excluded. 28 sinus cavernosus (14 heads, bilaterally) were analyzed. Analysis of these sinuses presented 5 different variations. Variation type a classified CN VI as a single trunk and entering a single dural space with 71% occurrence. Variation type b classified CN VI with 2 trunks running inside the sinus and entering a single dural space with 14% occurrence. Variation type c classified CN VI as 2 trunks and entering 2 separate but close dural spaces with 7% occurrence. Variation type d classified CN VI with 3 trunks and 2 separate but close dural spaces with 3% occurrence. Variation type e classified those nerves decussating with nervus oculomotorius with 3% occurrence.

Nervus Abducens plays a major role in the clinic of eye. Due to its intracranial and extracranial course, injuries to head and to the nerve may result in malfunctioning of the muscles of the eye. Therefore, the variations of branching, relations and its course were analyzed.

Poster 43:

Titel: Organotypic nigrostriatal slice cultures of mice as ex vivo model of the nigrostriatal pathway

Autoren/Adressen: Sarah Joost (Rostock University Medical Center), Andreas Wree (Rostock University Medical Center), Stefan Jean-Pierre Haas (Rostock University Medical Center); sarah.joost@med.uni-rostock.de

Abstract:
The nigrostriatal pathway as part of the basal ganglia is of high interest in medical research because the degeneration of dopaminergic neurons of the substantia nigra pars compacta projecting towards the striatum leads to Parkinson's disease. Research on the nigrostriatal pathway is usually conducted on animal models and inducing the degeneration of dopaminergic neurons in vivo is a severe animal experiment. In order to reduce the number of animal experiments and to generate an easily accessible model of the nigrostriatal pathway, we established an ex vivo approach utilising organotypical slice cultures.

Whole-brain sagittal organotypic slices containing all components of the nigrostriatal pathway were cultured on semiporous membranes for several weeks. A different experimental approach with sagittal-frontal co-cultures was used to observe outgrowth of dopaminergic fibres from the substantia nigra. For monitoring of dopaminergic fibres during culture, tissue from TH-eGFP mice, expressing a fluorescent reporter in dopaminergic neurons, was used. Different neuronal and glial cell populations were analysed by immunohistochemical stainings.

After long-term culture, nigrostriatal organotypic slice cultures show a high preservation of dopaminergic neurons in the substantia nigra pars compacta. Furthermore, glial cells show high overall survival and unimpaired morphology.

Organotypic nigrostriatal slice cultures are a promising ex vivo model for the nigrostriatal pathway, offering the opportunity to replace or reduce animal experiments in research on basal ganglia.

Poster 44:

Titel: The role of trafficking of fibroblast growth factor receptor 1 in axon elongation versus axon branching

Autoren/Adressen: Barbara Hausott (MUI), Lars Klimaschewski (MUI); lars.klimaschewski@i-med.ac.at

Abstract:
Fibroblast growth factors (FGFs) and their receptors play an important role in axon growth during brain development and regeneration in the adult nervous system. FGF-2 is up-regulated in response to nerve injury and has been shown to promote neuronal survival and neurite outgrowth mainly via activation of FGF receptor 1 (FGFR1).

Adult dorsal root ganglion (DRG) cultures were transfected with mutants of FGFR1 with reduced numbers of lysines in the intracellular domain or with wild-type FGFR1 (FGFR1-WT). DColocalization with transferrin and the fluorescence intensity of FGFR1 mutants fused to EGFP in the plasma membrane versus the cytoplasm (Fm/Fc) were analyzed after 30 minutes of FGF-2 treatment.

Overexpression of FGFR1 promotes FGF-2-induced elongative axon growth of adult DRG neurons in vitro. This effect is further enhanced by leupeptin, an inhibitor of lysosomal proteases. Leupeptin inhibits degradation of FGFR1 and promotes recycling of the receptor back to cell surface. Furthermore lysine-deficient mutants of FGFR1, which are preferentially recycled back to the cell surface, strongly promote elongative axon growth of adult DRG neurons compared to wild-type FGFR1. In contrast to enhanced receptor recycling, inhibition of endocytosis of overexpressed FGFR1 by methyl-ß-cyclodextrin (MßCD) or chlorpromazine enhances FGF-2-induced axonal branching.

Taken together, enhanced recycling of FGFR1, which allows transfer of activated receptors into signaling endosomes, promotes elongative axon growth, whereas inhibition of FGFR1 endocytosis induces axonal branching.

Poster 45:

Titel: Stimulation of Wnt signaling by R-Spondin enhances proliferation of neonate and adult ENS progenitors

Autoren/Adressen: Melanie Scharr (University of Tübingen), Peter Neckel (University of Tübingen), Karin Seid (University of Tübingen), Bernhard Hirt (University of Tübingen), Lothar Just (University of Tübingen); scharr.melanie@gmail.com

Abstract:
Increasing evidence indicates that the Wnt signaling pathway has a regulatory effect on the proliferation of enteric neuronal progenitor cells in vitro. Therefore, we systematically evaluated the influence of R-Spondin1 and Wnt3A on the proliferation of cultured enteric progenitor cells from neonate and adult mice.

Wnt signaling pathway was analyzed using cultured murine neonate and adult ENS progenitor cells which were characterized by BrdU incorporation assays, RT-PCR, and immunocytochemistry.

Gene expression analysis of proliferating enterospheres verified mRNA expression of Wnt-receptors and upregulation of known Wnt-target genes axin2, lef1, and lgr5 after activation of Wnt signaling pathway. Our cell culture experiments revealed that the total cell mass of proliferating enterospheres enlarged after R-Spondin1 and Wnt3A treatment. Furthermore, total number of isolated neurons (HuC/D+) as well as the amount of BrdU co-labeled neurons strongly increased after activation of Wnt signaling pathway.

Our results give first insights in the Wnt dependent regulation of neuronal progenitor cells derived from the neonate and adult ENS. In ongoing experiments, we are testing the intrinsic Wnt regulation of the enteric nervous system.

Poster 46:

Titel: Effects of estrogen and progesterone on local inflammasome regulation in a contusion model of spinal cord injury

Autoren/Adressen: Adib Zendedel (neuroanatomie), Cordian Beyer (Klinikum Aachen); azendedel@ukaachen.de

Abstract:
Progesterone (P) and 17β-estradiol (E2) are neuroprotective steroid hormones with a high anti-inflammatory potential. Inflammatory responses triggered by spinal cord injury (SCI) involve the processing of interleukin-1beta (IL-1b) and IL-18 mediated by caspase-1 which is under the control of an intracellular multiprotein complex called inflammasome. We recently demonstrated in a SCI model that inflammasome components including IL-18, IL-1b, NLRP3, AIM2, NLRC4, ASC, and caspase-1 are upregulated between 24 and 72 h post-injury. In the current study, we investigated the influence of E2 and P treatment on inflammasome regulation.

Male Wistar rats (300–320 g)at the age of 12 weeks were used for these experiments. After laminectomy, according to the Holtz model contusion lesions were made at the T9 spinal segment by placing a 50g weight on the intact dura for 5 min. Afterwards animals were treated subcutaneously with E2 or P immediately after injury and every 12 h for the next 3 days.

Behavioral scores were significantly improved in E2- and P-treated animals compared to the vehicle groups. Functional steroid-mediated improvement was paralleled by an attenuated expression of certain inflammasome components such as ASC, NLRP1b, and NLRP3 together with IL1b, IL- 18, and caspase-1. On the histopathological level, microgliosis and oligodendrocyte injury were also dampened by steroids.

These findings support and extend the knowledge of the steroid-mediated neuroprotective function. The control of the inflammasome machinery might be a missing puzzle piece to understand the anti-inflammatory potency of both steroid hormones.

Poster 47:

Titel: Cellular and sub-cellular localization of plasticity-related gene 5 in the brain

Autoren/Adressen: Isabel Gross (Rostock University Medical Center), Wree Andreas (Rostock University Medical Center), Anja Bräuer (Carl von Ossietzky University Oldenburg); isabel.gross@med.uni-rostock.de

Abstract:
The investigation of fundamental synapse formation and stabilization processes is of vital importance for the understanding of neurodegenerative diseases. The recently identified neuronal membrane protein, Plasticity-related gene 5 (PRG5) induces the formation of dendritic spine-like structures in primary hippocampal neurons, and therefore could play a significant role in synapse formation. For a more comprehensive understanding of the PRG5 function in the central nervous system, we performed immunohistochemical localization analysis in adult mice brains.

Vibratome sections of P25 to P35 mice brains were prepared and immunohistochemical staining with a polyclonal PRG5 antibody was performed. To identify PRG5 expressing cell types, co-immunostaining with different neuronal marker proteins were carried out.

Pyramidal cells of hippocampal Cornu ammonis regions and granula cells of the dentate gyrus strongly express PRG5. Co-localization with the somato-dendritic marker MAP2 showed a dendrite-specific expression pattern. High amounts of PRG5 were also found in cell bodies and dendrites of cerebellar Purkinje cells, as well as in interneurons and mitral cells of the olfactory bulb. GFAP-positive glial cells were not detected by our PRG5 antibody.

PRG5 is predominantly expressed by neuronal cells in the brain and no glial localization was shown. Its expression is restricted to somatic and dendritic parts of hippocampal, cerebellar and olfactory neurons. Consistent with PRG5 in vitro function in primary neurons, our localization study indicates a dendrite-specific in vivo function of PRG5, possibly in synapse formation and stabilization processes.

Poster 48:

Titel: DNA-repair mechanisms in spinal cord of wobbler mice

Autoren/Adressen: Felix John (Institute of Anatomy, Ruhr-University Bochum), Carsten Theiss (Institute of Anatomy, Ruhr-University Bochum), Veronika Matschke (Institute of Anatomy, Ruhr-University Bochum); veronika.matschke@rub.de

Abstract:
Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects upper and lower moto neurons in the spinal cord and motor cortex. The vast majority of sporadic and familial cases of ALS are still of unknown origin. It is known that multiple cellular events, including oxidative stress, mitochondrial dysfunction, excitotoxicity and protein aggregation are involved in the pathogenesis of ALS.

The wobbler mouse is a useful tool for the investigation of molecular factors of sporadic ALS. High levels of the reactive oxygen species (ROS) in the spinal cord of the wobbler mouse have been previously shown. Possible consequences of high ROS-levels are DNA double-strand breaks (DSB) and the oxidation of deoxyguanosin to 8-Oxo-2'-deoxyguanosine (8-oxo-dG). DSB and 8-oxo-dG might have an impact on replication of proteins and intrinsic apoptosis.

This opens the question how moto neurons react to a high level of ROS and do ROS have an impact on DSB. gH2AX and p53bp1 are one of the most important cell instruments to detect DSB and to initiate different pathways of repair. This study investigates the quantity and quality of this repair mechanisms at different stages of the disease of the wobbler mouse compared to the wild type.

The deregulation of DNA repair mechanisms could be one aspect in ALS pathogenesis.

Poster 49:

Titel: Induction of degenerative processes in motor neurons of wobbler mice

Autoren/Adressen: Mareike Zwilling (Institute of Anatomy, Ruhr-University Bochum), Carsten Theiss (Institute of Anatomy, Ruhr-University Bochum), Veronika Matschke (Institute of Anatomy, Ruhr-University Bochum); veronika.matschke@rub.de

Abstract:
Amyotrophic lateral sclerosis (ALS) is a degenerative disease characterized by a common decrease of motor neurons in the spinal cord. The more prevalent sporadic form is not only primarily caused by genetic factors but by several other environmental factors as well. With the aid of the wobbler mouse as a model for the sporadic form of ALS, there were already found to be various morphological and molecular deregulations within cells of the nervous system. Nevertheless, there are no studies dealing with the induction of these processes in affected cells. Accordingly, the knowledge about the inductor is missing so far. To understand whether there is an endogenous factor triggering the typical cell death or whether there are indispensable exogenous factors which are responsible for the alterations and degeneration of motor neurons of wobbler mice, we intend to work with dissociated motorneuronal cultures.

We culture early postnatal and adult dissociated cells over a fixed period of time after dissecting them form the spinal cord of diseased mice and healthy mice. Subsequently, the cells are dyed with a specific neuronal marker to analyse them in view of morphological characteristics.

The morphological comparison between in-vitro sprouted diseased and healthy cells as well as a comparison to their in-vivo grown complement showed distinct differences and morphological changes. These results give first indications of the period of induction of degenerative processes in cells of the spinal cord within the wobbler mice.

Pursuing these approaches, we start to understand the induction of the motor neuronal degeneration within the spinal cord of wobbler mice.

Poster 50:

Titel: A defect molecular clockwork affects intrinsic properties of neural progenitor cell migration

Autoren/Adressen: Beryl Schwarz-Herzke (Heinrich Heine University), Amira A.H. Ali (Heinrich Heine University), Charlotte von Gall (Heinrich Heine University); Beryl.Schwarz-Herzke@med.uni-duesseldorf.de

Abstract:
Disturbances of the circadian system lead to disintegration of daily body rhythms, reduced life span, cognitive impairment and presenile senescence. Circadian rhythms in organ and tissue function are controlled by a molecular clockwork. The transcription factor BMAL1 is an essential component of the core molecular clockwork. BMAL1-deficient mice (Bmal1-/-) show altered ROS homeostasis, impaired glutamate signal transduction as well as various signs of premature aging and cognitive deficits. We have shown earlier that BMAL1-deficiency affects proliferation and migration of neural progenitor cells (NPCs) in the subgranular zone of the hippocampus as well as the subventricular zone.

In order to dissect the effect of BMAL1-deficiency on the intrinsic properties of NPC migration, we have used an in vitro migration assay.

In isolated Bmal1-/- NPCs, not only migration velocity and expression pattern of genes involved in detoxification of reactive oxygen species were affected but also RNA oxidation of catalase was increased and catalase protein levels were decreased. The Bmal1+/+ migration phenotype could be restored by treatment of Bmal1-/- NPCs with catalase. Moreover, treatment of Bmal1+/+ -NPCs with hydrogen peroxide mimicked the Bmal1-/- migration phenotype.

Thus, we conclude that a defect molecular clockwork affects NPC migration as a consequence of dysregulated detoxification of reactive oxygen species.

Poster 51:

Titel: Trehalose-mediated autophagy does not protect hippocampal HT22 neurons against er-stress-induced cell death

Autoren/Adressen: Luisa Halbe (Klinikum der Johann Wolfgang von Goethe-Universität), Erik Maronde (Klinikum der Johann Wolfgang von Goethe-Universität), Abdelhaq Rami (Klinikum der Johann Wolfgang von Goethe-Universität); rami@em.uni-frankfurt.de

Abstract:
ER-stress leads to the activation of two protein degradation pathways, the ubiquitin-proteasome and lysosome-mediated protein degradation via autophagy. The ubiquitin-proteasome involves translocation of unfolded ER proteins to the cytosol where they are ubiquitinated and degraded. Autophagy can be activated as a secondary response to degrade accumulated proteins and thus attenuate ER stress. Because autophagy plays a major role for cellular homeostasis, we examined the effects of autophagy modulation on ER stress-induced cell death in HT22 neurons.

ER-stress in HT22 neurons was induced by tunicamycin (TM), an inhibitor of glycosylation that disturbs protein folding machinery in eukaryotic cells. We investigated the effects of autophagy-inhibition by 3-methyl-adenine (3-MA) and autophagy-activation by trehalose (TRE) under ER-stress. The dynamics in the expression of a variety of autophagy- and ER-stress-markers were analysed by immunoblotting and immunocytochemistry.

ER-stress induced cell death in HT-22 cells. Cell death was potentiated by 3-MA, a widely used inhibitor of autophagy. In addition, we demonstrated that TRE activated hallmarks of the autophagy machinery in HT-22 cells during ER-stress, but was insufficient to ultimately prevent TM-induced cell death.

Our data show (1) that disturbance of autophagy rendered cells vulnerable to ER stress and (2) that autophagy seems per se to be a protective rather than a deleterious mechanism. Both autophagy and ER stress have been implicated in neurodegenerative diseases, such as Parkinson’s and Huntington’s disease and exploration of the novel signaling pathways relevant to ER stress and autophagy could lead to the development of new therapeutic strategies for these conditions.

Poster 52:

Titel: Cholinergic purinergic cotransmission of the cholinergic parasympathetic detrusor innervation of mice demonstrated by using optogenetic tool

Autoren/Adressen: Amir Rafiq (Justus Liebig University Giessen), Nodir Mirsaidov (Justus Liebig University Giessen), Florian Wagenlehner (Justus Liebig University Giessen), Wolfgang Kummer (Justus Liebig University Giessen); amir.rafiq@anatomie.med.uni-giessen.de

Abstract:
Parasympathetic cholinergic neurons control detrusor contraction. Only 50% of neuronal induced contraction can be inhibited by cholinergic receptor blockers. The rest is accomplished by purinergic receptors. It is unclear, whether purinergic ligands are released together with acetylcholine from the same or another type of nerve fibers. We answered this question by using transgenic mice and optogenetics tool.

We have generated a transgenic mouse line expressing ChR2 (Channel rhodopsin-2) in the cholinergic neurons by crossbreeding ChAT-cre driver line with Ai27D mice. Detrusor contraction induced by the electrical field or by light stimulation of nerve fibers was recorded in an organ bath. A mouse strain (ChAT-eGFP) lacking ChR2 expression was used as a control.

EFS (1-32 Hz) of all nerve fibers led to detrusor contraction in both mouse strains. Optical stimulation by LED (460 nm) caused a contraction (44.2% of EFS) only in urinary bladders expressing ChAT-ChR2, thereby ruling out thermic effects. Atropine (2 µM) completely abolished muscarine (10 µM) induced contraction. However, the optically induced contraction was reduced only by 35.1%. The collective application of purinergic receptor antagonists (suramin 300 µM, PPADS 100 µM) resulted in 33% reduction of light stimulated contraction, while only 9.22% of contraction remained after simultaneous application of atropine, suramin, and PPADS.

We showed that purinergic ligand(s) are released together with acetylcholine from same nerve fiber population resulting in detrusor contraction. Our results indicate that further neurotransmitter(s) contribute to detrusor contraction. The identity of these neurotransmitter(s) needs to be clarified by further investigations.

Poster 53:

Titel: Differential expression of the protein NDRG2 as well as the neurodegeneration-associated microRNA-375 in the spinal cord of the wobbler mouse

Autoren/Adressen: Marlena Rohm (Institute of Anatomy, Ruhr-University Bochum), Carsten Theiss (Institute of Anatomy, Ruhr-University Bochum), Veronika Matschke (Institute of Anatomy, Ruhr-University Bochum); Veronika.Matschke@rub.de

Abstract:
Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease in humans, however the pathogenesis of ALS is poorly understood. To gain insight into the development and course of this disease a detailed analysis on the molecular level is an important approach in current ALS research. The wobbler mouse is considered as a model for the sporadic form of ALS due to its spontaneous mutation in the Vps-54 gene.

The protein NDRG2 and the microRNA miR-375 are analysed regarding their expression in the spinal cord of the wobbler mouse. NDRG2 is associated with cell proliferation, differentiation, transmembrane transport and stress response. Interestingly, NDRG2 is already associated with neurodegenerative diseases. Since miRNAs are directly related to neurodegeneration, and especially miR-375 is linked to NDRG2 expression, they are presumed to be involved in the pathomechanisms in the wobbler mouse.

To investigate the relevance of NDRG2 as well as miR-375 we used qPCR and Western Blot to compare the expression of these molecules in the spinal cord of wobbler mice to wildtype mice. Additionally, in situ hybridization was performed to investigate the localization of NDRG2 mRNA and miR-375 in the spinal cord.

We could show that NDRG2 as well as miR-375 are dysregulated in the wobbler mouse at different developmental stages in the spinal cord. Both genes were detected predominantly in motor neurons.

NDRG2 and miR-375 is likely to play an important role in the pathomechanism in the wobbler mice and hence in ALS.

Poster 54:

Titel: Morphological changes and differential expression of S1PR5 in NPC mice

Autoren/Adressen: Eric Tönnies (Universitätsmedizin Greifswald), Viola von Bohlen und Halbach (Universitätsmedizin Greifswald), Anne Gläser (Universitätsmedizin Rostock), Andreas Wree (Universitätsmedizin Rostock), Anja Bräuer (Universitätsmedizin Rostock), Oliver von Bohlen und Halbach (Universitätsmedizin Greifswald); oliver.vonbohlen@uni-greifswald.de

Abstract:
Niemann-Pick type C1 (NPC1) is induced by a mutation of the NPC1 gene. This rare autosomal-recessive neurodegenerative disease causes an intracellular accumulation of lipids (e.g. sphingomyelin, sphingosine) in the endosomal-lysosomal system followed by severe neurodegeneration. We analyzed the NPC1 mouse model for morphological changes induced by the absence of functional NPC1. Based on the disturbances in the sphingosine system we focused on the expression of sphingosine-1-phosphate receptor 5 (S1PR5).

For the analysis, brains derived from mutated NPC and respective age-matched control mice were analyzed. First of all, brain weight and volume were determined. Thereafter, serial coronal sections throughout the whole brain were made and the thickness of the corpus callosum was analyzed. Furthermore, the ventricles were reconstructed and the volume was estimated. Finally, S1PR5 immunohistochemistry in combination with other markers was performed.

The measurements show a higher total brain mass and volume of wild type (WT) mice compared to mutated animals. Also, the volume of the ventricular system in WT animals is different to the mutated mice. The main S1PR5 distribution in WT is located in myelinated fiber tracts and areas which are associated with the olfactory system. By using double-labelling immunohistochemistry we could show that S1PR5 expression is restricted to oligodendrocytes. RT-PCR experiments demonstrate that S1PR5 is lower expressed in NPC1 mice as compared to WT.

NPC1 deficient mice display severe disturbances in brain architecture and since S1PR5 is differentially in NPC1, S1PR5 may represent a potential regulator of the sphingolipid metabolism in NPC1 mouse model.

Poster 55:

Titel: Impact of VEGF to synaptic plasticity in dissociated Purkinje cells

Autoren/Adressen: Jonas Tjaden (Faculty of Anatomy Ruhr-University Bochum), Carsten Theiss (Faculty of Anatomy Ruhr-University Bochum), Verena Theis (Faculty of Anatomy Ruhr-University Bochum); Verena.Theis@rub.de

Abstract:
Recent studies have identified neuroprotective and neurotrophic functions of the vascular endothelial growth factor (VEGF) in the central nervous system. It revealed, that the somato- and dendritogenisis is enhanced by the exogenous application of VEGF in neonatal Purkinje cells (PCs). Three different transmembrane receptors that bind VEGF are established: VEGF-receptor 1, 2 and 3 (VEGFR-1, 2 and 3) whereas the observed effects in the CNS are mostly mediated by VEGFR-2.

Beside the increased dendritogenesis, an increased growth and new formation of dendritic spines (spinogenesis) caused by VEGF are discussed. Dendritic spines are essential for synaptic transmission and a morphological correlate for synaptic plasticity, which also takes place as a functional change in ion-channel density at the postsynaptic membrane. The experiments focus on T-type-Ca2+-channels as they are expressed in dendritic spines of PCs and play a key role in the stimulus conduction. Therefore, they serve as a sufficient target to evaluate functional changes

In the current study, we cultivate dissociated Purkinje cells derived from postnatal 0 (p0) rats to study, with the help of qPCR, electron microscopy and fluorescence microscopy, the effects on synaptic plasticity of exogenous VEGF application.

We will show, whether T-type-Ca2+-channels Cav3.1 and Cav.3.2 expression is increased and if there is a morphological alteration of the spines after exogenous VEGF administration.

An increased synaptic plasticity after VEGF application might help to understand learning and aging processes in more detail.

Poster 56:

Titel: Effects of progesterone on synaptic plasticity in Purkinje cells

Autoren/Adressen: Annika Eickhoff (Institute of Anatomy), Carsten Theiss (Institute of Anatomy), Verena Theis (Institute of Anatomy); verena.theis@rub.de

Abstract:
The development of cerebellar Purkinje cells (PCs) in the neonatal but not in the mature stage is, with regard to dendritogenesis, spinogenesis and synaptogenesis, physiologically influenced by progesterone via the classical progesterone receptors PR-A and PR-B.

In our current research, we examine effects of exogenous progesterone application on synaptic plasticity of dissociated PCs at the molecular level.

At the center of our investigations are two specific Ca2+-Channels (Cav3.1 and Cav3.2), which are of crucial importance for the communication of neurons, particular in PCs.

We cultivated dissociated Purkinje cell culture from neonatal rat cerebellums (p0-1) with high purity using an immuno-panning-method, followed by cultivation with addition of progesterone.

On mRNA level expression and localization of Cav3.1 and Cav3.2 are analyzed using qPCRs and in situ hybridization. With the help of electron microscopy we reveal changes at pre- and postsynaptical dendrites.

Additionally cell communication between PCs using will be checked by life-cell-imaging.

We will show significant results about targeted expansion of Cav3.1 and Cav3.2 in dissociated PC induced by an exogenous stimulation with progesterone.

On pre- and postsynaptical level exogenous progesterone application will lead to an extended communication between neighboured PCs.

The regenerative effects of progesterone on synaptic plasticity might be of great interest to understand the mechanism of learning or aging and the therapy of neurodegenerative diseases.

Poster 57:

Titel: Regulatory influence of mirnas on VEGF-triggered development of purkinje cells.

Autoren/Adressen: Julian Gehmeyr (Institute of Anatomy, Ruhr-University Bochum), Carsten Theiss (Institute of Anatomy, Ruhr-University Bochum), Verena Theis (Institute of Anatomy, Ruhr-University Bochum); verena.theis@rub.de

Abstract:
Vascular endothelial growth factor (VEGF) is well known as a growth factor with wide-ranging functions not only in the vascular system, but also in the central (CNS) and peripheral (PNS) nervous system. Recently, a lot attention is given to the investigation of its role in neuronal protection, growth and maturation processes. These effects are mainly mediated through VEGF receptor 2 (VEGFR-2). Current studies have shown the age-dependent expression of VEGFR-2 in Purkinje cells (PCs), promoting dendritogenesis and somatogenesis in neonatal but not in mature stages. We hypothesize that microRNAs (miRNAs) are involved in the regulation of VEGFR-2 expression during development of PCs. Therefore, we performed a miRNA profiling and identified two miRNAs that are closely connected to VEGFR-2. These miRNAs are likely to modulate VEGFR-2 expression.

In order to reveal the impact of these miRNAs on VEGFR-2 expression, organotypic slice cultures of postnatal 1 (p1) and 9 (p9) rat cerebellar are cultivated. Synthetic constructs, so-called miRNA-mimics and -inhibitors, are microinjected into PCs to analyze their effects by using confocal laser scanning microscopy and morphometric analysis.

Preliminary results show that the injection of mimics in neonatal developmental stages result in a lack of sensitivity of PCs to VEGF, which inhibits somato-, dendrito- and spinogenesis. The microinjection of inhibitors, on the other hand, has the opposite effect on cell development in adult PCs.

If the positive impact of VEGF can be achieved in adult stages of neuronal development by manipulating the miRNA expression, this could pioneer new ways to treat neuronal injuries.

Poster 58:

Titel: The influence of microRNA on progesterone stimulated Purkinje cells in the cerebellum

Autoren/Adressen: Frederique Wach (Institute of Anatomy), Carsten Theiss (Institute of Anatomy), Verena Theis (institute of Anatomy); verena.theis@rub.de

Abstract:
The steroidogenic enzyme progesterone is known to be expressed in neuronal cells of vertebrates. In cerebellar Purkinje cells (PC) the exposure with progesterone has a positive effect on dendrite-, spino- and synaptogenesis, however this is seen exclusively in neonatal stages. We assume that miRNAs play a key role in this limitation as miRNAs inhibit the biogenesis and functionality of progesterone receptor during maturation. If there is a chance to overcome this limitation treatment with progesterone could be a strategy for a forced regeneration of matured PC e.g. after damage.

To specify the causal relationship of miRNAs and neuronal cell growth organotypic cerebellar slice-culture are used. With aid of microinjection of mimics and antagomirs of miRNAs the influence on progesterone receptor expression and at least on dendrite-, spino-, and synaptogenesis can be tested.

Mimics should imitate the effect miRNA, which implicates a reduced postnatal growing of PC. The effect of inhibiting miRNAs by antagomirs and therefore increasing progesterone receptor activity should cause enhanced dendrito-, spino- and synaptogenesis in matured PC. Both is visualized by pEYFP-actin, localised in PC dendrites and spines.

The prospects of influencing the growth of matured PC will be of significant therapeutic interest for the treatment of traumatic brain injury or neurological diseases like cerebral ischemia.

Poster 59:

Titel: Bmal1-deficiency accelerates migration of neural progenitor cells to the olfactory bulb

Autoren/Adressen: Amira A. H. Ali (Heinrich-Heine-University), Beryl Schwarz-Herzke (Heinrich-Heine-University), Charlotte von Gall (Heinrich-Heine-University); Charlotte.vonGall@med.uni-duesseldorf.de

Abstract:
We have shown earlier that proliferation and distribution pattern of neural progenitor cells (NPCs) in the neurogenic niche of the subgranular zone of the dentate gyrus (SGZ) are affected in mice with a targeted deletion of the core clock gene Bmal1 (Bmal1-/-). In this study, we attempted to address the question whether this change in distribution is a consequence of a change in Migration.

We have analyzed migration of NPCs from the subventricular zone via the rostral migratory stream (RMS) to the olfactory bulb (OB) using BrdU assay and immunohistochemistry.

Proliferation of NPCs in the RMS of Bmal1-/- was decreased, consistent with our previous observations in the SGZ. However, a higher number of NPCs reached the OB in Bmal1-/- , indicating an increase in migration velocity. Marker for oxidative stress and the glial tube surrounding the RMS as well as Reelin immunoreaction in the OB were increased in Bmal1-/-.

Increased migration velocity of NPCs in Bmal1-/- might be a consequence of oxidative stress and paracrine factors from astrocytes and neurons.

Poster 60:

Titel: The important role of micrornas in the regulation of VEGFR2 in the peripheral nervous system

Autoren/Adressen: Kevin Gläsel (Ruhr-University Bochum), Carsten Theiss (Ruhr-University Bochum), Verena Theis (Ruhr-University Bochum); verena.theis@rub.de

Abstract:
The wide-ranging functions (e.g. axonal growth, neuronal cell survival etc.) of vascular endothelial growth factor (VEGF) in the central nervous system (CNS) and peripheral nervous system (PNS) are well known. Most effects are mediated by VEGF receptor 2 (VEGFR-2). However, the regulation of VEGFR-2 is not well understood. MicroRNAs are well discussed as key players in the maturation processes and the development of different diseases. We identified three miRNAs, miR-204-5p, miR-129-5p and miR-130a-3p, that might have an impact on the regulation of VEGFR-2 expression in the sensory as well as in the motoric system in the PNS.

The expression level of VEGFR-2 was analysed by using immunohistochemistry, in situ hybridization and RT-qPCR. To get a deeper insight into the regulation of VEGFR-2 by miRNAs the latter were identified and quantified within neurons.

For the first time we could show an age-dependent VEGFR-2 expression in sensory neurons as well as in motoneurons. This can be directly correlated with the expression of miR-204-5p, miR-129-5p and miR-130a-3p in these neurons.

MiRNAs play a key role in the up- and downregulation of VEGFR-2 expression in neurons of the motoric and sensory system. Revealing the mechanisms of this receptor expression is important to promote new strategies for the treatment of neurological diseases.

Poster 61:

Titel: Cellular and subcellular localization of sphingosine-1-phosphate receptor 5

Autoren/Adressen: Anne Gläser (University Medical Center Rostock), Eric Tönnies (University Medical Center Greifswald), Viola von Bohlen und Halbach (University Medical Center Greifswald), Oliver von Bohlen und Halbach (University Medical Center Greifswald), Andreas Wree (University Medical Center Rostock), Anja Ursula Bräuer (Carl von Ossietzky University Oldenburg); anne.glaeser@med.uni-rostock.de

Abstract:
The sphingosine-1-phosphate receptors (S1PR1-5) are G-protein-coupled receptors, consisting of 7 transmembrane domains, activated by sphingosine-1-phosphate (S1P). They function as regulators of multiple signalling pathways, activating various cell responses e.g. proliferation, migration and apoptosis. Meng et al. (2009) shows regulated S1PR expression during embryonic development via in situ hybridization and our previous expression analysis shows different S1PR expression in the adult mouse brain via qRT-PCR. Based on the expression patterns we focus on the S1PR5.

For celltype-specific analysis of S1PRs, we used primary cells (neurons, astrocytes, microglia and oligodendrocytes), confirmed their purity and determined the expression level via qRT-PCR. The verification of S1PR expression at protein level required cloning of S1PR1-5 plasmids, followed by transfection in HEK293H cells, confirming the functionality of the plasmids used for western blot analysis. Furthermore, we made vibratom sections of wildtype mouse brain for immunhistological detection of S1PR5.

The expression analysis shows an exclusive localization of S1PR5 in oligodendrocytes, confirmed by immunhistological detection in the mouse brain. The functionality of S1PR1-5 plasmids is proved and will be used to verify the antibody specificity.

Based on embryonic expression patterns we will investigate the S1PR5 expression in the postnatal developing nervous system. The localization of S1PR5 just as the verification at the protein level via western blot and further functional experiments concerning the influence of the ligand S1P at S1PR5 expression helps to characterize the regulating functions of S1PR5 in the sphingolipid metabolism.

Poster 62:

Titel: VEGFR2 mediated signaling protects against light-induced photoreceptor degeneration

Autoren/Adressen: Christina Ecker (University of Regensburg), Sabrina I Schmitt (University of Regensburg), Anita Grundl (University of Regensburg), Herbert Jägle (University Clinic Regensburg), Barbara M Braunger (University of Regensburg); Barbara.Braunger@ur.de

Abstract:
Vascular endothelial growth factor receptor 2 (VEGFR2) is broadly expressed in the eye. To learn about its function, we conditionally deleted VEGFR2 in the entire eye and used the light damage paradigm to induce photoreceptor degeneration.

Floxed VEGFR2 mice were crossed with CAG-Cre mice, expressing the Cre recombinase under control of a tamoxifen-responsive chicken actin promoter. Light damage was applied with white light (5000 Lux, 30 min) and apoptotic cell death in the retina was analyzed using TUNEL- labeling. Retinal structure and function were studied by light and electron microscopy, ERG analyses, immunohistochemistry, real time RT-PCR and western blot analyses.

Western blot analyses, real time RT-PCR and immunohistochemistry confirmed the successful deletion of VEGFR2. Retinal structure and function of VEGFR2 deficient mice did not show obvious alterations. However, following light exposure, VEGFR2 deficient mice had a significant higher number of apoptotic TUNEL-positive cells, concomitant with an impaired phosphorylation of proteinkinase B (AKT) compared to control animals. This resulted in a significantly elevated mRNA expression of the pro-apoptotic factor Bad in VEGFR2 deficient animals compared to controls.

VEGFR2 deficiency enhances the vulnerability of photoreceptors following light induced apoptotic cell death, indicating a neuroprotective role of the VEGFR2 signaling pathway.

Poster 63:

Titel: How to make a synaptic ribbon: ribeye deletion abolishes ribbons in retinal synapses and impairs neurotransmitter release

Autoren/Adressen: Stephan Maxeiner (Saarland University), Fujun Luo (Stanford University), Frank Schmitz (Saarland University), Thomas C. Südhof (Stanford University); stephan.maxeiner@uni-saarland.de

Abstract:
Synaptic ribbons are large proteinaceous scaffolds at the active zone of ribbon synapses in the retina and inner ear that are specialized for rapid sustained synaptic vesicle exocytosis. They are associated with large numbers of synaptic vesicles. At the base of the ribbon, voltage-gated Ca2+-channels are enriched. Ribbons are considered to facilitate vesicle release for sustained periods, thereby maintaining a large pool of release ready-vesicles. How synaptic ribbons work at a molecular level is largely unknown. We aim at understanding the function of RIBEYE as a scaffolding protein of synaptic ribbons by analyzing RIBEYE deficient mice.

We have generated RIBEYE deficient mice and subjected them to vigorous electrophysiological analysis. RIBEYE knockouts and controls have been studied by quantitative RT-PCR, immunofluorescence analysis and electron microscopy to assess potential differences reflected in the protein composition at the synapse and on the ultrastructural level in regard to ribbon synapse integrity.

RIBEYE deficiency in the retina resulted in complete loss of synaptic ribbons in photoreceptor and bipolar cell synapses. The number of docked and tethered vesicles was significantly reduced at the active site. Fast and sustained release was strongly reduced in the RIBEYE knockouts. Spontaneous release, however, persisted but was disconnected from the Ca2+ influx sites, which is in line with the observation of dispersed Cav1.4 channels at knockout synapses.

RIBEYE is the key organizer of the synaptic ribbon. Ribbons are needed for fast phasic and sustained release. Ribbons may organize presynaptic nano-domains that position release-ready vesicles adjacent to Ca2+-channels.

Poster 64:

Titel: Regulation of the mossy fibre-CA3 connectivity by Bcl11b/Ctip2

Autoren/Adressen: Elodie De Bruyckere (Ulm University), Ruth Simon (Ulm University), Sigrun Nestel (University of Freiburg), Bernd Heimrich (University of Freiburg), Dennis Kätzel (Ulm University), Herbert Schwegler (Otto-Von-Guericke University), Stefan Britsch (Ulm University); elodie.de-bruyckere@uni-ulm.de

Abstract:
The hippocampus is an important brain structure involved in spatial memory, learning processes and emotional behaviours. Granule cell neurons of the dentate gyrus form the primary gateway for information entering the hippocampus. These neurons project their axons, the mossy fibres, toward the CA3 region where they form highly specialized synapses on large dendritic spines, the thorny excrescences of CA3 pyramidal cells. The hippocampal mossy fibre system has been well established to be critical for the processing of information in the hippocampus.

The zinc-finger transcription factor Bcl11b/Ctip2 is expressed throughout life in post-mitotic granule cells of the dentate gyrus, but is absent from CA3 pyramidal neurons. Previously, we demonstrated that Bcl11b/Ctip2 is a key regulator of postnatal hippocampal development and is important for the differentiation and maturation of new-born neurons during adult hippocampal neurogenesis. The selective ablation of Bcl11b/Ctip2 in the adult forebrain results in a reduced number of thorny excrescences, ultrastructural changes of the mossy fibre bouton, as well as a strong and progressive loss of glutamatergic synapses in the CA3 stratum lucidum. Moreover, we observe a dramatic decrease of mossy fibre LTP in the CA3 and impaired spatial learning after mutation of Bcl11b/Ctip2 in adulthood. Together, our data provide strong evidence of the essential role of the transcription factor Bcl11b/Ctip2 for the regulation of the hippocampal mossy fibre-CA3 connectivity in adult mice.

Poster 65:

Titel: Impact of the peroxisomal tethering protein ACBD5 on motility and positioning of peroxisomes in hippocampal neurons

Autoren/Adressen: Yunhong Wang (Universität Heidelberg), Joseph L Costello (University of Exeter), Jeremy Metz (University of Exeter), Michael Schrader (University of Exeter), Christian Schultz (Universität Heidelberg), Markus Islinger (Universität Heidelberg); Yunhong.Wang@medma.uni-heidelberg.de

Abstract:
Neurons are highly polarized cells which have to regulate positioning and transport of organelles in a tightly controlled manner. To gain further knowledge on the regulation of these processes we analyzed peroxisome localization and motility in hippocampal neurons.

we applied live cell imaging expressing fluorescent marker proteins in primary cultures from mouse hippocampus.

Peroxisomes were preferentially found in the perinuclear region of the soma, with a limited number of peroxisomes located in neurites. Under life conditions multiple peroxisomes performed oscillating movements in the low micrometer range, while a small number were rapidly transported across long distances (>10 µm) in neurites reaching velocities up to 3 µm/sec. Recently, we described that the peroxisomal membrane protein Acyl-CoA binding domain containing protein 5 (ACBD5) and the endoplasmic reticulum (ER) protein vesicle-associated membrane protein-associated protein-B (VAPB) facilitate ER-peroxisome contact zones thereby influencing organelle motility. ACBD5 overexpression in neurons reallocated peroxisomes to the periphery of the soma. Moreover an increased number of peroxisomes were found in neurites. These organelle reallocations were accompanied by significant changes in peroxisome motility: ACBD5 overexpression was found to inhibit both, short- and long-range peroxisomal movements, whereas other organelles showed no changes in motility. Surprisingly, expression of a mutated ACBD5, which is not able to interact with VAPB, still inhibited peroxisomal movements.

Our findings might point to an additional function of ACBD5 in the regulation of peroxisomal motility in neurons, which might be involved in orchestrating organelle interactions.

Poster 66:

Titel: Phenotyping of calcium channel (CACN) subunit α2δ3 knockout mice

Autoren/Adressen: Julia Landmann (University of Leipzig), Franziska Richter (University of Leipzig), Joseph Classen (University of Leipzig), Angelika Richter (University of Leipzig), Josef Penninger (IMBA, Institute of Molecular Biotechnology of the Austrian Academy of Sciences), Ingo Bechmann (University of Leipzig); julia.landmann@medizin.uni-leipzig.de

Abstract:
Sensory impairment or loss has consequences for the affected sensory modality as well as for remaining modalities. Sensory deprivation may alter functional connectivity due to cross-modal processes and can be associated with hypersensitivity in the remaining senses. The consequences of sensory deprivation and cross-modal activation on normal brain function and their physiological underpinnings are still ill-defined.

Here, we phenotyped the calcium channel (CACN) subunit ALPHA2DELTA3 knockout (KO) mouse, which exhibits heat-pain deficiency and pain-induced cortical cross-modal activation (Neely et al., 2010) using various behavioral tests and neurohistological stainings.

Quantification of the expression pattern of Ca2+ channels revealed modifications of the N- and R-type channels suggesting an altered cortical excitability in ALPHA2DELTA3 KO animals. Further, L1-stainings exhibited alterations of thalamocortical fibers reaching the somatosensory/motor cortex, perhaps explaining reduced pain perception in ALPHA2DELTA3 KO mice. Additionally, we found differences in intra- and intercortical fibers, which may also account to cross-modal sensations. Knockout mice showed a constant “head tremble”, an enhanced response upon touch of the pinna and impaired audition. Furthermore, several tests revealed evidence for increased anxiety-like behavior of ALPHA2DELTA3 KO animals. In contrast, olfaction, vision, somatosensation and motor function did not appear to be altered. Interestingly, in a task challenging multiple modalities, mutant mice displayed superior performance compared to wildtype mice.

In summary, loss of the calcium channel subunit ALPHA2DELTA3 results in alterations in neuroanatomy and electrochemical properties of cortical neurons, which might be the cause for hypersensitivity and cross-activation of cortical sensory regions observed in these animals.

Poster 67:

Titel: The role of Bcl11b/Ctip2 in postnatal hippocampal mossy fiber development

Autoren/Adressen: Claudia Soi (University of Ulm), Ruth Simon (University of Ulm), Herbert Schwegler (Otto-von-Guericke-University), Stefan Britsch (University of Ulm); claudia.soi@uni-ulm.de

Abstract:
Hippocampal mossy fibers, the axons of dentate gyrus granule cells projecting to CA3 play a crucial role in memory and learning processes. However, the precise molecular mechanisms that regulate mossy fiber development, their wiring and spatial organization, are incompletely understood. The zinc finger transcription factor Bcl11b/Ctip2 is essential for postnatal development of the dentate gyrus. Loss of Bcl11b causes a reduction of progenitor cell proliferation and differentiation as well as the disorganization of mossy fiber projections resulting in impaired spatial memory and learning. To determine functions of Bcl11b/Ctip2 in mossy fiber development with single axon resolution we genetically labelled small amounts of dentate granule neurons by in vivo electroporation of a GFP reporter. Analyzing control and Bcl11b;Emx1-Cre mutant animals revealed a higher number of mossy fiber boutons in the CA3 region of mutant animals. In addition, a spatial misdistribution of the mossy fiber axons as well as of mossy fiber boutons was observed. Differential transcriptome analyses revealed Slit1, an axon guidance molecule, as a putative target gene of Bcl11b/Ctip2 in this process. Reintroduction of Slit1 in Bcl11b;Emx1-Cre mutant animals by in vivo electroporation was able to rescue the mossy fiber bouton phenotype but did not correct for the misdistribution of mossy fiber axons. This suggests Bcl11b/Ctip2 to control hippocampal mossy fiber development in part through its downstream target Slit1. Furthermore, our data suggest additional downstream pathways of Bcl11b/Ctip2 to be involved in this process.

Poster 68:

Titel: Enteric glial cells express the GDNF receptors GFRA1, GFRA2 and ncam in vivo and in vitro but display limited response to GDNF.

Autoren/Adressen: Marie Möding (Anatomisches Institut der CAU Kiel), Martina Barrenschee (Anatomisches Institut der CAU Kiel), Christina Lange (Anatomisches Institut der CAU Kiel), Michael Ebsen (Städtisches Krankenhaus Kiel), Ilka Vogel (Städtisches Krankenhaus Kiel), Thilo Wedel (Anatomisches Institut der CAU Kiel), Martina Böttner (Anatomisches Institut der CAU Kiel), Francois Cossais (Anatomisches Institut der CAU Kiel); f.cossais@anat.uni-kiel.de

Abstract:
The neurotrophic GDNF/Ret signaling pathway is a key regulator of enteric nervous system (ENS) development and maintenance. Besides enteric neurons, enteric glial cells (EGC) represent the main cellular compartment of the ENS. Whereas GDNF directly regulates plasticity parameters of enteric neurons, less is known about the impact of GDNF on EGC. Therefore, we characterized the glial expression of the GDNF-receptors within the human colonic ENS, and analyzed the impact of GDNF on EGC markers in vitro.

Co-stainings for the GDNF receptors Ret, GFRa1, GFRa2 and NCAM and the glial marker S100ß was performed on human colonic specimen. Impact of GDNF on the expression of the glial markers S100ß and GFAP, and on the expression of the GDNF-receptors Ret, GFRa1, GFRa2 and NCAM was analyzed using RT-qPCR in EGC lines and murine primary ENS cultures in vitro.

Expression of the GDNF receptors GFRa1, GFRa2 and NCAM but not Ret co-localized with the glial marker S100ß in the human colonic myenteric and submucosal plexus. Expression of GFRa2 was induced by treatment with GDNF both in EGC lines as well as in primary cultures of ENS, whereas GDNF did not impact the expression of the GDNF receptors GFRa1 and NCAM or the expression of the glial markers GFAP and S100ß in vitro.

Although EGC express important receptors for GDNF, our preliminary results indicate that GDNF exerts only little influence on EGC in vitro. Further work is required to determine the functional relevance of these receptors in EGC under physiological and pathological conditions.

Poster 69:

Titel: Site-specific gene expression and localization of HSPA8 and HSPA1a in the human enteric nervous system (ENS) in diverticular disease.

Autoren/Adressen: Andrea Preuße-Prange (Christian-Albrechts University Kiel), Martina Barrenschee (Christian-Albrechts University), Christina Lange (Christian-Albrechts University Kiel), Francois Cossais (Christian-Albrechts University Kiel), Bodo Kurz (Christian-Albrechts University Kiel), Thilo Wedel (Christian-Albrechts University Kiel), Martina Böttner (Christian-Albrechts University Kiel); a.preusse@anat.uni-kiel.de

Abstract:
Heat shock proteins (HSPs) are a family of proteins that are synthesized by cells in response to exposure to stressful conditions (e.g. inflammation). Besides the chaperone function HSPA8 (constitutive form) and HSPA1a (inducible form) also fulfill an important role in chaperone-mediated autophagy (CMA). Since diverticular disease (DD) is associated with intestinal hypoganglionosis and inflammation, the aim of our study was whether fluctuations in the mRNA expression of HSPA8 and HSPA1a play a role in the pathogenesis of DD.

Segments of the distal colon of patients with DD (n = 10) and controls (n = 10) were assessed for investigation of HSPA8 and HSPA1a. In localization studies we performed dual-label immunohistochemistry of HSPA8 and HSPA1a with PGP 9.5 and Hu C/D as pan-neuronal markers and S100β and GFAP as glial cell markers. Furthermore site-specific expression of HSPA8 and HSPA1a was examined by real-time quantitative polymerase chain reactions (qRT-PCR).

In the localization study, both proteins showed the strongest signals in the neuronal somata of the myentric plexus and submucous plexus. Compared to the control samples, the samples of patients with DD showed an up-regulation in the mRNA expression of HSPA8 and HSPA1a.

To our knowledge, this is the first study on HSPs in the ENS and their role in DD.

Since the task of the HSPs is the protection of the neurons in stress situations, further studies should clarify whether an altered mRNA expression of the HSPs in DD is responsible for the development of intestinal hypoganglionosis.

Poster 70:

Titel: Functional relevance of local endocannabinoid signalling in the hypothalamic arcuate nucleus

Autoren/Adressen: Henrike Horn (University of Leipzig, Medical Faculty), Beatrice Schützelt (University of Leipzig, Medical Faculty), Marco Koch (University of Leipzig, Medical Faculty); marco.koch@medizin.uni-leipzig.de

Abstract:
In order to maintain energy homeostasis, peripheral humoral signals and local synaptic input organisation in the hypothalamic arcuate nucleus (ARC) provide reciprocal activity of anorexigenic proopiomelanocortin (POMC) and orexigenic Agouti-related peptide (AgRP)/ neuropeptide Y (NPY) neurons. Interestingly, chronic overeating finally leading to obesity is strongly associated with malfunction of the local neuronal network in the ARC. Whether physiological control of energy homeostasis principally depends on local ARC lipidergic signalling molecules, such as endocannabinoids (ECs) remains elusive. Moreover, it is of significant interest to reveal whether imbalanced EC signalling in the ARC stimulates overeating and contributes to onset and maintenance of obesity.

Prandial state and diet-dependent dynamics of EC synthesizing/degrading enzymes in the ARC are determined by qPCR and Western Blot techniques, while their distribution is analysed by use of immunofluorescence and electron microscopy. EC levels are quantified by use of HPLC/MS-MS. Short-term and chronic experimental manipulation of local ARC EC signalling is determined by region-specific pharmacological and chemogenetic interventions in mice under normal and high fat diet. Finally, the relevance of cell type specific EC signalling in POMC and AgRP/NPY neurons is determined by electrophysiology and by use of the cre/loxP system.

Our results provide mRNA and protein expression in the ARC of all relevant members of the EC system. Moreover, morphological characterisation reveals a predominant retrograde mode of EC signalling at AgRP/NPY neurons, while POMC neurons also show non-retrograde modes of action.

Together, our data point toward a significant contribution of EC in the ARC for retention of energy homeostasis.

Poster 71:

Titel: Different roles of the small GTPases Rac1, Cdc42, and RhoG in CALEB/NGC-induced dendritic tree complexity

Autoren/Adressen: Jana Schulz (Ulm University), Kristin Franke (Ulm University), Manfred Frick (Ulm University), Stefan Schumacher (Ulm University); jana.schulz@uni-ulm.de

Abstract:
Rho GTPases are fundamental for cytoskeletal reorganizations. Much is known about the individual functions of Rho GTPases in distinct signaling pathways leading to cytoskeletal rearrangements. However, major questions addressing the integration and the signaling hierarchy of different Rho GTPases in regulating the cytoskeleton in physiological events like neuronal process differentiation have yet to be answered. Here, we investigate the roles of the small GTPases Rac1, Cdc42 and RhoG in defining dendritic tree complexity evoked by the EGF family member CALEB/NGC.

To elaborate the effects of these small GTPases on dendritic branching, we combined gain-of-function and loss-of-function analysis in primary hippocampal neurons. Furthermore, we performed Rac activation assays, FRET analysis, and cell-surface localization studies to investigate the functional interaction between CALEB/NGC and the small GTPases with respect to dendritic tree complexity.

We find that CALEB/NGC-mediated dendritic branching is differentially affected by Rac1, Cdc42, and RhoG. Rac1 is essential for dendritic tree complexity induced by CALEB/NGC, whereas the Rac1-related GTPase RhoG adversely affects dendritic branching as an upstream regulator of CALEB/NGC. In contrast, Cdc42 is not directly interconnected to CALEB/NGC with regard to dendritic tree complexity. Interestingly, we find that only the palmitoylated, but not the prenylated isoform of Cdc42 reduces dendritic branching.

Mechanistically, CALEB/NGC activates Rac1 while RhoG reduces the amount of CALEB/NGC that is located at the right site for Rac1 activation. Thus, Rac1, Cdc42, and RhoG perform very specific and non-redundant functions at different levels of hierarchy in regulating dendritic tree complexity induced by CALEB/NGC.

Poster 72:

Titel: R-smad-dependent TGFβ signalling mediates TGFβ-induced effects on microglia

Autoren/Adressen: Phani Sankar Potru (University of Freiburg), Tanja Zöller (University of Freiburg), Björn Spittau (University of Freiburg); phani.sankar.potru@anat.uni-freiburg.de

Abstract:
Microglia are the resident immune cells of the central nervous system (CNS) and are exclusive conciliators of immune responses in CNS. TGFβ1 signalling is crucial for the induction of a microglia-specific molecular signature as well as maintaining an immunologically inactive microglia state. However, the molecular mechanisms of microglial TGFβ1 signalling are not well understood. It is believed that TGFβ1 signalling transduction requires the formation of a complex containing SMAD2 and/or SMAD3 (R-SMADs) and SMAD4 (Co-SMAD). However, several studies have proven that TGFβ signalling is possible in a SMAD-independent manner.

In the present study, we addressed the question whether TGFβ-induced effects on microglia are mediated in a SMAD-dependent and/or SMAD-independent manner. Using primary mouse microglia and BV2 cells we performed subcellular fractionations, western blotting, Co-IPs as well as PathScan Intracellular Signalling Arrays.

Here, we demonstrate impaired phospho-SMAD2-mediated transcriptional regulation is observed in microglia-specific deletion of Tgfbr2 but not of Smad4. Moreover, we revealed that SMAD2 and SMAD4 translocated independent of each other into the nucleus and SMAD4 was undetectable in chromatin fractions despite the presence of SMAD2/3. Furthermore, Co-IP experiments revealed that SMAD2 and SMAD4 do not seem to interact upon TGFβ treatment in microglia. Finally, SMAD-independent pathways were not activated after stimulation of microglia with TGFβ1.

Taken together, our data indicate that SMAD2/3 and SMAD4 do not necessarily interact upon stimulation with TGFβ1 and that SMAD-independent TGFβ signalling might be dispensable in microglia.

Poster 73:

Titel: Functional analysis of novel alternatively spliced transcripts of rat and human aquaporin-4 in the sensory domain of the cochlear duct

Autoren/Adressen: L. Garcia Pradas1, F. Sigg1, A. Wagner1, H. Kalbacher2, A.F. Mack1, B. Hirt1, C. Gleiser1 ; 1 Institute of Clinical Anatomy and Cell Analysis, University of Tübingen, Germany;2 Medical and Natural Sciences Research Centre, Tübingen, Germany; kristin.jaeger@anatom.uni-tuebingen.de

Abstract:
Objective: The supporting cells of the cochlea are exposed to high K+ levels during mechano-electrical sound transduction. This results in rapidly changing transmembranous osmotic gradients, which force the epithelium of the cochlear duct to equilibrate its cell volume. The osmotic volume equilibration of the supporting cells in the context of K+ recycling is mediated by the water channel aquaporin-4 (AQP4) in co-localization with the potassium-channel Kir4.1. Interestingly, the supporting cells at the transduction sites of the K+ recycling routes lack AQP4, and this counteracts the necessity of rapid osmotic equilibration of the supporting cells.
Methods: We used 3'RACE- and qPCR to identify novel splice variants of AQP4. Using immunofluorescence confocal microscopy, we analyzed the expression of the AQP4 splice variants in the cochlear duct. Moreover, a fluorescence quenching-based assay was used to measure the volume changes of transfected cells after osmotic stimulation, revealing the water transport capability of the AQP4 splice variants.
Results: We identified two alternatively spliced transcipts of human and rat AQP4, where exon 4 is replaced. We found a predominantly cytoplasmic expression of these splice variants in the supporting cells directly adjacent to the hair cells using specific anti-AQP4 antibodies. Water transport measurements of transiently transfected cells showed different osmotic cell responses compared with wild-type cells.
Conclusion: The distinct expression of the novel AQP4 splice variants in the supporting cells of the Organ of Corti may enable them to maintain their intracellular volumes against the exceptionally high ionic gradients that emerge in the cortilymph during sensory transduction.

Poster 74:

Titel: Investigating the barrier function of cultivated cells isolated from dissociated endolymphatic sac tissue of the inner ear.

Autoren/Adressen: JW. v. d. Ruhr, A. Wagner , L. Garcia Pradas; B. Hirt, L. Just, C. Gleiser; Institute of Clinical Anatomy and Cell Analysis, University of Tübingen, Germany;
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Abstract:
Objectives: Correct hearing as well as the sense of balance is based on homeostasis of the endolymphatic fluid in the inner ear. A disturbed homeostasis leads to severe pathologic effects like endolymphatic hydrops, which are typical for Morbus Menière. Partly responsible for this, is the endolymphatic epithelium of the inner ear acting as a regulation barrier.
Methods: To investigate this barrier function cells were dissociated from isolated endolymphatic sac tissue of rat inner ears. The isolated cells were cultivated prior to analyzation by qPCR and immunocytochemistry and then compared to native endolymphatic sac tissue. Since a recent study suggests that arginine vasopressin (AVP) is a potential regulator of endolymphatic fluid and ion homeostasis, cells and isolated inner ear tissue were osmotically challenged using AVP to test for the barrier function in vitro and in vivo.
Results: In this study, we identified specific tight junction (TJ) molecules and ion channel proteins in epithelial cell preparations of the rat endolymphatic sac tissue by qPCR and immunofluorescence confocal microscopy. Further, by measuring the osmotic induced volume changes, we showed that proteins involved in the barrier function of epithelial cells originating from endolymphatic sac tissue are relevant targets of arginine-vasopressin (AVP).
Conclusions: Our results indicate a high relevance of tight junction (TJ) molecules and water respective ion channel proteins as important barrier molecules in the inner ear. To some extent, it is also possible to verify these molecules in vitro in cell preparations from inner ear tissue, indicating that cell culture systems could be used to investigate the pathophysiology of Menière’s disease.

Poster 75:

Titel: Analysis of the tissue quality of post-mortem human cochlea

Autoren/Adressen: A. Wagner, L. Garcia Pradas, K. Jäger, B. Hirt, C. Gleiser; Institute of Clinical Anatomy and Cell Analysis, University of Tübingen, Germany; kristin.jaeger@anatom.uni-tuebingen.de

Abstract:
Objective: Otology is unique in that the inner ear is mostly inaccessible during life. Only two centers worldwide were able to take human cochlea out in occasional surgeries. Hence, insights into the basis of the inner ear function can be obtained only by postmortem studies of human temporal bones. However, the human cochlea is one of the most difficult tissues to study due to the bony capsule and its vulnerable contents. In addition, the inner ear tissue undergoes quick autolytic changes, making investigations difficult.
Methods: By building an efficient organizational structure, we obtained human inner ear tissue in a very short post mortem period using an endaural access procedure. We extracted mRNA of cochleae obtained from body donors in various post mortem times. We estimated the RNA integrity scores and analyzed the marker expression of cochlea by qRT-PCR. In addition, we examined the expression of markers on the human cochlea by immunofluorescence confocal microscopy.
Results: We isolated intact total mRNA of cochlea tissue within a post mortem period of up to 12h. We showed that this mRNA is suitable for gene expression analysis. Our immunofluorescence analysis using functional and molecular markers showed that the morphology of the ductus cochlearis' cells and the ease of immunostaining is preserved over 12h.
Conclusion: Our organizational structure and the endaural access to the inner ear offers the possibility to isolate intact mRNA for gene expression analysis and the advantage of good preservation of morphology and ease of immunostaining.

Poster 76:

Titel: TFF1 overexpression reduces tumorigenic potential of human retinoblastoma cells involving p53-caspasis pathway and MiR-18a regulation

Autoren/Adressen: Maike Busch (University of Duisburg-Essen, Medical Faculty), Jan Große-Kreul (University of Duisburg-Essen, Medical Faculty), Janina Jasmin Wirtz (University of Duisburg-Essen, Medical Faculty), Harald Stephan (University of Duisburg-Essen, Medical Faculty), Manfred Beier (Heinrich-Heine University, Medical Faculty), Brigitte Royer-Pokora (Heinrich-Heine University, Medical Faculty), Klaus Metz (University of Duisburg-Essen, Medical Faculty), Nicole Dünker (University of Duisburg-Essen, Medical Faculty); maike.busch@uk-essen.de

Abstract:
Trefoil factor family (TFF) peptides play a pivotal role in oncogenic transformation, tumorigenesis and metastasis by changing cell proliferation, apoptosis, migration and invasion behavior of cancer cell lines. We investigated the effects of TFF1 overexpression in different retinoblastoma (RB) cell lines.

Effects of TFF1 overexpression on RB cell growth and viability were revealed by WST-1 and TUNEL assays as well as BrdU and DAPI cell counts. A caspase inhibitor and capase-3 immunocytochemistry were used to investigate the involvement of caspases in apoptosis induction. p53 activity was measured by pG13-luciferase reporter assays and WB analysis. Gene expression changes were analysed by expression array analysis and Q-RT-PCR. Effects of TFF1 on tumorigenicity and migration were analysed using in ovo chicken chorioallantoic membrane (CAM) and soft agarose assays.

TFF1 overexpression decreases RB cell viability, proliferation and growth and significantly increases apoptosis. Apoptosis is executed through cleaved caspase-3 activation. TFF1 induced apoptosis is mediated through transcriptional activity of p53 with concurrently downregulated miR-18a expression. CAM assays revealed that TFF1 overexpression significantly decreases the size of tumors forming from RB cells as well as reduces the migration potential. Differentially expressed genes and pathways involved in cancer progression were identified after TFF1 overexpression in Y79 cells, underlining the effects on reduced tumorigenicity. TFF1 KD revealed caspase-3/7 independent apoptosis induction.

In summary, the in vitro and in vivo data demonstrate for the first time a tumor suppressor function of TFF1 in RB cells which is at least partly mediated by p53 activation and miR-18a downregulation.

Poster 77:

Titel: News on function of PRG5

Autoren/Adressen: Anja U. Bräuer (Carl von Ossietzky Universität Oldenburg); anja.braeuer@uni-oldenburg.de

Abstract:
The investigation of fundamental synapse formation and stabilization processes is of vital importance for the understanding of neurodegenerative diseases since they often include spine loss or other dendritic changes. Overexpression of the recently identified neuronal membrane protein, Plasticity-related gene 5 (PRG5) induces the formation of dendritic spine-like structures in primary immature hippocampal neurons and leads to filopodia outgrowth in different non-neuronal cell lines.

For a more comprehensive understanding of PRG5 function in the brain, we performed immunohistochemical localization analysis in adult mice brains with a polyclonal PRG5 antibody and different neuronal marker proteins.

PRG5 is predominantly expressed by neuronal cells and no glial localization was shown. Its expression is restricted to somatic and dendritic parts of hippocampal, cerebellar and olfactory neurons. Consistent with PRG5 function in primary neurons, our localization study indicates a dendrite-specific in vivo function of PRG5, possibly in synapse formation and stabilization processes.

To examine the impact of PRG5 loss in the cultured cells, we chose HAP1 cells with a loss of function mutation in the PRG5 gene. Overexpression of PRG5 in both, mutated and wildtype HAP1 cells, provokes intense filopodia outgrowth, indicating an involvement of PRG5 in general membrane modification processes. Compliant with these results, affymetrix transcriptome analysis of the knockout cell line revealed several differentially expressed genes. For basic examination of cell properties, a colony formation test was performed, and resulted in differential colony growth patterns of PRG5 knockout cells.

Results could provide further insight on possible signaling pathways of PRG5 induced mechanisms.

Poster 78:

Titel: A retrospective study realised on 218 cases of spinal meningiomas

Autoren/Adressen: Anca Sava ("Gr. T. Popa" University of Medicine and Pharmacy Iaşi), Gabriela Florenţa Dumitrescu (“Prof. Dr. Nicolae Oblu” Emergency Clinic Hospital Iasi), Cristinel Ionel Stan ("Gr. T. Popa" University of Medicine and Pharmacy Iaşi), Delia Hînganu ("Gr. T. Popa" University of Medicine and Pharmacy Iaşi), Lucia Indrei ("Carol Davila" University of Medicine and Pharmacy Bucharest), Claudia Florida Costea ("Gr. T. Popa" University of Medicine and Pharmacy Iaşi), Lucian Eva (“Prof. Dr. Nicolae Oblu” Emergency Clinic Hospital Iaşi); dr.anca.sava.68@gmail.com

Abstract:
The aim of this study is to emphasize the histopathological type of meningiomas, which are predominantly benign tumors of adults, usually attached to the dura, that arise from the meningothelial cells of the arachnoid.

Our article studied the histopathological types of 218 spinal meningiomas from patients which were treated in the Emergency Hospital ,,Prof. Dr. Nicolae Oblu’’ Iaşi during two years: 2015 and 2016. The tumours were examinated both from a histopathological and immunohistochemical point of view.

From these 218 cases, 138 were females and 80 males. Regarding the age, the majority of the pacients were situated in the 7-th decade of age. We observed atypical and anaplastic meningioma too in the cases presented, but they did not occur as frequently as cerebral meningiomas. We noticed two cases of meningiomas associated with hemangiomas or glioblastoma of the brain.

The majority of these meningiomas were psammomatous type (42%), followed by meningothelial type (21%) and fibroblastic (16%).

Poster 79:

Titel: ATF4 promotes angiogenesis and confers ferroptosis in a xCT-dependent manner

Autoren/Adressen: Nicolai Savaskan (Universitätsklinikum Erlangen & BiMECON), Daishi Chen (Universitätsklinikum Erlangen FAU), Zheng Fan (ETH Zürich), Manfred Rauh (Universitätsklinikum Erlangen FAU); savaskan@gmx.net

Abstract:
Activating transcription factor 4 (ATF4) is a critical mediator of metabolic and oxidative homeostasis and cell survival and is elevated under starvation, ER stress damages and exposure to toxic factors.

We facilitated human brain specimens and performed the whole range of biomedical methods and assays.

Here we show that ATF4 expression fosters the malignancy of primary brain tumors (WHO grade III and IV gliomas) and increases proliferation and tumor angiogenesis. Hence, ATF4 expression promotes cell migration and anchorage-independent cell growth, whereas siRNA-mediated knockdown of ATF4 attenuates these features of malignancy in human gliomas. Further experiments revealed that ATF4-dependent tumor promoting effects are mediated by transcriptional targeting the glutamate antiporter xCT/SCL7A11 (also known as system Xc-). Moreover, increased xCT levels ameliorate sorafenib and erastin-induced ferroptosis. Conversely, ATF4 knockdown renders cells susceptible for erastin, sorafenib and RSL3-induced ferroptosis. We further identified that ATF4 promotes tumor-mediated neuronal cell death which can be alleviated by xCT inhibition. Moreover, elevated ATF4 expression in gliomas promotes tumor angiogenesis. Noteworthy, ATF4-induced angiogenesis could be diminished by ferroptosis inducers erastin and by GPx4 inhibitor RSL3.

Our data provide proof-of-principle evidence that ATF4 fosters proliferation and induces a toxic microenvironmental niche. Furthermore, ATF4 increases tumor angiogenesis and shapes the vascular architecture in a xCT-dependent manner. Thus, inhibition of ATF4 is a valid target for diminishing tumor growth and vasculature via sensitizing tumor cells for ferroptosis.

Poster 80:

Titel: Increased ROS-level in spinal cord of wobbler mice due to NMNAT2-downregulation

Autoren/Adressen: Pascal Röderer (Ruhr University Bochum), Lara Klatt (Ruhr University Bochum), Verena Theis (Ruhr University Bochum), Konstanze F. Winklhofer (Ruhr University Bochum), Carsten Theiss (Ruhr University Bochum), Veronika Matschke (Ruhr University Bochum); Veronika.Matschke@rub.de

Abstract:
Amyotrophic lateral sclerosis is a devastating motor neuron disease and to this day not curable. While 5-10% of patients inherit the disease (familiar ALS) up to 95% of patients are diagnosed with the sporadic form (sALS). ALS is characterized by the degeneration of upper motor neurons in the cerebral cortex and of lower motor neurons in the brainstem and spinal cord. The Wobbler mouse resembles almost all phenotypical hallmarks of human sALS patients and is therefore an excellent motor neuron disease model. The motor neuron disease of the Wobbler mouse develops over a time course of around 40 days and can be divided into three phases: p0 – presymptomatic, p20 early clinical and p40 stable clinical phase. Recent findings suggest an essential implication of the NAD+-producing enzyme Nmnat2 in neurodegeneration as well as maintenance of healthy axons.

The expression level of mRNA and protein was quantified by qPCR and western blot. Localization studies were performed using fluorescence microscopy. NAD+ and ROS levels were measured using cell-based assays.

Here, we could show a significant downregulation of both gene and protein expression of Nmnat2 in the spinal cord of the wobble mice at the stable clinical phase. The product of the enzyme, NAD+, is also significantly reduced and the values of the reactive oxygen species are significantly increased in the spinal cord of the wobbler mouse at p40.

Thus, the deregulated expression of Nmnat2 appears to have a great influence on the cellular stress in the spinal cord of wobbler mice.

Poster 81:

Titel: Oligodendrocyte-derived IL-6 as a modulator of microglia activation

Autoren/Adressen: Miriam Scheld (RWTH Aachen University), Tim Clarner (RWTH Aachen University), Athanassios Fragoulis (RWTH Aachen University), Markus Kipp (Ludwig-Maximilians-University of Munich), Cordian Beyer (RWTH Aachen University); mscheld@ukaachen.de

Abstract:
MS pathogenesis is only partly understood. Preactive lesions precede active inflammatory demyelination and might develop into full-blown MS lesions. Preactive lesions are characterized by focal microglia activation in close spatial relation to stressed oligodendrocytes. In this study we aimed to investigate the mechanisms involved in initial microglia activation. Therefor we investigated the signaling of stressed oligodendrocytes towards resting microglia cells in vitro and additionally in a relevant MS-animal model in vivo.

In vitro oligodendrocyte stress was induced by moderate sodium azide treatment. Conditioned oligodendrocyte medium was utilized to stimulate a resting microglia cell line. PCR Arrays, ELISA and RT-qPCR were performed to (A) identify oligodendroglial signaling pathways towards microglia cells and (B) characterize microglia activation state triggered by stressed oligodendrocytes.

Conditioned medium of stressed oligodendrocytes triggered the activation of microglia cells. Among various chemokines and cytokines, oligodendrocyte derived IL-6 was identified as a major regulator of this microglia activation. Relevance of this finding for lesion development was further investigated by RT-qPCR and immunostaining on short term (2 days) cuprizone fed mice. In these animals IL-6 expression in oligodendrocytes was found in close vicinity of activated microglia cells.

Taken together we provide evidence that stressed oligodendrocytes actively participate in early lesion formation by activating microglia cells via IL-6 production. Further studies using conditional IL-6 knock-out animals will have to show the precise value of this novel inter-glial signaling for lesion formation.

Poster 82:

Titel: Indications for cellular migration from the central nervous system to its draining lymph nodes in CD11c-GFP+ bone-marrow chimeras following EAE

Autoren/Adressen: Kerstin Immig (University of Leipzig), Fridtjof Schiefenhövel (University of Leipzig), Carolin Prodinger (University of Leipzig), Ingo Bechmann (University of Leipzig); kerstin.immig@medizin.uni-leipzig.de

Abstract:
The immune privilege of the brain has initially been based on the lack of classical lymph vessels and later, the absence of dendritic cells (DC). This view has been challenged by demonstrating drainage/migration of injected tracers and cells into cervical lymph nodes (CLNs) and the presence of brain antigens in CLNs in the course of various brain pathologies. Using CD11c-diphtheria toxin receptor-green fluorescent protein (GFP) transgenic mice (CD11c-GFP mice), we have shown CD11c+ cells within the brain parenchyma.

We have now transplanted wild type (wt)-bone marrow (BM) to lethally irradiated CD11c-GFP mice to restrict the CD11c-GFP+ population to the brain and induced experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). Further, we avoided possible artifacts which might be caused due to injections of tracers or cells.

Our findings indicate that radio-resistant cells, expressing CD11c-GFP+ and located in the CNS, irrespective of their origin, which might be the yolk sack or the periphery, show a phenotype capable of antigen presentation under neuroinflammatory conditions following induction of EAE.

Furthermore, our data supports the hypothesis that these CD11-GFP+ cells are capable of leaving the CNS and migrate to specific LNs. It seems likely that this cellular migration of CD11c-GFP+ cells might be responsible for the presence of CNS-associated antigens in CNS-draining LNs.

Poster 83:

Titel: Niacin treatment significantly reduces the expression of proinflammatory cytokines in primary microglia

Autoren/Adressen: Isabel Demmer (Christian-Albrechts-University, Kiel), Uta Rickert (Christian-Albrechts-University, Kiel), Ralph Lucius (Christian-Albrechts-University, Kiel); u.rickert@anat.uni-kiel.de

Abstract:
Microglia are the immunocompetent cells of the central nervous system (CNS). They play an important role in the cellular defense and are also associated with neuroinflammation in neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD).

Niacin, a member of the vitamin B family, exerts anti-inflammatory effects in a number of tissues/macrophages, but direct evidence in modulating microglial cell functions is lacking.

We carried out the investigations on LPS-activated primary rat microglia and measured Nitrite oxide (NO) release as a pro-inflammatory metabolite (Griess reagent). The influence of niacin was further analyzed via quantification of mRNA synthesis of iNOS, IL-1 beta, IL-6 and TNF-alpha (qPCR). We also investigated the protein secretion of TNF-alpha and IL-6 via ELISA. Intracellular signaling mechanisms were detected by Western blotting and immunofluorescence staining.

Treatment with niacin significantly downregulates NO-synthesis and reduces iNOS-, IL-1 beta-, IL-6-, and TNF-alpha mRNA-synthesis after 3h and 6h in LPS-stimulated cells. Moreover, niacin influences the secretion of TNF-alpha and IL-6 after 6h and 24h in activated microglia. Western blotting data and immunofluorescence demonstrated an interaction with MAP-kinases respectively NF-kappa B pathway.

These results indicate that activated microglia decrease pro-inflammatory mediator- and cytokine synthesis in the presence of niacin. This immune-modulatory effect of niacin might be a potential therapy for neurodegenerative diseases.

Poster 84:

Titel: Analysis of TGF-beta1-regulated expression of Mrc1 in primary mouse microglia

Autoren/Adressen: Alexander von Ehr (Institute of Anatomy und Cell Biology), Nico Neidert (Institute of Anatomy und Cell Biology), Björn Spittau (Institute of Anatomy und Cell Biology); alexander.vonehr@icloud.com

Abstract:
Microglia are the immune cells of the central nervous system and play important roles under physiological and pathological conditions. TGF-BETA1 is a pleiotropic growth factor being expressed in several neurodegenerative diseases and capable to downregulate microglia activation. The main objective of the present study is to examine the influence of TGF-BETA1 on the expression of the Mannose-receptor-1 (Mrc1) in microglia. Mrc1 is a transmembrane receptor that is mainly expressed by tissue macrophages and microglia playing important roles during phagocytosis and cell homeostasis. However, its primary function in microglia has yet to be determined.

Primary mouse microglia were treated with recombinant TGF-BETA1 and a TGF-BETA-receptor-inhibitor. qPCR, Western Blot, Flow Cytometry and immunocytochemistry were performed to analyze the expression of Mrc1. Moreover, chromatin immunoprecipitation (ChIP) using BV2 cells, was performed to verify putative SMAD-binding-sites in the promotor region of Mrc1.

Mrc1-expression was downregulated after treatment with TGF-BETA1 and inhibition of the TGF-BETA1 signaling pathway resulted in increased Mrc1-expression. Several SMAD binding sites in the Mrc1 promoter indicate that Mrc1 might be a direct TGF-BETA1 target gene in microglia.

Taken together, our data introduce Mrc1 as a new TGF-BETA1-regulated gene in primary mouse microglia. Nevertheless, further studies are required to understand the functional impact of TGF-BETA1-regulated Mrc1-exprresion for microglia functions under physiological und pathological conditions.

Poster 85:

Titel: Mechanisms and molecules involved in ectopic lymphoid tissue formation in the CNS in a mouse model of multiple sclerosis

Autoren/Adressen: Verena Schropp (Friedrich-Alexander University Erlangen-Nürnberg), Damiano Rovituso (University of Würzburg), Stefanie Kürten (Friedrich-Alexander University Erlangen-Nürnberg); verena.schropp@fau.de

Abstract:
In a subgroup of patients suffering from progressive multiple sclerosis (MS), which is an inflammation-mediated neurodegenerative disease of the central nervous system (CNS), B cell follicles were discovered within the meninges. Occurrence of these follicles was associated with a more severe disease course and cortical histopathology. Here we set out to investigate the mechanisms, in particular the role of lymphoid tissue inducer (LTi) cells and Th17 cells, underlying the formation of lymphoid tissue in the CNS.

For our analysis, we employed MP4-induced experimental autoimmune encephalomyelitis (EAE) as a B cell-dependent mouse model of MS. This model is characterized by the development of B cell aggregates, which then evolve into lymphoid structures in the chronic stage of the disease. Using flow cytometry, we determined the presence of $CD5^-CD3^-CD4^+ROR\gamma t^+$ LTi cells and $CD5^+CD3^+CD4^+ROR\gamma t^+$ Th17 cells in the CNS at different disease stages.

While we were able to detect LTi cells in the embryonic spleen, which served as a positive control, there was no evidence for the existence of such a population in acute or chronic EAE. However, we observed a significant increase in the number of Th17 cells in the CNS, especially in chronic EAE.

We conclude that LTi cells are not relevant for the formation of lymphoid structures in EAE mice, but the presence of Th17 cells suggests an important role of this cell type and will be subject of further investigation.

Poster 86:

Titel: The CNS vasculature: a fountain of youth for microglia?

Autoren/Adressen: Tobias Königer (University of Würzburg), Süleyman Ergün (University of Würzburg), Stefanie Kürten (University of Erlangen-Nuremberg); tobias.koeniger@uni-wuerzburg.de

Abstract:
Over the last decade our understanding of the development and the maintenance of tissue macrophages such as microglia has dramatically changed. As it becomes increasingly clear that these resident phagocytes maintain themselves independently of monocytes in the bloodstream, a new question arises regarding the exact mechanism of their regeneration. Outside of the central nervous system (CNS), macrophage progenitors have been demonstrated to reside within the walls of blood vessels. This study aims to examine the existence of a comparable niche within the CNS, where it could contribute to the maintenance and expansion of specialized CNS macrophage populations in health and disease.

To study CNS macrophage regeneration in vivo, pharmacological csf1-r inhibition using the small molecule PLX5622 was employed to transiently deplete tissue macrophages from the brains of living mice.

Within 7 days of treatment, csf1-r inhibition removed 80% of CNS macrophages. Following withdrawal of the drug, a rapid repopulation of microglia/macrophages was observed within one week. Depletion and repopulation varied considerably in distinct regions of the brain, revealing differences in self-renewal activity. Intriguingly, the repopulation pattern resembled the arterial network spanning the CNS.

While it is discussed that CNS macrophages themselves might be universally capable of self-renewal, our study shows that the potential for microglia/macrophage regeneration could indeed be differentially distributed over the CNS, and even in relation to the vasculature. Localized progenitor activity is a possible explanation for this observation, which we aim to examine in further detail.

Poster 87:

Titel: Chemosensory cholinergic signaling network in the thymic medullary epithelium

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Abstract:
A subset of medullary epithelial cells in the thymus (mTECs) was previously shown to be cholinergic and to express components of the bitter taste transduction cascade. In this study we set out to further characterize these cholinergic chemosensory cells (CCC).

Reporter mice expressing EGFP under choline-acetyltransferase (Chat) or TRPM5 (the key component in taste transduction cascade) promoters were used to pick individual CCC or to sort all CCC in a given thymus. Individually picked cells were subjected to deep sequencing and the sorted cells were subjected to RT-PCR with the primers for a set of taste and olfactory receptors. To test the functionality of Chat, thymi were lysed and intracellular acetylcholine was measured by HPLC.

While pooled cholinergic chemosensory cells express the majority of known taste receptors and some of olfactory receptors, the expression pattern is differential at the single cell level. Surprisingly, neither of tested single cells expressed adenylate cyclase III and Golf, the key components in the odorant signal transduction. Thymic lysates showed the presence of acetylcholine.

We demonstrate here the presence of an array of taste and olfactory receptors on the novel epithelial cell type in the thymus. It seems that each of these cells has its “own” repertoire of taste and olfactory receptors. Notably, CCC differ from olfactory sensory neurons in that they express several olfactory receptors at one time.

Funded by DZL and SFB-TR 84.

Poster 88:

Titel: Homeostasis in the brain: differential activation of microglia

Autoren/Adressen: Max Brück (University of Kiel), Uta Rickert (University of Kiel), Francois Cossais (University of Kiel), Katja Schröder (University of Kiel), Christina Lange (University of Kiel), Peter Behrend (University of Kiel), Ralph Lucius (University of Kiel); m.brueck@anat.uni-kiel.de

Abstract:
Microglia constitute up to 20% of the resident glia cell population within the central nervous system. By taking on diverging states of activation microglia adapt to different ambient stimuli thereby contributing significantly to the formation of the immune defense system of the brain. Nevertheless it is not yet fully understood how microglia are able to accomplish the task of changing their state of activation. In accordance to the related peripheral macrophages it has recently been suggested that microglia do so by altering the expression of certain genes.

However, the determination of a distinct set of expression markers representing a certain type of activated microglia remains unclear and is subject of an ongoing scientific discussion.

In our work we performed an unbiased qRT-PCR assay to reveal a pattern of gene expression representing the two states of activation M1 and M2. Therefor we used 48 potential M1 and M2 markers. The primary cell cultures of rat microglia used were driven towards their activation states by stimulation with LPS, IL-4, IL-13 and TFF3.

The analysis of the qRT-PCR revealed a set of pro- and anti-inflammatory genes such as ARG1, MRC1, CD163, IFN-GAMMA, IGF-ALPHA and FCGR2A. Depending on the stimulating substance and the incubating time the quantity of gene expression changed correspondingly.

In conclusion we suggest that there is not a certain gene or set of genes representing a distinct subtype of microglial activation. Instead we postulate that rather the composition of pro- and anti-inflammatory gene expression defines the state of microglial activation.

Poster 89:

Titel: Depletion of NLRP3 fails to mitigate inflammasome-mediated inflammation in BV-2 cells after in vitro hypoxia

Autoren/Adressen: Isabelle Schöllwer (RWTH Aachen), Cordian Beyer (RWTH Aachen), Alexander Slowik (RWTH Aachen); aslowik@ukaachen.de

Abstract:
Acute stroke is a cerebrovascular event leading to reduced blood flow and oxygen supply, which is paralleled by inflammatory processes. Neuronal loss during growth of the necrotic core lead to release of damage-associated molecular patterns (DAMPs), which are sensed by intracellular receptors, termed inflammasomes. In the inflammatory cascade one particular inflammasome, namely NLRP3, mediates the interleukin (IL)1BETA and IL18 maturation. Therefore, we investigated if IL1BEAT maturation in the murine microglia-like BV-2 cell line is prejudiced by NLRP3 depletion.

BV-2 cells were transfected with a shNLRP3 clone to suppress NLRP3 expression. Hypoxic impact on BV-2 cells lacking NLRP3 was investigated by inducing in vitro hypoxia in a nitrogen-flooded hypoxia chamber. Afterwards gene and protein expression studies of inflammasome-related cytokines (IL1BETA or IL18) or inflammasome components (NLRP3, ASC, AIM2, NLRC4) were performed using semi-quantitative real time PCR or Western Blot analysis, respectively. Active BV-2 parameters were determined by quantification of caspase-1 activity by the caspase-1 Glo luminescence kit and IL1BETA release by using the ELISA technic. Additionally, phagocytic capabilities were determined.

We found that, depletion of NLRP3 neither effects IL1BETA maturation and secretion nor impact of hypoxia on BV-2 cells. Caspase-1 activity was elevated after hypoxia in all groups.

Our data indicate that NLRP3 plays a minor role in mediation of hypoxia in BV-2 cells. We suggest, that hypoxia is mediated through the other inflammasomes, namely AIM2 and NLRC4, respectively. Future investigations in deletion of these both inflammasome components will reveal their importance in hypoxia.

Poster 90:

Titel: Effect of prostaglandine application on ACAID in the mouse eye

Autoren/Adressen: Dr. Thomas Buder (Friedrich-Alexander-University of Erlangen-Nürnberg), Prof. Elke Lütjen-Drecoll (Friedrich-Alexander-University of Erlangen-Nürnberg); Thomas.Buder@fau.de

Abstract:
To assess the early and long term effect of prostaglandine application on anterior chamber associated immune deviation (ACAID) in mouse eyes.

A single 150ng (3.µl) dose of latanoprost was applied daily to the right eye of 36 Balb/c mice. After 4, 8, 12, 16, 20 and 24 weeks of treatment ACAID was performed in 6 mice of each group. A group of 12 untreated mice for each timespan (in total 72) served as a control for the ACAID experiments.

After four weeks of daily treatment the mice showed loss of ACAID. The same results were found after 8 and 12 weeks of latanoprost treatment. After 20 and 24 weeks the treated mice like the controls showed ACAID. After 16 weeks of treatment there were individual differences in the ACAID reaction between the animals.

These data indicate that the early effect of latanoprost treatment in the mouse eye is comparable to that previously shown in primate eyes following short term treatment with PGF2alpha-isopropylesther. In addition the results reveal that under long term treatment ACAID is regained.

Therefore the mice provide a suitable model for further studies of the mechanisms

underlying the latanoprost effect on ACAID.

Poster 91:

Titel: Modulation of astrocyte reactivity by lipocalin-2 in MS animal models

Autoren/Adressen: Tim Clarner (RWTH Aachen University), Miriam Scheld (RWTH Aachen University), Stella Nyamoya (Ludwig-Maximilians-University of Munich), Markus Beissel (RWTH Aachen University), Fatemeh Ranjbar Taklimie (RWTH Aachen University), Adib Zendedel (RWTH Aachen University), Markus Kipp (Ludwig-Maximilians-University of Munich), Cordian Beyer (RWTH Aachen University); tclarner@ukaachen.de

Abstract:
Astrocytes are a fundamental part of the brains barriers and the guardians of the CNS biochemical homeostasis. They get activated as a response to many pathogenic stimuli and might critically influence the recruitment of peripheral immune-cells into the CNS during Multiple sclerosis (MS) lesion formation. Lipocalin-2 is a molecular switch for determining the phenotypic fate of astrocytes under inflammatory conditions. In this study, we investigated the role of lipocalin-2 for astrocyte reactivity and the recruitment of peripheral immune-cells into the CNS.

The cellular and quantitative expression of lipocalin-2 was investigated by means of RT-qPCR and immunohistochemistry in three different MS animal models: (A) Cuprizone intoxication (Cup; primary neuroinflammation without BBB breakdown) (B) Autoimmune encephalomyelitis, (EAE; autoimmune response without invasion of immune-cells into the cerebrum). (C) A combination of both Cup and EAE (CupEAE; primary neuroinflammation followed by perivascular invasion of immune-cells into the CNS).

Additionally, astrocytic activation by lipocalin-2 treatment was characterized in vitro by means of RT-qPCR, Western blot and ELISA.

Lipocalin-2 expression was significantly induced in CupEAE in comparison to control, Cup and EAE. On a cellular level, lipocalin-2 was expressed by astrocytes in close spatial relation to perivascular cuffs and damaged brain regions in this model. In response to lipocalin-2, primary astrocytes expressed chemokines and cytokines that are potentially involved in the invasion of peripheral immune cells into the CNS.

Our results indicate a possible role of astrocytic expression of lipocaline-2 in immune-cell infiltration in MS.

Poster 92:

Titel: The role of CEACAM1 in lymph node metastasis of prostate cancer

Autoren/Adressen: Verena Pfeiffer (Institut of Anatomy and Cell Biology), Patrick Vix (Institut of Anatomy and Cell Biology), Bernhard B. Singer (Institut of Anatomy and Cell Biology), Derya Tilki (Martiniklinik UKE-Eppendorf), Süleyman Ergün (Institut of Anatomy and Cell Biology); verena.pfeiffer@mail.uni-wuerzburg.de

Abstract:
Here we show that CEACAM1 (Cc1) is upregulated in lymph node (LN) sinus of a part of prostate cancer (PCa) patients who's LNs were diagnosed to be free of tumor cells.

Double immunostainings revealed an inverse relation between Cc1 and prostate-specific antigen (PSA) expression in cell clusters or glandular structures of PCa regardless of their location in prostate and LNs. Breading of TRAMPC1 mice (PCa model) with Cc1-/- and Cc1-/-/EC+/+ (endothelial Cc1 rescue), we generated TRAMPC1/Cc1-/- and TRAMPC1/Cc1-/-/EC+/+ to assess lymphatic metastasis of PCa in vivo.

Similar to human prostate, epithelial Cc1 was downregulated in PIN stage of TRAMPC1 mice with concurrently up-regulation of Cc1 in blood vessels and lymphatics. Remarkably, some LN sinuses displayed Cc1 expression in absence of PCa metastasis. In comparison to TRAMPC1, TRAMPC1/Cc1-/- mice showed an accelerated onset of single LN PCa metastasis already at HGPIN stage. In TRAMPC1/Cc1-/-/EC+/+ mice, mimicking the endothelial up-regulation of Cc1 in PCa-associated blood and lymphatic vessels, we observed earliest onset of LN metastasis.

Our data suggest that TRAMPC1-PCa model displays features similar to human PCa which is highly suitable for mechanistic analysis. Quantitative analyses of our data show that the epithelial down- versus endothelial up-regulation of Cc1 increases tumor growth and accelerates tumor cell spreading into LN. Furthermore, the inverse expression pattern of Cc1 and PSA in certain Gleason stages of human PCa could be of clinical relevance for diagnosis and therapeutic stratification in PCa.

Poster: 93

Titel: Valid gene expression normalization and analysis by RT-qPCR in in-vitro studies on human periodontal ligament fibroblasts (hpdl) with a focus on orthodontic tooth movement and periodontitis

Autoren/Adressen: Christian Kirschneck (University Medical Centre of Regensburg), Peter Proff (University Medical Centre of Regensburg), Sarah Batschkus (University of Göttingen), Gerrit Spanier (University Medical Centre of Regensburg), Agnes Schröder (University Medical Centre of Regensburg)

Abstract:
Meaningful, reliable and valid relative gene/mRNA-expression analyses by RT-qPCR can only be achieved, if suitable reference genes are chosen for normalization and if appropriate RT-qPCR quality standards are met (MIQE). The human periodontal ligament fibroblast (hPDL) plays a major mediating role during orthodontic tooth movement and periodontitis. Despite corresponding in-vitro gene-expression studies being a focus of interest for many years, no information is yet available on suitable reference genes and quality control.

Pooled hPDL (four patients, 16-23 years, 6th passage) were incubated under physiological conditions for 48h, either untreated, stimulated by compressive orthodontic forces of 2g/cm2 for 24h or by toxins of Aggregatibacter actinomycetemcomitans (Agac). RNA-extraction, cDNA-synthesis and RT-qPCR were performed according to MIQE guidelines. 13 candidate reference genes were screened and nine ranked according to their relative expression stability by four different algorithms (geNorm/NormFinder/BestKeeper/comparativeΔCq).

PPIB/TBP were the most stable reference genes overall, whereas RPL22/EEF1A1 were expressed most stably in untreated hPDL, EEF1A1/PPIB during orthodontic stimulation and TBP/PolR2A in presence of Agac-toxins. Considerable differences in gene-stability ranking were observed between experimental groups and individual algorithms. Two reference genes in combination were determined to suffice for normalization in all experimental conditions.

The variation observed across the various experimental conditions and even individual algorithms stresses the necessity of identifying valid reference genes for normalization, if reliable results are to be obtained. Our investigation should provide a valid basis for optimized future gene expression studies on hPDL fibroblasts, particularly in context of orthodontic tooth movement and periodontitis.

Poster 94:

Titel: TNF-β- induced inflammation in articular chondrocytes

Autoren/Adressen: Constanze Buhrmann (Ludwig-Maximilian-University Munich), Bastian Popper (Ludwig-Maximilian-University Munich), Bharat B Aggarwal (Anti-inflammation Research Institute), Mehdi Shakibaei (Ludwig-Maximilian-University Munich); constanze.buhrmann@med.uni-muenchen.de

Abstract:
TNF-β (Lymphotoxin α) is involved in inflammatory joint environment and the natural polyphenol resveratrol has been shown to exert anti-inflammatory and chondroprotective effects via activation of histondeacetylase Sirt1. In the herein presented study we investigated the anti-inflammatory effects of resveratrol on TNF-β-induced inflammation in primary human chondrocytes (PCH) as a potential novel therapeutic approach for the treatment of inflammatory joint diseases such as rheumatoid arthritis (RA).

Investigation where performed in adhesion-assay (PCH and T lymphocytes), monolayer and 3D-alginate microenvironment culture of PCH.

TNF-β-induced inflammatory microenvironment in adhesion-asssay was suppressed by resveratrol via Sirt1-activation and suppression of NF-kB-activation. Ultrastructural investigations in 3D-alginate microenvironment culture revealed that resveratrol revoked TNF-β-induced dose-dependent degenerative and apoptotic morphological changes in PCH. In inflammatory microenvironment adhesion-assay with T Lymphocytes, resveratrol inhibited TNF-β-induced down-regulation of collagen type II, Ki67, Sirt1, Sox9, β1-Integrin, up-regulation of p-FAK and NF-κB signalling pathway and NF-κB-dependent gene end products in PCH. Knock-down of Sirt1 with ASO against Sirt1 or nicotinamide revealed that resveratrol-blocked TNF-β-induced degeneration and inflammation in PCH is dependent on Sirt1-activation, mediated by β1-Integrin signaling but not by activation of FAK. Long term alginate cultures revealed that dose-dependently TNF-β-induced inflammatory and apoptotic effects in PCH can be partially revoked by resveratrol.

Chondroprotective effects of resveratrol/Sirt1 by supression of TNF-β-induced inflammatory microenvironment in chondrocytes might be a novel therapeutic approach for targeting inflammation during RA.

Poster 95:

Titel: Expression of stem cell markers in beta cell of the human

Autoren/Adressen: Luis Filgueira (University of Fribourg), Karl Link (University of Zurich), Samuel Natzeder (University of Zurich), Ahmaed Bashar (University of Western Australia), Luca Martinelli (University of Fribourg), Alexander Rubin (University of Fribourg), Elisabeth Eppler (University of Basel; luis.filgueira@unifr.ch

Abstract:
The purpose of this study was to assess the expression of embryonic stem cell and pancreas-specific markers in insulin producing beta cells in adult human islets, including Nanog, Oct4, Sox2, menin, Ngn3, FZD2 and PDX-1.

An indirect immune staining was done on archival formalin-fixed human pancreatic samples.

Expression of the three beta cells precursor markers menin, Ngn3 and PDX-1 were identified in the islets, always co-localized with insulin. Expression of Nanog, Oct4 and Sox2 were distinctly identified in the islets of the human pancreas. Oct4 and Sox2 expression was always co-localized with insulin, indicating that all insulin secreting beta cells do express these two markers. Nanog was mainly co-expressed with insulin in beta cells, but there was a small number of cells in the islets expressing Nanog, but no insulin, demonstrating that Nanog might be expressed by the others endocrine cells or early precursors or tissue-specific stem cells. A majority of islet cells also expressed FZD2, the WNT5a receptor, which has been shown to be expressed in islet precursor cells.

These findings demonstrate that the three embryonic stem cell markers Nanog, Oct4 and Sox2, as well as FDZ2, are expressed in the islets of the human pancreas. The precursor markers of beta cells such as menin, Ngn3 and PDX-1 are always localized with insulin producing beta cells as well. Consequently, the presence of embryonic stem cell markers in the human adult pancreas may offer a new principle for regenerating and replacing ageing or damaged beta cells.

Poster 96:

Titel: Human meibomian gland epithelial cell line (HMGEC) as a model to study meibomian gland dysfunction.

Autoren/Adressen: Fabian Garreis (Friedrich-Alexander University Erlangen-Nürnberg (FAU)), Susanne Adelung (Friedrich-Alexander University Erlangen-Nürnberg (FAU)), Daniel B. Abrar (Friedrich-Alexander University Erlangen-Nürnberg (FAU)), Ulrike Hampel (University Medical Center of the Johannes Gutenberg University Mainz), Martin Schicht (Friedrich-Alexander University Erlangen-Nürnberg (FAU)), Friedrich Paulsen (Friedrich-Alexander University Erlangen-Nürnberg (FAU); fabian.garreis@fau.de

Abstract:
Meibomian gland dysfunction is considered the most common cause of dry eye disease. The pathogenesis of MGD is not completely understood. We characterized culture conditions and analyzed the impact of MGD-associated factors such as expression of sex hormone receptors and central enzymes of sex hormone biosynthesis in cultivated HMGECs.

The impact of culture conditions, sex hormones, follicle-stimulating hormone and aromatase inhibitor letrozole to HMGEC was analyzed by means of transmission electron microscopy, Sudan III lipid staining, cell proliferation and vitality assays. Moreover, expression of MGD-associated markers for keratinization, proliferation and lipid synthesis, sex hormone receptors and enzymes were analyzed by real time RT-PCR. The expression of cell-cell junction members was investigated on mRNA and protein level.

Western blot analysis revealed presence of sex hormone receptors as well as aromatase in HMGEC. PR, ERα and ERβ expression was significantly induced by serum-induced differentiation, whereas stimulation with sex hormons showed no further effect. Our results showed no impact of MGD-associated sex hormones to cellular morphology or lipid accumulation in HMGEC. Cell proliferation was slightly induced through application of sex hormones and supplementation of calcium. However, dihydrotestosterone, β-estradiol and calcium altered gene expression of MGD-associated markers, especially keratinization genes were induced. Furthermore, expression of cell adhesion proteins and linking-protein desmoplakin was increased by serum and calcium application.

Sex hormones and culture conditions alter cell morphology of HMGEC and induce gene expression of MGD-associated genes. It seems that HMGEC are a good in vitro model for further studies of (hyper)keratinization process that occur during MGD.

Poster 97:

Titel: Contribution of the signaling molecules during vascular development in tooth germ

Autoren/Adressen: Masataka Sunohara (The Nippon Dental University), Shigeru Morikawa (National Institute of Infectious Diseases), Kenzo Noguchi (The Nippon Dental University), Iwao Sato (The Nippon Dental University); ma-suno@tky.ndu.ac.jp

Abstract:
Angiogenesis and vasculogenesis are essential for fetal development. However, the mechanisms of formation of blood vessels remains poorly understood in tooth germ.
The aim of this survey was to clarify signaling molecules involved in initial formation of blood vessels during dental embryo-morphogenesis.

By using the probes of the the signaling molecules involved in blood vessels formation during tooth development, we performed in situ hybridization analysis and then immunohistochemically we stained serial sections of mouse embryos with the antibody against them.

In situ hybridization and immunohistochemical analysis showed that localizations of the signaling molecules were observed in peripheral dental mesenchyme, dental papilla and enamel organ and the stage-specific expression patterns of them were observed vicinity of the blood vessel.

In our survey, we detected the key signaling molecules may have involved in formation of blood vessels at the different stages of dental embryo-morphogenesis.

*This work was supported by JSPS KAKENHI Grant Numbers 22592052, 26462800.

(COI：) In this research, we do not have the conflict of interest that we should disclose.

Poster 98:

Titel: The renal phenotype of CLN7 knockout mice

Autoren/Adressen: Sara Afonso (University of Regensburg), Julia Wiesner (University of Regensburg), Heinl Elena (University of Regensburg), Hannes Doellerer (University of Regensburg), Ines Tegtmeier (University of Regensburg), Tatyana Danyukova (University Medical Center Hamburg-Eppendorf), Stephan Storch (University Medical Center Hamburg-Eppendorf), Markus Reichold (University of Regensburg); markus.reichold@ur.de

Abstract:
The Neuronal Ceroid-Lipofuscinoses (NCL, Batten disease) are a group of rare autosomal-recessive neurodegenerative disorders that display hallmarks of lysosomal storage diseases. Mutations in thirteen different genes are known to cause NCL, most of which are lysosomal proteins of unknown function. The most common shared phenotype among the NCL forms is the accumulation of ceroid-lipofuscin deposits in cells of different tissues. The symptoms normally start during childhood and comprise of retinal degradation, a progressive mental and motor degeneration, dementia and seizures. CLN7 disease with variant late-infantile phenotype is caused by mutations in the CLN7/MFSD8 gene. Currently, no cure exists for any of the forms.

Kidney morphology of CLN7 knockout mice were histologically evaluated using electron microscopy as well as different kinds of chemical and immunohistochemical stainings.

Primary investigations show that the CLN7 knockout mice have massive accumulation of ceroid-lipofuscin deposits, which are restricted to cells of the early proximal tubule. The mostly circular deposits can get up to one µm in diameter and electron microscopic pictures show a layered, fingerprint-like composition. Interestingly, these deposits can also be found in the tubular lumen, which might result from proximal tubular cell death.

Although the neuronal phenotype of CLN7 patients is a dominate feature, a reduced kidney function might aggravate the course of the disease. While the function of the CLN7 protein is unknown, it is speculated that it may be a lysosomal transporter. Further studies in the kidneys may help determine its function as the kidney is very convenient to study transport processes.

Poster 99:

Titel: The chondroprotective effect of GDF-15 in a pro-inflammatory in vitro model

Autoren/Adressen: Sam Razaeian (Christian-Albrechts-Universität zu Kiel), Andrea Preuße-Prange (Christian-Albrechts-Universität zu Kiel), Martina Böttner (Christian-Albrechts-Universität zu Kiel), Bodo Kurz (Christian-Albrechts-Universität zu Kiel);
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Abstract:
Osteoarthritis (OA) is not seen any more as a strictly non-inflammatory degenerative disease, and previously underestimated inflammatory mediators such as TNF-α play a key role in disturbing the metabolic balance through promoting catabolic and destructive progress in cartilage. However, disease-modifying drugs which are well established in the treatment of rheumatoid arthritis and could address here, unfortunately proved disappointing so far.
Growth and differentiation factor 15 (GDF-15) is a promising member of the transforming growth factor beta superfamily that has already shown modulating effects on inflammatory and apoptotic pathways in other tissues. Therefore we investigated the chondroprotective influence of GDF-15 in a pro-inflammatory in vitro model.

Cartilage explant disks (3 mm diameter x 1mm thickness) were isolated from 2-year-old cattle. After 3 days of TNF-α (10ng/ml) and GDF-15-treatment (low-dose 10ng/ml; high-dose 100ng/ml) glycosaminoglycane (GAG)-release (DMMB assay), gene expression of matrix protein and degrading enzymes (quantitative RT-PCR) and number of cells showing signs of apoptosis (nuclear blebbing, NB) were determined. In a secondary study the TNF-α-induced GAG-release was examined 3 days after 24 h pre-incubation with GDF-15.

GDF-15 significantly reduced the TNF-α-mediated apoptosis rate in high-doses and showed a tendency in low-doses. On gene expression levels no significant effect of GDF-15 could be stated.
Both concentrations showed stronger effects to reduce TNF-α-induced GAG-release, if explants are pre-incubated 24 h with GDF-15.

GDF-15 is a promising chondroprotective candidate for the treatment of TNF- α-involving joint diseases such as OA.

Poster 100:

Titel: Alarin in human gastrointestinal epithelium

Autoren/Adressen: Samir Jabari (Friedrich Alexander Universität), Schrödl Falk (Paracelsus Medizinische Universität), Kofler Barbara (Paracelsus Medizinische Universität), Brehmer Axel (Friedrich Alexander Universität); samir.jabari@fau.de

Abstract:
Alarin (AL), a member of the galanin family, acts antimicrobiotically, has vasoactive effects and has been localized in various CNS-regions of rodents. Here, we analyzed epithelia of twelve human intestinal samples (6 small, 6 large bowel) as to the immunohistochemical distribution pattern of AL.

Two sets of cryosections were quadruple-stained immunohistochemically either for AL, Chromogranin, Synaptohphysin and Somatostatin or for AL, Chromogranin, Peptid Y and 5-Hydroxytryptamin. For quantitative evaluation, ten high power fields (HPF) of each section were obtained. Care was taken that the HPFs included all epithelial regions.

Small bowel:
The majority of AL positive cells (47%) were identified as Paneth cells lying within the base of the crypts. Additionally, in the first set, about 23 % of labelled cells were positive for Synaptophysin, Chromogranin and Alarin and were most obviously entero-endocrine cells. In the second set, most Alarin positive cells were not colocalized with any of the other markers applied (39%).
Large bowel:
In the first set, AL was mostly co-localized with all markers applied (61%). In the second set, AL was co-localized with 5-Hydroxytryptamin and Chromogranin positive cells (16%) as well as with peptid Y and 5-Hydroxytryptamin (11 %). Various combinations of enteroendocrine specific markers were colocalized with Alarin but accounted for small numbers each.

The presence of AL in various enteroendocrine cells of human intestines indicates complex physiological roles: regulation of local blood flow or fluid distribution or food uptake. Due to its presence in Paneth-cells, it may also be involved in defense mechanisms.

Poster 101:

Titel: Bitter taste receptor agonists mediate contraction and relaxation in murine gallbladder smooth muscle

Autoren/Adressen: Maryam Keshavarz (Justus Liebig University Giessen), Petra Hartmann (Justus Liebig University Giessen), Burkhard Schütz (Philipps University), Wolfgang Kummer (Justus Liebig University Giessen); maryam.keshavarz@anatomie.med.uni-giessen.de

Abstract:
Taste receptors have extra oral functions. Previously we identified numerous chemosensory cholinergic cells (brush cells) in the gallbladder epithelium, supposed to sense chemical irritants and bacteria through bitter taste receptors (TAS2Rs). Moreover, TAS2Rs are also expressed on smooth muscle cells (SMC) where they are not linked to the canonical taste transduction cascade.

Expression of TAS2Rs in the murine gallbladder was assessed by RT-PCR. Contractile responses to TAS2R agonists were recorded in wild-type and trpm5-/- (cation channel being essential for canonical bitter taste transduction) mice using organ-baths.

Tas2R108, Tas2R126, Tas2R135, Tas2R137, Tas2R138 and Tas2R143 were expressed in the murine gallbladder. The bitter compounds denatonium, noscapine and quinine induced strong relaxation, whereas dextromethorphan causes contraction in a dose-dependent manner at 1-100 µM and relaxation at 1-5 mM. All these responses were unaffected in gallbladders from trpm5-/- mice and were not sensitive to the cholinergic muscarinic blocker atropine (1 µM), muscarinic and purinergic blockers (suramin 300 µM + PPADS 100 µM + atropine 1 µM), or blockade of neural action potential generation (TTX 10 µM). Contraction and relaxations to dextromethorphan were, however, markedly impaired after the selective L-type Ca2+ channel inhibitor nifedipine (10 µM).

TAS2R agonists have profound effects on gallbladder SMC mainly through a pathway that is independent of cholinergic brush cells and nerve fibers and utilizes a signaling cascade that differs from that in oropharyngeal taste cells.

Poster 102:

Titel: HspB5/alphab-crystallin protein amount is negatively regulated by microRNA 129-2-3p

Autoren/Adressen: Britta Bartelt-Kirbach (University of Ulm), Lara Barteczko (University of Ulm), Nikola Golenhofen (University of Ulm); britta.bartelt@uni-ulm.de

Abstract:
Heat shock proteins are upregulated by cells to survive pathophysiological conditions. The small heat shock protein HspB5 is known to be neuroprotective. We found previously that HspB5 is posttranscriptionally regulated after heat shock in hippocampal neurons. Therefore, we investigated the possible involvement of microRNAs in the regulation of HspB5.

Candidate microRNAs for HspB5 regulation were identified by in silico search for miRNA binding sites and by microarray data on the expression of rat microRNAs in hippocampal neurons subjected to stress. They were tested for their activity on the HspB5 UTRs in a luciferase reportergene assay. Amount of microRNA in cells was assessed by TaqMan realtime PCR. Overexpression and inhibition of miR129 in C6 rat glioma cells was achieved by transfection of microRNA mimic and inhibitor, respectively, or by transfection of a miR129-shRNA vector.

Three of the five candidate microRNAs were able to bind to the HspB5-UTR determined by luciferase reportergene assay. Of these, we found only miR129-2-3p consistently downregulated after stress in neurons, thus, being the most promising candidate for stress-dependent upregulation of HspB5. To investigate if miR129 is able to regulate HspB5 in a cellular context we inhibited and overexpressed miR129 in C6 glioma cells and measured HspB5 protein content. Inhibition of miR129 resulted in increased HspB5 protein amount while overexpression had the opposite effect.

We could show that translation of HspB5 can be regulated by miR129-2-3p. Our data suggest that stress-induced downregulation of miR129 might contribute to HspB5 upregulation in the cellular stress response.

Poster 103:

Titel: Effects of the extracorporeal shock wave on cell-vitality and expression of cell-cell contact proteins of equine mesenchymal stem cells

Autoren/Adressen: Laura Konarek (Justus-Liebig Universität Gießen); Laura.M.Konarek@vetmed.uni-giessen.de

Abstract:
In equine veterinary medicine extracorporeal shock wave therapy (ESWT) is used to optimize healing processes of orthopedical injuries. The intention of this examination was to explore the influence of focused ESWT on the viability and proliferation of mesenchymal stem cells (MSCs) and to investigate its effects on cell-cell- communication.

The MSCs were harvested from subcutaneous adipose tissue and isolated by standard methods. MSCs were treated with different levels of focused ESWT and prepared for further investigations. Cells were plated in a monolayer and incubated at 37 C° with 5% CO2. To evaluate cell viability a colorimetric MTT assay was used 72h after ESWT. To visualize the expression of cell-cell contacts OB-Cadherin and β1-Integrin, an immunfluorescence staining was performed. Furthermore a wound healing assay was performed for 72h to investigate the migratory potential.

Treated cells showed an increase in cell-viability and proliferation capacity, as well as an acceleration of cell movement after the shock wave treatment. The immunfluorescence revealed an increased accumulation of cell-contact proteins on the cell surface of the treated cells. Furthermore, the morphology slightly changed, especially at the higher levels of treatment.

The present results show that the shock wave has a positive impact on viability and proliferation of MSCs. It would be interesting to see, how an ESW- treatment of MSCs before implantation would influence the therapeutical results of orthopedic injuries in horses.

Poster 104:

Titel: Generation of neural precursor cells from canine adipose tissue derived mesenchymal stem cells (ADMSCS)

Autoren/Adressen: Laura Heilen (Justus-Liebig-University), Stefan Arnhold (Justus-Liebig-University); Laura.B.Heilen@vetmed.uni-giessen.de

Abstract:
In the field of veterinary medicine the need for an appropriate stem cell source for the treatment of nervous system lesions increases. As the bioavailability of neural stem cells is limited, in our approach we investigated the suitability of canine AdMSCs instead of neural precursor cells from the brain.

Canine fat tissue was collected during abdominal surgery. CAdMSCs were isolated and cultured according to standard protocols. After testing different coatings to avoid cell- attachment, cells were seeded in 24 well plates with inlaying silicon pads. The pre induction medium contained DMEM/F-12 supplement with 2% B27 supplement, 1% N2 supplement, bFGF (10ng/ml) and EGF (20ng/ml). Spheres were harvested after 3 days of incubation (37°C; 5% CO2).

For further differentiation cells were plated in gelatin coated wells using a medium consisting of DMEM/F-12 supplement, 2% B27 supplement, 1% N2 supplement, BDNF (10ng/ml) and NGF (100ng/ml). Nestin expression was detected as a marker for neural precursor cells.

Already after 24 hours small spheres were generated. After 48 hours spheres with a diameter of 26µm to 195µm were formed. The immunofluorescence shows a strong Nestin expression within the spheres. On gelatin-coated wells cells attached immediately and began to extend neurite-like processes within a few hours.

Generation of neural-like cells from canine adipose tissue is feasible. It should be further investigated whether the basal Nestin expression is upregulated in the spheres and whether detection of further neural characteristics is possible.

Poster 105:

Titel: Characterization of endothelin receptor type b mediated signalling in Müller cells and photoreceptors

Autoren/Adressen: Sabrina I. Schmitt (University Regensburg), Antje Grosche (University Regensburg), Andreas Neueder (University College London), Barbara M. Braunger (University Regensburg); sabrina.schmitt@vkl.uni-regensburg.de

Abstract:
Müller cells and retinal neurons express endothelin receptor type B (Ednrb) that is activated after binding of its ligands endothelin (Edn) 1-3. In this study, we characterized the role of Ednrb in retinal development and in regulating the expression of neuroprotective factors in vivo and in vitro.

We generated mutant mice with a conditional deletion of Ednrb in neurons and Müller cells (α-Cre;EDNRBfl/fl). We analysed the retinal morphology, performed morphometric analyses and investigated the expression levels of neuroprotective factors like Edn2, fibroblast growth factor 2 (Fgf2) and leukaemia inhibitory factor (Lif) in the retina. To examine the function of Ednrb/Edn2 signalling selectively in photoreceptors, we generated a Crispr/Cas mediated deletion of Ednrb in the photoreceptor derived cell line 661W and analysed the mRNA expression levels of neuroprotective factors.

Deletion of Ednrb was confirmed by western blotting and realtime RT-PCR. The retinal morphology was regular in α-Cre;EDNRBfl/fl mice and controls. Retinal mRNA expression levels of Edn2 and Fgf2 were increased in mice carrying the deletion of Ednrb in Müller cells and retinal neurons (α-Cre;EDNRBfl/fl) compared to controls. However, deleting Ednrb in the photoreceptor derived cell line 661W resulted in decreased mRNA levels of Edn2 and Lif.

Deletion of Ednrb in Müller cells and retinal neurons versus photoreceptors only influences the expression levels of Edn2, Fgf2 and Lif in a cell type specific manner. Our data highlight the importance of Ednrb-mediated signaling in regulating the expression of neuroprotective factors.

Poster 106:

Titel: Influence of carprofen, firocoxib and meloxicam on mesenchymal stem cells for the treatment of canine osteoarthritis

Autoren/Adressen: Michele Christian Klymiuk (Justus-Liebig-University Giessen), Kathrin Wolf-Hofmann (Justus-Liebig-University Giessen), Stefan Arnhold (Justus-Liebig-University Giessen); michele.klymiuk@vetmed.uni-giessen.de

Abstract:
Osteoarthritis is a common orthopedic disease in dogs. A marginal part of the therapeutic plan is the usage of non-steroidal anti-inflammatory drugs (NSAIDs), to reduce severe pain and to support the healing process. Due to the fact that mesenchymal stem cells play an increasing role as therapeutic approach in orthopedic diseases, the aim of this study was to evaluate the influence of common NSAIDs (Meloxicam, Firocoxib and Carprofen) on the growth and differentiation behavior of canine adipose derived mesenchymal stem cells (cAdMSC) in vitro.

For cAdMSC isolation small pieces of fat tissue was collected while surgery in pet dogs and the desired cells were isolated and cryopreserved by standard lab procedure. Passage 3 cells of cAdMSC were cultured in standard medium with addition of different concentrations of the above mentioned NSAIDs to determinate cell proliferation (MTT Assay) and a pellet culture was performed with a chondrogenic differentiation medium to evaluate the influence of typical NSAIDs concentrations used for therapeutic applications. Sections of fixated and paraffin embedded pellets were stained with Alcian blue after 2 weeks of incubation to determine formation of chondrogenic matrix.

Only in NSAIDs concentrations above therapeutic relevance, a reduced cell proliferation was detectable. In addition, no influence was detectable after 2 weeks' of incubation time regarding the formation of chondorgenic matrix.

Usage of the tested NSAIDs does not interfere the growth and differentiation of cAdMSC and is therefore reliable for the treatment of canine orthopedic diseases referring cAdMSC.

Poster 107:

Titel: The role of fibronectin in the renal interstitium

Autoren/Adressen: Silke Eggerstorfer (University of Regensburg), Leonie Herrnberger (University of Regensburg), Ernst Tamm (University of Regensburg); silke.eggerstorfer@vkl.uni-regensburg.de

Abstract:
There is increasing evidence that the renal interstitium – defined as the extratubular, extraglomerular and extravascular space of the kidney – plays an important role during renal development and renal function in the adult organism. A major component of the renal interstitium is the extracellular matrix molecule fibronectin (FN). Here we studied the role of FN in the renal interstitium by investigating mice with a conditional deletion of FN.

Kidneys were studied by light and transmission electron microscopy (TEM), immunohistochemistry and quantitative real-time RT-PCR.

By end of the first postnatal week, the expression of FN mRNA was reduced to 5 % when compared to littermate controls. In addition the amounts of FN seen by immunohistochemistry were dramatically decreased. At the same time we observed a distinct widening of the interstitial spaces between the tubuli at the cortical-medullary boundary. Distinct cysts formed in those areas in the subsequent weeks. The cysts were lined by flattened cells and were localized at the cortical-medullary boundary, quite comparable to the changes seen in nephronoschisis in humans. The cells lining the cysts were immunoreactive for PDGFR-beta, a specific marker of interstitial cells, but negative for markers of tubular epithelial cells such as Calbindin and Megalin or collecting ducts such as Aquaporin-2.

Our data indicate an important role of FN for maintenance of kidney structure and function. This role may be attenuated during the pathogenesis of nephronoschisis.

Poster: 108

Titel: Effect of modified beta tricalcium scaffold (β TCP) on tissue and bone regeneration by critical size fracture (in vivo study)

Autoren/Adressen: Diana Roch (RWTH University Clinic), Nazanin Barahmand Pour (RWTH University Clinic), Philipp Lichte (RWTH University Clinic), Christian Bergmann (RWTH University Clinic), Michaela Bienert (RWTH University Clinic), Tobias Heigl (RWTH University Clinic), Hans-Christoph Pape (RWTH University Clinic), Sabine Neuß-Stein (RWTH University Clinic), Horst Fischer (RWTH University Clinic), Thomas Pufe (RWTH University Clinic), Mersedeh Tohidnezhad (RWTH University Clinic); mtohidnezhad@ukaachen.de

Abstract:
Large bone defects still challenge the orthopaedic surgeon. Combination of tissue engineering and cell therapy represents a promising approach for bone regeneration.

Aim of the present study is the investigation of critical size fracture healing in a transgenic NFκB2 luc mouse.

Critical size fracture healing was performed using calcium phosphate scaffolds with a defined porosity. These scaffolds were seeded at the beginning with

osteogenic differentiated mesenchymal stem (OIM) cells and endothelial cells (EC) or were doped with Strontium (Sr) or a combination of them (Sr/OIM/EC). Groups without a scaffold serve as control group. After two month, animals were euthanized and the fracture sites were histologically examined.

Groups without scaffold showed a 100 % tissue formation, but no renewal of bone tissue. All animals treated with scaffolds showed a significant higher rate of bone formation. The Sr/OIM/EC group showed the lowest rate of bone renewal compared to other groups with scaffold. There was no significant difference between Sr group and OIM/EC group. The addition of strontium in scaffolds influences inflammation in various stages of the healing and leads to an increase of tissue Formation.

These effects might influence the healing process and may prove to be advantageous for osteoporosis fracture healing.

Poster 109:

Titel: The caries-degree dependent phosphorylation of ERK1/2 in inflamed human odontoblasts

Autoren/Adressen: Marleen Kufahl (University of Cologne), Hubert Christian Roggendorf (University of Cologne), Isabelle Graf (University of Cologne), Doychin Angelov (University of Cologne), Wilhelm Bloch (German Sport University), Bert Braumann (University of Cologne), Yüksel Korkmaz (University of Cologne); yueksel.korkmaz@uk-koeln.de

Abstract:
The inflammation stage in the dental pulp is dependent on carious lesion degrees. Different degrees of carious lesion require appropriate different caries therapies. Using odontoblast-like cells it was revealed that the phosphorylated MAPK ERK1/2 translocates into the nucleus inducing expression of dentin matrix proteins by activation of the transcription factors Runx2 and Osterix. However, the caries lesion degree-dependent effects of different inflammatory mediators on phosphorylation of ERK1/2 in human odontoblasts are unknown.

Due to the orthodontic treatment extracted healthy and carious third molars with dentin caries, deep-dentin caries and exposed pulp with mixed inflammation were immersion-fixed, decalcified and frozen-sectioned. For quantitative and double-immunohistochemistry the free floating sections were incubated with antibodies against total (t) ERK1/2 and phosphorylated (p) ERK1/2.

In the healthy odontoblasts, t-ERK1/2 was detected. Only a small subpopulation of the healthy odontoblasts revealed strong staining intensity for p-ERK1/2. A non-significant increase of p-ERK1/2 was detected in odontoblasts beneath the dentin caries region, while deep dentin caries induced a significant increase of p-ERK1/2 in cytoplasm and nuclei of the odontoblasts. In odontoblasts of mix-inflamed pulp, a localization of p-ERK1/2 was not detected.

The caries-degree dependent release of inflammatory mediators may induce nuclear translocation of p-ERK1/2 in inflamed odontoblasts. Subsequent expression of dentin matrix protein genes by Runx2 and Osterix might result in the formation of tertiary dentin matrix in dependence of caries degree. The activation of ERK1/2 in inflamed human odontoblasts depends on the extent of the carious lesion and on the inflammation stage in the dental pulp.

Poster 110:

Titel: The irreversible inflammation of the human dental pulp decreases phosphorylation of eNOS at Ser1177 in pulp blood vessels

Autoren/Adressen: Özlem Erdek (University of Cologne), Hubert Christian Roggendorf (University of Cologne), Wilhelm Bloch (German Sport University), Doychin Angelov (University of Cologne), Jörg Neugebauer (University of Cologne), Yüksel Korkmaz (University of Cologne); yueksel.korkmaz@uk-koeln.de

Abstract:
The formation of the tertiary dentin in response to carious lesion requires terminal differentiation of odontoblasts and intact circulation of the dental pulp. In the case of caries, circulation of the dental pulp is affected by numerous inflammatory mediators depending on the stage of pulp inflammation and caries degree. In endothelium, endothelial nitric oxide synthase (eNOS) generates NO that inhibits leucocyte adhesion and vascular inflammation. Under inflammatory conditions, eNOS becomes uncoupled form generating O2- instead of NO. The reciprocal phosphorylation (p) of eNOS at Ser1177 increases and at Thr495 decreases activity of eNOS. However, the in vivo effects of inflammation on the p-eNOS at Ser1177 and Thr495 are unknown.

Due to the orthodontic treatment extracted third healthy and carious molars (deep dentin caries) were immersion-fixed, decalcified and free floating frozen-sections were incubated with antibodies against t-eNOS, p-eNOS at Ser1177 and at Thr495 using quantitative and double immunohistochemical methods.

In healthy and inflamed blood vessels, eNOS was detected. In comparison to the healthy pulpal blood vessels, inflammation induced significant decrease in staining intensity of Ser1177 and an increase in staining intensity of Thr495 in blood vessel walls.

Our results indicate, that inflammation induces a decrease in formation of bioactive NO by reduction of p-eNOS at Ser1177 in the blood vessels of the dental pulp. In the case of deep dentin caries, the prevention of eNOS uncoupling and induction of phosphorylation of eNOS at Ser1177 in the dental pulp blood vessels may have therapeutic potential.

Poster 111:

Titel: Pharmacological modulation of the morpho-functional recovery of the ischemic murine retina

Autoren/Adressen: Jesus Eduardo Rojo Arias (TU Dresden, Faculty of Medicine), Matina Economopoulou (TU Dresden, University Hospital), Henning Morawietz (TU Dresden, University Hospital), Richard H.W. Funk (TU Dresden, Faculty of Medicine), József Jászai (TU Dresden, Faculty of Medicine); jozsef.jaszai@tu-dresden.de

Abstract:
The primary objective of the study was to investigate the efficacy of Aflibercept (AFL; VEGF-Trap), a recombinant decoy receptor recognizing ligands of VEGFR-1 and VEGFR-2, in promoting microvascular regeneration and morpho-functional recovery of the ischemic- hypoxic retinal tissue in the murine oxygen-induced retinopathy (OIR) model. This model is the most widely studied paradigm of retinal hypoxic stress being characterized by an exuberant vaso-proliferative response mimicking retinal neovascular diseases, e.g. retinopathy of prematurity and late stages of proliferative diabetic retinopathy.

Vaso-obliteration (VO) and pathologic neovascularization (NV) was induced exposing post-natal mice to hyperoxia followed by normoxia (relative hypoxia). Retinae from mice subjected to OIR (control) and such after receiving VEGF-Trap were analyzed morphological, biochemical and electrophysiological methods, and a microarray gene expression profiling was performed from whole retinas at time-points of maximum hypoxia and neovascularization (i.e. post-natal days 14 and 17, respectively).

Our results indicate that AFL strongly inhibits neovascularization while promoting a tip-cell driven ordered revascularization. AFL reduces inflammatory responses by decreasing vasopermeability and shifting the activation status of microglia/macrophages. Moreover, AFL improves responses to visible light. At the transcriptional level our analysis revealed a marked normalization effect on the expression of a group of genes delineating an angiogenic signature at both time-points. Moreover, an increased expression of genes associated with neurotransmission was found at P17 as revealed by the enrichment of the gene ontology (GO) term “synapse”.

Overall, our study indicates that AFL is beneficial for the treatment of ischemic retinopathies and it might be relevant for the maintenance of neuronal connectivity.

Poster 112:

Titel: The degeneration-degree dependent increase in elongation of the human odontoblast nucleus: an objective criterion for classification of the odontoblasts degeneration

Autoren/Adressen: Patrycja Chlopek (University of Cologne), Lena Austermann (University of Cologne), Luise Kuithan (University of Cologne), Wolfram Fröhling (University of Cologne), Suzan Kaya (University of Cologne), Arzu Akpur (University of Cologne), Tzvetelina Marinho de Barros (University of Cologne), Kalbie Emin (University of Cologne), Daniel R. Fuhrmann (University of Cologne), Wilhelm Bloch (German Sport University), Hubert Christian Roggendorf (University of Cologne), Jörg Neugebauer (University of Cologne), Yüksel Korkmaz (University of Cologne); yueksel.korkmaz@uk-koeln.de

Abstract:
The odontoblast degeneration is very often neglected, although it has been known for a long time. Because there is no generally accepted degree-dependent classification criterion for odontoblast degeneration, we examined degenerated odontoblasts to find parameters which allow an objective assessment of the degree of odontoblast degeneration and its subsequent classification.

Due to the orthodontic treatment extracted third molars were immersion-fixed, decalcified, cryoprotected, frozen-embedded and frozen-sectioned. The free-floating sections (30 µm) were histopathologically characterized by H&E staining and the consecutive sections of healthy and degenerated odontoblasts were embedded in resin for the semi-thin sections (1 µm). Semi-thin sections were stained with methylene blue. The free-floating sections were stained by chromosomal marker DRAQ5 and analyzed in a confocal laser scanning microscop.

In the healthy dentin-pulp complex, dentin, predentin, odontoblast layer, cell-free zone, cell-rich zone and subodontoblastic plexus were detected in a cellular order. In the degenerated dentin-pulp complex multiple degeneration vacuoles of different sizes were detected in the odontoblasts layer. The sections with degenerated odontoblasts were divided into three main-groups as ODI-III depending on the degree of degeneration. In ODII and especially in ODIII, the cell layers of the dentin-pulp complex were not found in the cellular order. In comparison to the round-oval nuclei in healthy odontoblasts, the nuclei in degenerated odontoblasts were elongated depending on the degree of degeneration.

The degeneration-degree dependent increase in elongation of the odontoblast nucleus may be considered as an objective criterion for classification of degree-dependent odontoblast degeneration.

Poster 113:

Titel: Contribution of keratin retraction and Dsg3 depletion to autoantibody-induced cell dissociation in pemphigus vulgaris

Autoren/Adressen: Elisabeth Schlögl (Ludwig-Maximilians-Universität München), Mariya Radeva (Ludwig-Maximilians-Universität München), Jens Waschke (Ludwig-Maximilians-Universität München), Volker Spindler (Ludwig-Maximilians-Universität München); volker.spindler@med.uni-muenchen.de

Abstract:
Pemphigus vulgaris (PV) is a potentially lethal autoimmune disease characterized by blister formation of the skin and mucous membranes and caused by autoantibodies against desmogleins (Dsg)1 and Dsg3. Dsg1 and Dsg3 are linked to keratin filaments in desmosomes, adhering junctions abundant in tissues exposed to high degrees of mechanical stress. The binding of the antibodies leads to the internalization of Dsg3 and a collapse of the keratin cytoskeleton – yet the relevance of these changes for loss of cell-cell adhesion and blistering is poorly understood.

We performed live cell imaging of human keratinocytes expressing keratin5-YFP and Dsg3-mCherry to detect the temporal relationship between changes in the keratin network and the internalization of Dsg3.

Loss of the keratin network at the cell periphery was detectable as soon as after 60 min of incubation with autoantibody fractions of PV patients (PV-IgG), whereas a condensation of keratin filaments into thick bundles occurred after several hours. Dsg3 internalization started at 90 min of PV-IgG treatment, thus following the early keratin changes. By inhibiting casein kinase 1 using D4476, we provoked keratin alterations resembling the effects of PV-IgG. Although D4476-induced loss of the peripheral keratin network correlated with loss of cell cohesion, it was not sufficient to trigger the internalization of Dsg3. However, additional incubation with PV-IgG still promoted Dsg3 loss at the membrane.

Keratin changes appear very early after autoantibody binding suggesting a crucial role for loss of cell cohesion. Our data furthermore indicate that the internalization of Dsg3 may be independent from keratin alterations.

Poster 114:

Titel: Reduction of the formaldehyde concentration in the breathing air during the anatomical dissection course by a newly developed jet nozzle system

Autoren/Adressen: Hans Hieke (Justus-Liebig-Universität Gießen), Sonja Pfeil (Justus-Liebig-Universität Gießen), Monika Wimmer (Justus-Liebig-Universität Gießen); Hans.Hieke@admin.uni-giessen.de

Abstract:
The best conservation methods for body donors are embalming solutions containing formaldehyde. New EU legislation defined a much lower permissible exposure limit (PEL) for formaldehyde imposing the need to reduce formaldehyde in the breathing air. The newly developed system using precisely directed air flow had to be compatible with the given constructural premises in the dissection room and had to be cost effective

The existing air ventilation system with 12.000 m3/h and 7 air changes per hour was not able to reduce the formaldehyde concentration below the PEL value in the breathing air. With the new modification of the air flow, using three long throw nozzles arranged in series vertically above the middle of each dissection table the arising draft can be decreased to avoid impairment of the working students. These nozzles generate a down flow of 0.6 m/s. Thus, fresh air from the ceiling area is conveyed into the atmosphere in front of students and teachers. Ground-based exhaust vent installation with a performance of 12.500 m^3/h guarantees no increase in formaldehyde concentration in the ambient air in between the dissection tables.

The presented long throw nozzle air directing system is capable to reduce formaldehyde vapor by at least 75% in the breathing air. Nearly no formaldehyde vapor emitted by the cadavers pollutes the breathing air of students.

The acquired data constitute the basis for improving the breathing air by reduction of the formaldehyde vapor in any anatomical dissection room. The existing regular built-in ventilation systems can be upgraded with such a system of accurately adapted nozzles.

Poster 115:

Titel: Novel monolayer cultivation model of tenocyte in cell stretcher x6

Autoren/Adressen: Katja Goltz (RWTH University Clinic), Sara Tabea Schneider (RWTH University Clinic), Bernd Hoffmann (research center Jülich), Uwe Schnakenberg (RWTH Aachen University), Holger Jahr (RWTH University Clinic), Thomas Pufe (RWTH University Clinic), Mersedeh Tohidnezhad (RWTH University Clinic); mtohidnezhad@ukaachen.de

Abstract:
Almost 40 % of sport-injuries in Germany are related to muscles and tendons. The healing process takes a long time with increased risk of recurrence. The mechanical loading of the tendon cues seem to be essential for maintaining the stability of tendons. The aim of the study is a) use of a novel bioreactor for physiological cultivation of tenocytes and b) analysis of uniaxial mechanical stretch on tenocyte physiology and Differentiation.

We used primary tenocytes from mice in cyclic 2D tensile tests with the Cell stretcher X6 by 1 Hz and 2 Hz and an elongation of 5.5 %. Expression of differentiation markers as SCX, TMDN, COL1, COL2, MMP-1 and -13 were analyzed using immunohistochemistry and real-time RT PCR. ELISA was used for quantification of released VEGF

We were able to measure increased intensities of COL1, SCX at 1 Hz but no increase in TNMD protein expression. MMP1, MMP13 and VEGF protein expression remained unchanged. The results show that the tenocytes orient themselves at an angle of 60-90° to the pull direction. At a frequency of 2 Hz the expressions of SCX, MMP-1 and MMP-13 were measured. Decreasing of cell viability was associated with damage to the cytoskeleton.

We conclude that a) the cell stretcher can be used for in vitro cultivation and analysis of tenocytes and b) the elongation of 5.5 % and frequency of 1 Hz acts as a physiological model, whereas 2 Hz act as an overloading model for tenocytes cultures.

Poster 116:

Titel: DEHP effects on human adipogenesis in SGBS-derived adipocytes

Autoren/Adressen: Kristina Schaedlich (Martin Luther University Faculty of Medicine), Scarlett Gebauer (), Luise Hunger (), Laura-Sophie Beier (), Martin Wabitsch (), Bernd Fischer (), Jana Ernst ();kristina.schaedlich@medizin.uni-halle.de

Abstract:
DEHP is a common plasticizer which has been used in plastic products for decades. Since 2015 DEHP is on the REACH Authorisation List of the EU. Routes of exposure are through ingestion, inhalation, dermal contact and intensive medical care. Studies in mice and murine cell lines identified DEHP as an endocrine disruptor that may act as an obesogen. As this is of high concern in respect of the worldwide obesity epidemic, our aim is the translation of these findings into a human model system.

On the base of the DOHaD-hypothesis, we investigated the influence of DEHP [50 µg/ml] on adipogenesis in the human pre-adipocyte cell strain SGBS. During the induction of adipogenesis, we exposed pre-adipocytes to DEHP and subsequently differentiated the cells into mature adipocytes. We analyzed adipocyte markers like GLUT4, FABP4, LPL, CD36, HSL and PPARs, signaling pathways like AMPK/ACC2 and functional markers like adipokine secretion and triglyceride content as well as ROS production in mature adipocytes.

Reduced levels of adiponectin together with higher levels of leptin and a significant reduction in lipid accumulation of the whole adipocytes were detected. Furthermore, DEHP activated the AMPK/ACC2 signaling cascade and decreased the expression of FABP4 and LPL, pointing towards a higher beta-oxidation rate and a decreased rate of fatty acid binding and transport. The production of ROS was significantly elevated after DEHP-exposure, which may have led to a higher phosphorylation of ERK1/2.

In contrast to animal studies, we found significant anti-obesogenic effects after DEHP-exposure during the induction phase of adipogenesis.

Poster 117:

Titel: Training a sophisticated microsurgical technique: interposition of external jugular vein graft in the common carotid artery in rats

Autoren/Adressen: Maria Kokozidou (Paracelsus Medical University, Klinikum Nürnberg Nord, Nuerenberg, Germany), Stefan Langer (Marien Hospital, Witten, Germany), Thomas Andreas Koeppel (Asklepios Kliniken St. Georg und Wandsbek, Gefäßzentrum Ost, Hamburg, Germany), Karina Schleimer (Universitätsklinikum RWTH Aachen, Aachen, Germany); maria.kokozidou@pmu.ac.at

Abstract:
Neointimal hyperplasia is one the primary causes of stenosis in arterialized veins that are of great importance in arterial coronary bypass surgery, in peripheral arterial bypass surgery as well as in arteriovenous fistulas. The experimental procedure of vein graft interposition in the common carotid artery by using the cuff-technique has been applied in several research projects to examine the aetiology of neointimal hyperplasia and therapeutic options to address it. The cuff prevents vessel anastomotic remodeling and induces turbulence within the graft and thereby the development of neointimal hyperplasia. Using the superior caval vein graft is an established small-animal model for venous arterialization experiments.

To simplify the procedure and to minimize the number of experimental animals needed, a detailed operation protocol is presented. This should help the novice surgeon to learn both the cuff-technique and the vein graft interposition. Hereby, the right external jugular vein was grafted in cuff-technique in the common carotid artery of 21 female Sprague Dawley rats categorized in three equal groups that were sacrificed on day 21, 42 and 84, respectively. Notably, no donor animals were needed, because auto-transplantations were performed.

The survival rate was 100 % at the time point of sacrifice. In addition, the graft patency rate was 60 % for the first 10 operated animals and 82 % for the remaining 11 animals. The blood flow at the time of sacrifice was 8±3 ml/min.

In conclusion, this surgical protocol considerably simplifies, optimizes and standardizes this complicated procedure. It gives novice surgeons easy, step-by-step instruction, explaining possible pitfalls, thereby helping them to gain expertise fast and avoid useless sacrifice of experimental animals.

Poster 118:

Titel: CEACAM1 regulates endothelial adherens junctions and barrier function

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Abstract:
CEACAM1 is an important mediator of vascularization/ angiogenesis. However, its role in mature vessel homeostasis is largely unknown. Therefore, we analyzed the impact of CEACAM1 on an essential aspect of endothelial function: adherens junction integrity and barrier function.

We used aortic samples and endothelial cell lines derived from myocardial explants of WT and Cc1-/- mice (MyEnd cells). We analyzed protein tyrosine phosphorylation and protein-protein interaction (immunoprecipitation), quantified gene expression (Western Blot) and evaluated endothelial barrier function in situ (aortic dye deposition) and in vitro (transendothelial electrical resistance - TEER).

Aortic endothelial barrier function is reduced in Cc1-/- mice (3 months). Similarly, Cc1-/- MyEnd cells showed reduced basal TEER. This coincided with an increased caveolin-1 expression and tyrosine phosphorylation in Cc1-/- MyEnd cells known to destabilize endothelial adherence junctions. However, at 9 months endothelial barrier function is reduced in WT mice compared to Cc1-/- mice, indicating a differential regulation of barrier function by an age-dependent factor. Aortic TNF-α expression was only detectable in aged mice and was much higher in WT mice. Moreover, TNF-α reduced barrier function in WT but increases it in Cc1-/- aortic rings. Only in WT MyEnd cells, TNFα increased tyrosine phosphorylation of adherens junction (AJ) proteins VE-cadherin and β-catenin known to destabilize their interaction. This is putatively due to TNFα-induced association of CEACAM1 with VE-cadherin and subsequent Src kinase recruitment.

CEACAM1 deficiency affects endothelial barrier function implying a crucial role of CEACAM1 in the homeostasis of mature blood vessels.

Poster 119:

Titel: Characterization of iliotibial band-derived fibroblasts harvested by explant culturing

Autoren/Adressen: Gundula Schulze-Tanzil (Paracelsus Medical University, Salzburg and Nuremberg), Clemens Gögele (Paracelsus Medical University, Salzburg and Nuremberg), Benjamin Ondruschka (University of Leipzig), Niels Hammer (University of Otago), Silke Schwarz (Paracelsus Medical University, Salzburg and Nuremberg); gundula.schulze@pmu.ac.at

Abstract:
The iliotibial band (ITB) contains type I collagen fiber bundles, parallel running in longitudinal and crossing directions with few specialized fibroblasts arranged in longitudinal rows. This robust tissue is suitable for ligament reconstruction and attracts increasing interest for various tissue engineering purposes. Explant cultures were often used for harvesting fibroblasts from various origins. So far, the ITB fibroblasts are poorly characterized. Understanding the emigration from cultured explants might allow utilizing this process for seeding ITB fibroblasts on biomaterials. Therefore, the aim of the present study was to investigate the expression profile of ITB fibroblasts during emigration from ITB tissues in order to contribute to a deeper understanding of the emigration process.

Explant cultures were prepared from human ITB samples and cultured for 8-10 weeks. Cell vitality was visualized using live dead assay. The protein expression profile (types I-III collagen, elastin, lubricin, decorin, aggrecan, fibronectin, tenascin C, CD44, β1-integrins, scleraxis, vimentin, F-actin, α-smooth muscle actin (αSMA) and VEGFA) was assessed using confocal laser scanning microscopy.

ITB fibroblasts survived for several months in explant culture, continuously forming novel monolayers. Fibroblasts migrated along the longitudinal collagen fiber bundles or circled laterally the bundles and multiplied at the bottom of the explants. In explant and monolayer they expressed VEGF and the tested matrix components (except for elastin), β1-integrins, CD44, scleraxis, vimentin, αSMA and formed stress fibers.

Using self-assembled ITB fibroblast spheroids, it is possible to restore and utilize the migration capacity of the ITB cells for the recalibration of biomaterials for tissue engineering applications.

Poster 120:

Titel: Muscular changes in asymptomatic diverticulosis preceding the onset of diverticular disease

Autoren/Adressen: Christina Lange (Kiel University), Martina Barrenschee (Kiel University), François Cossais (Kiel University), Ines Hohmeier (Kiel University), Michael Ebsen (Städtisches Krankenhaus Kiel), Ilka Vogel (Städtisches Krankenhaus Kiel), Jan-Hendrik Egberts (University Hospital Schleswig-Holstein, Campus Kiel), Thomas Becker (University Hospital Schleswig-Holstein, Campus Kiel), Martina Böttner (Kiel University), Thilo Wedel (Kiel University); c.lange@anat.uni-kiel.de

Abstract:
Intestinal motility is controlled by the enteric nervous system communicating with smooth muscle cells, the effectors of intestinal movement.

As diverticular disease (DD) is associated with an impaired intestinal motility, we analyzed markers of smooth muscle cells in patients with asymptomatic diverticulosis and controls in order to enlighten the pathologic changes preceding the onset of DD.

Colonic samples obtained from patients with asymptomatic diverticulosis (n=12) and controls (n=19) were subjected to histological analyses. Furthermore, comparative site-specific gene expression analyses were performed for smooth muscle marker genes by real-time-qPCR analysis on mRNA samples extracted from tunica muscularis (TM). Protein expression was assessed and quantified by immunohistochemistry on full-thickness sections of patients and controls.

In contrast to our previous findings on DD, only moderate structural muscular alterations were observed in colonic full thickness sections from patients with asymptomatic diverticulosis compared to controls. Expression of HDAC8 and Tropomyosin 1 was significantly increased in the TM of patients with diverticulosis compared to controls.

Our data contribute to the clarification of the pathogenic changes leading to DD. We observed a disturbed expression of smooth muscle markers and moderate histological changes of the TM in asymptomatic diverticulosis during early stages of diverticula formation independent from inflammatory events. Besides enteric neuropathy also enteric myopathy appears to be a factor contributing to the pathogenesis and disturbed intestinal motility in DD.

Poster 121:

Titel: Oxygen-glucose deprivation alters astrocytic homeostasis and gap junction morphology

Autoren/Adressen: Anja Beckmann (Saarland University), Sandra Wolf (Saarland University), Alexander Grißmer (Saarland University), Carola Meier (Saarland University); anja.beckmann@uks.eu

Abstract:
Perinatal hypoxia in the immature brain results in neuronal cell death, activation of microglia and, subsequently, development of an astrocytic scar. Astrocytes are part of a functional network, mainly because of gap junctional coupling. The main astrocytic gap junction protein is Connexin (Cx) 43. In perinatal hypoxia-ischemia, Cx43 expression was previously shown to be upregulated, which was attributed to the glia scar and correlates with the opening of channels.

As gap junctions are dynamic structures within the cell membrane, we postulate that both composition and particle arrangement underlie functional changes. Here, cell death, release of reactive oxygen species and Cx43-gap junction ultrastructure were investigated in astrocytes after oxygen-glucose deprivation.

Neonatal murine astrocyte cultures were subjected to oxygen-glucose deprivation for 6 hours, followed by 2 hours reoxygenation. Cell death, reactive oxygen species and Cx43 expression were determined thereafter. Analysis of Cx43 gap junction ultrastructure was performed by freeze-fracture replica immunogold labeling.

Oxygen-glucose deprivation resulted in survival of the majority of cells and an increase of reactive oxygen species. Changes in Cx43 expression was altered and seemed to correlate to differences in the morphology of gap junction plaques and arrangement of channels within a gap junction.

The relatively short period of oxygen-glucose deprivation affected the viability of astrocytes minimally, however, the increase of reactive oxygen species revealed cell stress. The observed changes of Cx43 gap junction morphology are an intriguing finding, which should be investigated further to elucidate the role of gap junctions and its channels in the stroke-related pathophysiology.

Poster 122:

Titel: Atoh8 in reprogramming and maintenance of pluripotency

Autoren/Adressen: Divvela SSK, Balachandran Y, Balakrishnan-Renuka A, Boeing M, Napirei M, Zaehres H, Brand-Saberi B
Institute of Anatomy, Department of Anatomy and Molecular Embryology, Ruhr-University Bochum; satya.divvela@rub.de

Abstract:
Objective:
Atoh8 belongs to the atonal basic helix loop helix transcription factor family. Previous studies on Atoh8 have shown its significance in many developmental events. We have previously reported its regulatory role in skeletal myogenesis, whereas recently we have identified its expression in the inner cell mass of the blastocyst. However, the role of Atoh8 in the context of pluripotency and early differentiation remains to be resolved. In this study, we plan to decipher the potential role of ATOH8 in the context of reprogramming, pluripotency and early differentiation.
Methods:
As the first step, we decided to study the role of Atoh8 in cellular reprogramming and maintenance of pluripotency by comparing wildtype and Atoh8 knockouts.
Results:
The Atoh8 knockout fibroblasts cultured on feeders completely failed to reprogram, whereas the Atoh8 knockout fibroblasts cultured in feeder-free condition (Matrigel) were able to give rise to iPSCs, but with very low efficiency. Corresponding to the above results, qRT-PCR performed at different time points representing different phases of reprogramming revealed significant alterations in the mRNA levels of genes involved in mesenchymal epithelial transition (MET) and maintenance of pluripotency. Furthermore, in our preliminary studies, we have also observed that the iPSCs derived from Atoh8 knockouts were undergoing differentiation earlier than wildtype suggesting the essential role of Atoh8 in maintaining the pluripotent state.
Conclusion:
Altogether, our data point towards a crucial role of Atoh8 in reprogramming and maintenance of pluripotency.

Poster 123:

Titel: Tailored liposomes against bacterial toxins

Autoren/Adressen: Annette Draeger (Institute of Anatomy, Univ. of Bern), Eduard B. Babiychuk (Institute of Anatomy, Univ. of Bern); draeger@ana.unibe.ch

Abstract:
Gram-positive bacterial pathogens that secrete cytotoxic pore-forming toxins, such as Staphylococcus aureus and Streptococcus pneumoniae, cause a substantial burden of disease.Using a combination of life imaging and cryo-electron microscopy we show that pneumolysin, released by cultured bacteria, is capable of permeabilizing the plasmalemma of host cells. However, such permeabilization does not lead to cell lysis since pneumolysin is actively removed by the host cells. The process of pore elimination starts with the formation of pore-bearing plasmalemmal nanotubes and proceeds by the shedding of pores that are embedded in the membrane of released microvesicles. Pneumolysin prepores are likewise removed.

Inspired by the principles that govern natural toxin-host interactions, we have engineered artificial liposomes that are tailored to effectively compete with host cells for toxin binding. Liposome-bound toxins are unable to lyse mammalian cells in vitro. We use these artificial liposomes as decoy targets to sequester bacterial toxins that are produced during active infection in vivo.

Liposomes protect mice against death by pneumococcal septicaemia or pneumonia.

Composed exclusively of naturally occurring lipids, tailored liposomes are not bactericidal and could be used therapeutically either alone or in conjunction with antibiotics to combat bacterial infections

Poster 124:

Titel: Loss of Dsg2 results in partial EMT and an upregulation of cAMP levels

Autoren/Adressen: Lena Sachs (LMU), Julian Zeiler (LMU), Jan Hoffmann (LMU), Volker Spindler (LMU); volker.spindler@med.uni-muenchen.de

Abstract:
Pancreatic ductal adenocarcinoma (PDAC) is a highly invasive tumor prone to early metastasis, but the mechanisms underlying the invasive phenotype of pancreatic cancer cells are poorly understood. Epithelial tissues rely on desmosomes to maintain structural integrity through strong cell-cell adhesion and to modulate cellular behavior. Here, we studied the role of the desmosomal cadherin-type adhesion molecule Desmoglein 2 (Dsg2) for migration of PDAC cells.

We generated stable dsg2 knockout (KO) and knockdown (KD) AsPC-1 cell lines by introducing a sh-construct or using CRISPR/Cas9, respectively. Through a transcriptome analysis and qPCR we discovered upregulation of mesenchymal markers in dsg2-depleted cells. We assessed migration rate by wound healing assays and monitored protein levels by Western Blot.

KD or KO of dsg2 resulted in reduced cell cohesion, faster migration and upregulation of mesenchymal markers. Among them was Slug, which is a key regulator of Epithelial to mesenchymal transition (EMT). KD of slug caused a reduced migration rate in Dsg2-depleted cells, demonstrating its relevance for invasiveness of dsg2 KO/KD cells. Although cAMP signaling is associated with stronger cell-cell contacts, Dsg2-depleted cells displayed increased cAMP levels compared to controls. However, pharmacologic elevation of cAMP levels caused a reduction of migration rate as well as a reduction of Slug levels.

This suggests that loss of Dsg2 promotes invasiveness of PDAC cells through a partial EMT but may simultaneously lead to a compensatory upregulation of cAMP. Elevation of cAMP levels thus represents a promising approach to reduce the invasive behavior of PDAC cells.

Poster 125:

Titel: Changes in gene expression during neuroendocrine differentiation of PCA cell line LNCAP reveal new microrna target genes

Autoren/Adressen: Marc Wiesehöfer (University of Duisburg-Essen), Jaroslaw Szczyrba (University of Duisburg-Essen), Gunther Wennemuth (University of Duisburg-Essen); marc.wiesehoefer@uk-essen.de

Abstract:
Prostate carcinoma is still a leading cause of cancer related death in men worldwide. An increase of androgen-independent neuroendocrine-like (NE) cancer cells after neuroendocrine transdifferentiation (NEtD) correlates with a lower survival rate. NE-like cancer cells release, according to normal NE cells, a variety of diverse neuropeptides with mitogenic effects on adjacent cells. However, the mechanism of NEtD and a possible role of miRNAs have not been clarified yet. MiRNAs are small non-coding RNAs which regulate posttranscriptionally gene expression.

To investigate the expression changes of mRNAs and miRNAs and their role in NEtD, we differentiated LNCaP prostate cancer cells by androgen deprivation and performed Micro-Array analysis. Deregulated mRNAs were analyzed using different algorithms to predict new target sites for deregulated miRNAs. Subsequent luciferase reporter assays and Western Blots were performed to confirm predicted miRNA binding sites.

Micro-Array results reveal highly modified mRNA and miRNA expression profiles for transdifferentiated LNCaP cells compared to untreated LNCaP cells. CyclinD1 is one of several deregulated mRNAs and it could be confirmed as miRNA target gene for five different miRNAs. These five miRNAs also had a high influence on various aspects of cell behavior.

Our data demonstrate wide changes in gene expression after NEtD of LNCaP cells and show the importance of miRNAs in this process. In order to give these results a higher value, both in vivo studies and patient tissue will be analyzed. The project shall provide an explanation on the mechanism of NEtD, thus revealing opportunities for therapy and diagnosis.

Poster 126:

Titel: Pathomechanisms in Desmoglein 2-mutant hearts

Autoren/Adressen: Claudia A. Krusche, Florian Bruns, Muhammed Gercek, Ina-Maria Bezrucav, Nadine Lubos, Sebastian Kant, Rudolf E. Leube; Institute of Molecular and Cellular Anatomy, RWTH Aachen University, Aachen, Germany; ckrusche@ukaachen.de

Abstract:
We have established desmoglein 2 (Dsg2)-mutant mice as valuable models of arrhythmogenic right ventricular cardiomyopathy (ARVC). They are characterized by cardiomyocyte loss, inflammation, fibrosis and cardiac dilation. ARVC pathogenesis is divided into three consecutive phases: (i) concealed phase, (ii) acute structural disease onset phase, and (iii) chronic phase leading to heart failure. We aim to unravel pathomechanisms that are activated during the different disease phases using our murine ARVC models.

Heart chamber-specific morphology, gene transcripts and protein expression were analysed in Dsg2-mutant hearts derived from the three disease phases. The focus of our efforts was on alterations reflecting inflammation, fibrotic remodeling, metabolism, and hypertrophy.

During the concealed phase and at acute structural disease onset altered expression of glucose transporters and regulators of fatty acid metabolism was detected. The inflammatory response during the acute structural disease phase showed remarkable parallels to inflammation after ischemic heart infarction. Lesion formation was characterized by increased mRNA expression of the fibrotic remodeling markers Mmp12, Timp1 and Serpine1 in both ventricles and of Mmp2 exclusively in the right ventricle. Expression of the hypertrophy-associated Rcan1 mRNA started to be increased during the acute phase and was further increased in both ventricles during chronic disease progression. Inflammation and Mmp expression declined, but remained significantly higher than in the wildtype. Mutant atria presented with progressive dilation associated with local fibrosis and inflammation.

Taken together, our analyses revealed disease-phase and ventricle-specific pathological alterations in our ARVC mouse models. These insights will help to devise novel therapeutic treatment strategies.

Poster 127:

Titel: Hypoxia delays the myogenic differentiation and enhances the adipogenesis and osteogenesis of the muscle-derived stem cells

Autoren/Adressen: Mohamed I. Elashry (Justus Liebig University of Giessen), Manuela Heimann (Justus Liebig University of Giessen), Sabine Wenisch (Justus Liebig University of Giessen), Stefan Arnhold (Justus Liebig University of Giessen); Mohammed.elashry@vetmed.uni-giessen.de

Abstract:
Muscle-derived stem cells have become a promising approach for investigation of myogenesis and tissue regeneration under various conditions. Muscle regeneration is performed by muscle stem cells (SC), self-renewal population beneath the basal lamina of the muscle fibers. However, the impact of hypoxia on SC proliferation, migration and ectopic differentiation inductions are not fully characterized. Here we examined the effect of oxygen fluctuation on SC myogenesis, adipogenesis and osteogenesis.

Hind limb muscles of wild type mice were processed for both SC/fiber isolation and myoblasts extraction using magnetic beads. SC were induced for myogenic, adipogenic and osteogenic commitment under normoxic (21% O2) and hypoxic (3% O2) atmosphere for 48 and 72 h for SC/fiber and 1, 2 and 3 weeks for myoblasts. SC proliferation and differentiation were evaluated by Immunohistochemistry and qRT-PCR.

Hypoxia increases SC proliferation following myogenic induction compared to normoxia at 48 h. Hypoxia initially increased SC per cluster at 48 h followed by sharp decline at 72 h compared to myogenic normoxic. At 72 h hypoxia decreases the number of myogenin expressing cells and increases Pax7 positive cells compared to myogenic normoxia. Hypoxia caused persistent upregulation of MyoD and downregulation for myogenein expression following myogenic induction. Furthermore, hypoxia upregulated the expression of adipogenic markers FABP4 and PPARγ at day 7 and day 21 respectively compared to normoxia. Osteogenic induction showed early upregulation of osteocalcin and osteopontin expression at day 7 and 14 compared to normoxia.

To conclude hypoxia delays myogenesis and enhances the multipotency of muscle stem cells.

Poster 128:

Titel: Characterization of dermomyotomal filopodia-like protrusions (filips) in chicken embryos

Autoren/Adressen: Margarethe Draga (University of Cologne), Sagar (Max Planck Institute of Immunobiology and Epigenetics), Felicitas Pröls (University of Cologne), Martin Scaal (University of Cologne); martin.scaal@uk-koeln.de

Abstract:
The development of multicellular organisms is stringently controlled by intercellular communication and interaction. In vertebrates and invertebrates specialized cellular protrusions called signaling filopodia or cytonemes play an important role in cell-cell communication by carrying receptors and ligands to distant cells to activate various signaling pathways. We are investigating signaling filopodia, called Filopodia-Like Protrusions (FiLiPs) in the somite epithelia of the chicken embryo (Sagar et al., 2015). They are formed at the basal side of polarized epithelial cells of early and matured somites, i.e. the dermomyotomal cells. FiLiPs penetrate the basement membrane of the somite and contact the overlying ectoderm cells.

To find signaling pathways required for the formation of FiLiPs we investigate factors that regulate the reorganization of the actin-cytoskeleton. These proteins are over-expressed in the dorsal part of the somites in the chicken embryo using electroporation. The expression pattern and the role of the proteins in filopodia formation are analyzed by confocal microscopy.

We can show that overexpression of the constitutive active form of the RhoGTPase Rac1, which leads to inhibition of Rac1 activity, disrupts the formation of FiLiPs. Furthermore the actin-binding protein Cofilin is also required for the formation of FiLiPs.

The formation of FiLiPs in the dermomyotome of the chicken embryo involves reorganization of the actin cytoskeleton which is regulated by the RhoGTPase Rac1 and the actin binding protein Cofilin. We are presently searching further factors involved in FiLiPs formation to understand the role of FiLiPs in somite development.

Poster 129:

Titel: Divergent sonic hedgehog expression patterns in vertebrate prospective floor plate and notochord

Autoren/Adressen: Nikoloz Tsikolia (Universitätsmedizin Göttingen), Kristine Henningfeld (Universitätsmedizin Göttingen), Stanislav Kremnyov (Lomonosov Moscow State University), Christoph Viebahn (Universitätsmedizin Göttingen); nikoloz.tsikolia@med.uni-goettingen.de

Abstract:
The notochord is required for floor plate induction and establishment of the dorso-ventral patterning of the neural tube and is believed to have organizer properties. The inductive properties are related to the function of sonic hedgehog (shh) which diffuse from the notochord, forms a gradient and autoinduces shh expression in the floor plate. However reported data is inconsistent and detailed spatio-temporal pattern of shh expression has not been studied across species.

Therefore we studied dynamics of shh expression in Xenopus leavis, chicken the rabbit embryos using in situ hybridization.

Technovit sections reveal divergent pattern: in Xenopus shh is expressed at the beginning of gastrulation in distinct area above the dorsal blastopore lip adjacent to the prospective neuroectoderm whereas the floor plate is positive at the end of gastrulation, in the chick embryo shh is expressed in the prospective neuroectoderm prior to the notochord formation and even prior to mesoderm migration, whereas in the rabbit the classical pattern was found in that shh is first expressed in the notochord and the floor plate domain is then induced during somitogenesis stages.

These findings are discussed within the framework of loss of function experiments and known mutants and morphants with disturbed notochord development and shh expression. We propose that the mode of floor plate induction adapts to differences in topography of embryonic tissues during gastrulation and neural induction.

Poster 130:

Titel: Differential proteomics of the cerebral cortex of juvenile, adult and aged rats: an ontogenetic study

Autoren/Adressen: Michael Wille (University of Rostock), Antje Schümann (University of Rostock), Michael Kreutzer (University of Rostock), Michael O. Glocker (University of Rostock), Grit Mutzbauer (University of Würzburg), Oliver Schmitt (University of Rostock), Andreas Wree (University of Rostock); michael.wille@uni-rostock.de

Abstract:
The identification of up- and downregulated as well as absent proteins in the CNS is necessary to understand the interplay of migration, differentiation and integration of neuronal progenitor cells at different stages of development. In a first step, differentially expressed proteins of the cerebral cortex of the laboratory rat at three significant stages of development were identified. The cerebral cortex needs differential abundances of proteins during ontogenesis and uses its high plasticity postnatally to adapt to many types of intrinsic and extrinsic changes. This study focuses on the identification of differentially expressed proteins during postnatal development.

Cerebral cortices of P7, P90 and P637 old wistar rats were dissected and analyzed by two-dimensional polyacrylamide gel electrophoresis (2DE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS).

Proteins of the functional classes of the carbohydrate metabolism, structural, regulatory proteins and proteins involved in the energy metabolism show the highest differential abundance within the analyzed stages of development. Cytoskeleton proteins like neurofilaments and BETA-actin are downregulated in early development. In contrast, some proteins which are necessary for migration and motility are upregulated in P7 versus P90 animals. Furthermore, proteins for vesicular trafficking like drebrin and Gdi2 are upregulated in P7. In aged animals oxidative stress sensors, proteins necessary for autophagy of dysfunctional mitochondria, growth control and hypoxia tolerance (Ppp1ca, Eno1) turned out to be upregulated.

Overall, energy consumption and differentiation processes as well as specific regulatory mechanisms can be observed at least indirectly by differential abundances of proteins during the investigated stages of ageing.

Poster 131:

Titel: Staging E14.5 mouse embryos

Autoren/Adressen:
Stefan Geyer (Medical University of Vienna), Lukas Reissig (Medical University of Vienna), Julia Rose (Medical University of Vienna), Robert Wilson (The Francis Crick Institute), Fabrice Prin (The Francis Crick Institute), Dorota Szumska (Wellcome Trust Centre for Human Genetics), Ramiro Ramirez-Solis (Wellcome Trust Sanger Institute), Catherine Tudor (Wellcome Trust Sanger Institute), Jacqui White (Wellcome Trust Sanger Institute), Timothy Mohun (The Francis Crick Institute), Wolfgang Weninger (Medical University of Vienna); stefan.geyer@meduniwien.ac.at

Abstract:
Comparison of the phenotype of mutants with that of wild-types forms the basis for identifying the function of genes. The "Deciphering the Mechanisms of Developmental Disorders (DMDD)" programme studies embryonic lethal gene mutations in the mouse (dmdd.org.uk). Embryos are harvested at E14.5 and the phenotype of mutants is compared to that of controls, which exactly match in their developmental stage. Since existing staging systems proved to be inadequate for this task, we developed a novel, simple and quick method for staging E14.5 mouse embryos.

We produced and analysed digital volume data generated with the High resolution episcopic microscopy (HREM) method from 58 mouse lines of the C57BL/6 strain, comprising a total of 215 wild-type and 297 mutant embryos.

We came up with a simple method for defining 6 developmental sub-stages in E14.5 mouse embryos. It enabled us to detect a broad variety of stage-dependent differences in the structure of the organs of E14.5 embryos. Prominent examples are the appearance oft the palate, gut and heart. Furthermore, applying the new staging system to the DMDD embryos revealed that homozygous mutants are frequently delayed in development.

Together these results illustrate the importance of accurate staging of individual embryos used for assessing phenotype and the profound impact small differences in developmental stage can have. Comparison of mutant embryos with wild type littermates is likely to be seriously misleading, unless our system is used to ensure that only the phenotypes of embryos of exactly matching developmental stages are compared.

Poster 132:

Titel: Hemopexin induces proteinuria in zebrafish larvae

Autoren/Adressen: Ahmed M. Kotb, Antje Blumenthal, Florian Siegerist, Frances Kindt, Karlhans Endlich, Nicole Endlich; Institute of Anatomy and Cell Biology, University Medicine Greifswald, Greifswald, Germany; kotba@uni-greifswald.de

Abstract:
Introduction and aim
Hemopexin (HPX), a 60 kDa glycoprotein that has an extraordinary affinity to free plasma heme reduces the heme-induced oxidative stress of cells by elimination of heme. After binding of heme-HPX to its specific receptor, the heme-HPX-receptor complex becomes catabolized intracellularly. Since it is known that HPX has anti-inflammatory effects, it has been suggested to treat patients with HPX to ameliorate heme-induced effects. Further, it was observed that HPX is upregulated in response to acute kidney injury, in patients suffering from diabetic as well as minimal change nephropathy. Interestingly, it was found that the injection of HPX into rats induced proteinuria due to changes of the podocytes foot process morphology, an important cell type responsible for proper blood filtration in the glomerulus. To study the function of HPX directly in a living organism, we used transparent zebrafish larvae that develop a simplified kidney, the pronephros, composed of one glomerulus combined with a pair of tubules. This animal model enabled us to study the role of HPX not only by immunohistochemistry and RT-PCR/Western blot but also in living zebrafish larvae by 2-photon microscopy (2-PM).
Methods
Several zebrafish strains were used: The transgenic strain ET [Tg(wt1a:eGFP); Tg(mitfaw2/w2; roya9/a9)] expressing eGFP under the podocyte-specific wt1a promoter, Cade [Tg(l-fabp:eGFP-DBP); Tg(mitfaw2/w2; roya9/a9)] expressing the 78 kDa eGFP-vitamin D-binding protein in the blood plasma and Chipper [Tg(fli1a:eGFP); Tg(nphs2:Eco.NfsB-mCherry); Tg(mitfaw2/w2; roya9/a9)] expressing eGFP in the endothelial cells of the vessels and mCherry specifically in podocytes. The zebrafish larvae were studied by the 2-PM (Zeiss, Jena, Germany) as well as by immunohistochemistry and RT-PCR.
Results
At 3-4 dpf, HPX was injected intravenously into the zebrafish strain Cade that expresses the eGFP-labeled vitamin D-binding protein in the blood plasma, being unable to pass the healthy filtration barrier. However, 24 hours after the injection more than 60% of the larvae developed severe pericardial edema, a hallmark of kidney disease. Moreover, the zebrafish larvae showed a loss of the fluorescence intensity in the blood plasma indicating passage of eGFP-labeled vitamin D-binding protein through a leaky glomerular filtration barrier. By in vivo observation of HPX-injected Cade larvae using 2-PM, we were able to follow the decrease of the eGFP fluorescence in vessels over time and the increase of the fluorescence in the tubular epithelium due to endocytotic uptake of the eGFP-vitamin D-binding protein that had passed the filtration barrier. Analysis of the podocyte-specific proteins nephrin and podocin by RT-PCR and Western blot showed no significant changes. Cross sections of the zebrafish larvae revealed dilated glomerular capillaries. By electron microscopy we found an unchanged morphology of the podocyte foot processes, however the pores of the glomerular endothelium were enlarged.
Conclusion
We found and directly followed by 4D in vivo imaging that injection of HPX into zebrafish larvae induces a leaky glomerular filtration barrier by affecting the glomerular endothelium.

Poster 133:

Titel: Eye abnormalities in 70 genetically engineered mouse lines of the DMDD programme

Autoren/Adressen:
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Abstract:
Deciphering the Mechanisms of Developmental Disorders (DMDD) is a collaborative effort to systematically analyse embryonic lethal and subviable knockout mouse lines. At its core is a comprehensive screen of the morphological phenotype of embryos at E14.5, with all data freely available at https://dmdd.org.uk. Gathering data on embryonic lethal knockouts is a powerful way to gain understanding of the genetic mechanisms that underlie normal organ and tissue development, and to identify mutations that may lead to congenital abnormalities.

Multiple mutant embryos from each line are imaged in 3D at near-histological resolution with the High resolution episcopic microscopy (HREM) technique, and then undergo comprehensive morphological phenotyping.

Almost all embryos exhibit multiple phenotypes, many of which are related to the eye and eye muscles. Analysis of the first 70 lines (562 embryos in total) shows that 42 lines have at least one eye phenotype. The most abundant eye phenotype is ‘abnormal eye muscle morphology’, a term that encompasses many abnormalities of the muscles inside the orbit. These range from abnormal shape of the muscles and their connection to the optic cup, to the absence of single, several, or all muscles. Abnormal form and position of the optic cup, sometimes associated with abnormal optic stalk composition, is frequently diagnosed, as is the absence or dislocation of the lens.

Our results show that DMDD offers a valuable resource to identify mouse lines suited for studying abnormal eye development.

Poster 134:

Titel: Development of the so-called "mandibular symphysis" in humans

Autoren/Adressen: Bianca Prieth (Health University of Applied Sciences Tyrol), Elisabeth Pechriggl (Medical University of Innsbruck), Romana Urbas (Paracelsus Medical University), Michael Blumer (Medical University of Innsbruck), Erich Brenner (Medical University of Innsbruck); erich.brenner@i-med.ac.at

Abstract:
In clinical slang, but also in the TA98, the synostosis in the midline of the body-halves of the human mandible is called “mandibular symphysis”. A symphysis is defined as nonsynovial joint composed of fibrocartilage. In humans, this nonsynovial joint fuses and forms a synostosis within the first year of life. The aim of this study was to investigate if this nonsynovial joint is either a symphysis or a syndesmosis in prenatal life.

Human embryonal and fetal mandibles (11-28 gestational weeks; gw) were investigated by HE-staining, safranin/fast-green-staining and collagen-2-immunohistochemistry.

In gw 11, Meckels cartilages already reached the mental region, separated from each other by a tiny sheet of connective tissue. Initial bone lamellae are situated vestibularly, distinctly separated from Meckels cartilage. With increasing age the bony lamellae grew mesially. At gw 15, a real syndesmosis between the bony mandibular halves developed. This syndesmosis was consistent throughout all later stages, without containing any glycosaminoglycans or collagen 2. At the lingual side, “symphyseal chondriole” (islets of Meckel) could be identified in gw 22, but not later on. They must not be confounded with the cartilaginous anlagen of the mental ossicles, which appeared first in gw 23 at the inside of the developing mandible. In gw 28, the connective tissue also perforated the mesial bone-lamellae of the alveolar process connecting the syndesmosis with the periodontal apparatus of the growing first tooth.

In humans, the nonsynovial joint in-between the developing osseous mandibular halves is a true syndesmosis, at least between gw 11 and gw 28, but not a symphysis.

Poster 135:

Titel: Morphological and morphometrical analyses of pronephric and definitive kidney in Danio rerio regarding nephrogenesis

Autoren/Adressen: Jasmin Hochstuhl (Heidelberg University, Medical Faculty Mannheim), Markus Islinger (Heidelberg University, Medical Faculty Mannheim), Marlies Elger (Heidelberg University, Medical Faculty Mannheim); hochstuhl@stud.uni-heidelberg.de

Abstract:
The renal tubules were investigated in larval (72h), juvenile (26d) and adult (sexually mature) zebrafish. By using light and transmission electron microscopy as well as fluorescence labeled lectins, we established criteria for identification of nephron segments, cell types and their substructure.

The pronephric kidney consists of six segments: neck segment (NS), first proximal tubule (PI), second proximal tubule (PII), early distal tubule (EDT) and late distal tubule (LDT), followed by a short collecting tubule. The nephrons of the definitive kidney showed the same sequence of segments as found in the pronephric kidney. In fully developed nephrons the PII could be subdivided into two parts.
Based on segment location and cellular substructure, in most cases the major function of the segments could be assumed by comparison with physiological data of the respective segments mammals.
Measurements of segment length and epithelial height allowed to classify different developmental stages of the entire nephron and of individual nephron segments.
Nephron anlagen of different developmental stages were found exclusively apposed to the pronephric duct and the collecting duct. The prospective distal end of the anlagen invaded the pronephric duct or the collecting duct, respectively.

This study presents comprehensive morphological data of the tubular substructure of the pronephros and the definite kidney and may serve as a structural basis for the investigations dealing with transport mechanisms and renal disorders in this established model organism.

Poster 136:

Titel: Leflunomide-induced suppression of inflammation and osteoclastogenesis reduced orthodontic tooth movement and associated dental root resorptions in a rat model

Autoren/Adressen: Christian Kirschneck (University Medical Centre of Regensburg), Peter Proff (University Medical Centre of Regensburg); christian.kirschneck@ukr.de

Abstract:
Drugs may inadvertently affect orthodontic tooth movement (OTM). If applied locally at target teeth, they could also potentially aid orthodontic therapy by accelerating or reducing OTM velocity or prevent orthodontically-induced inflammatory dental root resorptions (OIIRR). In this study we investigated possible (side-)effects of the immunosuppressive anti-rheumatic drug leflunomide during OTM.

In three consecutive experiments of 21 animals each (A/B/C), 63 male Fischer344 rats were respectively assigned to three experimental groups (1-3;n=7): (A) CBCT-imaging; (B) histology/serology; (C) RT-qPCR/leflunomide-serology – (1) controls, (2) OTM of the upper left first/second molars, (3) OTM with 15 mg/kg/day p.o. leflunomide medication. After 14 days of OTM, we quantified leukocyte blood counts, serum leflunomide/CRP/IL-6 concentration, relative expression of inflammatory/osteoclast marker genes within the dental-periodontal tissue (RT-qPCR), extent of OIIRR and osteoclast activity (histology) as well as OTM velocity (CBCT, within 14/28 days of OTM).

Bioavailability of leflunomide in blood serum corresponded to therapeutical levels in man. Blood leukocyte counts were significantly reduced in rats treated with leflunomide. OTM-associated increase in serum IL-6/CRP concentration was inhibited as well as locally OTM-upregulated IL-1β/6/8 and CTSK/CLCN7 gene-expression within dental-periodontal tissue. We found significantly less periodontal osteoclast activity during OTM+leflunomide corresponding to a significant reduction in OTM velocity and OIIRR observed.

Leflunomide seems to inhibit OTM-associated pseudo-inflammatory reactions and osteoclastogenesis, resulting in OTM-reduction, but also prevention of undesired OIIRR. Prolonged orthodontic treatment is to be expected under leflunomide medication, while the drug may be suited for pharmacologically-induced tooth anchorage or OIIRR-prevention, if applied locally.

Poster 137:

Titel: Impaired tracheal cilia-driven transport, airway inflammation and balt formation in mice infected with bordetella pseudohinzii

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Abstract:
Characterization of the pathogenic potential of a newly described Bordetella strain which colonizes the respiratory tract of laboratory mice.

Bacterial genome was analyzed using Illumina´s NextSeq 500 next generation sequencing system. Particle transport speed (PTS) and ciliary beat frequency (CBF) were analyzed with a high speed camera and by tracking dynabeads. Cultured tracheal rings were infected with isolated Bordetella pseudohinzii for 4 or 24 h.

Four bacterial isolates from lung and trachea of SPF-kept mice were identified as Bordetella pseudohinzii by genomic sequencing, presenting as rod-shaped coccobacilli with peritrichous flagellae in electron microscopy. Community-acquired infection with B. pseudohinzii lead to decreased number of tracheal ciliated cells (42.4±2; n=8 uninfected to 30.6±1.7% infected; n=5; mean±SEM), reduced CBF (18.21± 0.60; to 12.12±1.48 Hz; n=7) and PTS (baseline: 60.7±2.76; n=19 to 24.51±2.79 µm/s; n=11). B. pseudohinzii-positive animals showed no clinical signs of infection but neutrophils in BAL were highly increased (0.24±0.04 to 14.39±4.6%; n=6). Histopathological analysis revealed tracheitis, interstitial pneumonia, and formation of tertiary lymphoid follicles (bronchus-associated lymphoid tissue = BALT) along the main and secondary bronchi. Scanning electron microscopy of in vitro-infected tracheal rings demonstrated that B. pseudohinzii attaches to ciliated cells, forms biofilms and damages the epithelium.

Colonization of mice with B. pseudohinzii leads to BAL neutrophilia, inflammation of the respiratory tract and impaired mucociliary clearance due to reduction and damage of ciliated cells. B. pseudohinzii may represent a novel mouse specific model organism to study closely related human lung pathogens like Bordetella hinzii and pertussis.

Poster 138:

Titel: HER2 and epidermal growth factor receptor expression - practical tool to characterize invasive urothelial carcinoma of urinary bladder

Autoren/Adressen: Alina Maria Sisu (Victor Babes University of Medicine and Pharmacy), Marius Raica (Victor Babes University of Medicine and Pharmacy), Sorin Lucian Bolintineanu (Victor Babes University of Medicine and Pharmacy), Amalia Raluca Ceausu (Victor Babes University of Medicine and Pharmacy), Valeria Tarlui (Victor Babes University of Medicine and Pharmacy), Anca Maria Cimpean (Victor Babes University of Medicine and Pharmacy); alinasisu@gmail.com

Abstract:
HER2 is the receptor for epidermal growth factor 2, a transmembrane protein, well known in oncology, particularly because of its overexpression in breast cancer. HER2 overexpression induces proliferation, growth and cell survival, and these properties become more evident in tumour cells, including urothelial carcinomas.

There were investigated 27 cases with T2-T4 invasive bladder cancer, and the diagnosis was based on clinical, imagistic, endoscopic and pathological data. All patients underwent radical cystectomy and multiple biopsies.

Cases with score 0 (n=10) highlighted no tumour cells colouring or under 10%, and no intense membrane intensification. Cases with score +1 (n=7) had over 10% coloured tumour cells, reduced intensity, and discontinuous membrane intensification. Cases scored +2 (n=6) showed over 10% of cells membrane intensification, but they had weak or moderate intensity. Cases with score +3 (n=4) had most of cells intense coloured, and a continuous membrane pattern. Out of 27 cases, 17 were negative (score 0 and +1) and 10 were positive (score +2 and +3), 37.037%. Sarcomatoid urothelial carcinomas, squamocellular and clear cells carcinomas were negative. From the adenocarcinomas, 2 were positive, showing HER2 overexpression. EGFR expression was identified in 82.22% of invasive bladder carcinoma cells.

The normal urothelium did not express HER2. In cases with overexpression, the final product of reaction had a membrane pattern, continuous, with strong intensity.Present study was supported by funds kindly provided by Victor Babes University of Medicine and Pharmacy Timisoara, Romania, through P-III-C5-PCFI-2017/2018-03 Internal grant-acronym UROVESSELS.

Keywords: HER2 receptor, overexpression, urothelial carcinoma.

Poster 139:

Titel: Antimicrobial immune proteins in human milk

Autoren/Adressen: Gwendoline K Kueffer (University of Fribourg), Alecia Jane Twigger (University of Western Australia), Donna T Geddes (University of Western Australia), Michael Walch (University of Fribourg), Luis Filgueira (University of Fribourg); luis.filgueira@unifr.ch

Abstract:
Human milk (HM) contains many immune components, including leucocytes in colostrum or during infections of the mother or the child. Immune Proteins, such as perforin (Per), granulysin (Grly) and granzymes (Grzm), expressed in lymphocytes, are known to induce apoptosis of infected host cells and to kill bacteria and parasites. However, the presence these proteins has not yet been shown in HM cells.

Presence of Per, Grly and Grzm(A, B, H and M) was examined in cells isolated from samples of prepartum secretions (PS) and HM collected longitudinally from participants (n=31) during the first year postpartum, including HM samples from 3 women with breast infection. Gene expression was analyzed in RNA sequencing data and further using qRT-PCR. Linear modeling and principle component analysis between the genes was conducted. Flow cytometry (n=5) was performed to investigate the presence of these immune proteins in HM cells.

Gene expression of all immune proteins was confirmed in PS and in HM during month 1 of lactation. Strong correlations were found between granzyme A – perforin (r2=0.86), granzyme A – CD45 (r2=0.76), perforin – CD45 (r2=0.68) and perforin – granzyme B (r2=0.67). Principle component analysis showed a cluster of all immune proteins, in addition to the biggest variation between CD45 and EPCAM. Comparison between healthy and mastitis samples showed a higher expression of CD45 and all immune proteins in mastitis.

The presence of Per, Grly and Grzms has been confirmed in HM cells. An increase in gene expression of these immune proteins has been confirmed in 3 participants suffering from breast infection.

Poster 140:

Titel: Level of CEACAMs in plasma of sepsis patients and its clinical relevance

Autoren/Adressen: Verena Schmitt (Institute of Anatomy - University Hospital Essen), Simon Schäfer (Clinic for Anasthesiology - University Hospital Munich), André Scherag (Clinical Epidemiology - University Hospital), Gunther Wennemuth (Institute of Anatomy - University Hospital Essen), Michael Adamzik (Clinic for Anasthesiology, Intensive Care and Pain Therapy - University Hospital), Bernhard B. Singer (Institute of Anatomy - University Hospital Essen); verena.schmitt@uk-essen.de

Abstract:
Sepsis is a life threatening overreaction of the immune system caused by pathogens. This leads to organ damage or even multi organ failure. Most sepsis infections end deadly because of a lack in earliest diagnostic.

The CEA-related cell adhesion molecule 1 (CEACAM1) is a homophilic cell-cell adhesion molecule expressed on epithelium, endothelium and various leukocyte-subpopulations. It acts as co-modulator of receptors such as T- or B cell receptor and supports proper immune response.

Since membrane anchored CEACAM1 can interact with CEACAM1 as well as with CEACAM6 and CEACAM8 it is tempting to speculate that potentially altered CEACAM-levels in the serum contribute to the overreaction of the immune system during sepsis.

For analyzing the CEACAM1-, 6- and 8-levels in serum we established a Sandwich-ELISA using specific monoclonal mouse antibodies against human CEACAM1, 6 and 8. We analyzed CEACAM-levels of 130 patients that developed sepsis after surgery, 101 post-OP patients that did not developed sepsis after surgery and 77 healthy donors.

In 83,1% of the sepsis patients the CEACAM1-level, in 87,7% the CEACAM6-level and in 23,8% the CEACAM8-level was increased. In 27,7% of the post-OP patients the CEACAM1-level, in 20,8% the CEACAM6-level and in 11,4% the CEACAM8-level was increased.

The CEACAM1 and CEACAM6 serum level is significantly increased in sepsis patients. In some severe cases also CEACAM8 was detected. We conclude that the increased CEACAM-levels could indeed be involved in the overreaction of immune cells during sepsis. Thus our data imply CEACAMs as novel diagnostic and therapeutic target to combat sepsis.

Poster 141:

Titel: Electric stimulation of the medial forebrain bundle is able to modulate prepulse inhibition. A pilot study in Alzheimer´s disease

Autoren/Adressen: Patricia Panther (University Hospital of Magdeburg), Maria Kühne (University Hospital of Magdeburg), Jörn Kaufmann (University Hospital of Magdeburg), Hans-Jochen Heinze (University Hospital of Magdeburg), Andreas Kupsch (Academic Neurology Practice), Tino Zaehle (University Hospital of Magdeburg), Jürgen Voges (University Hospital of Magdeburg), Sven Nullmeier (Ulm University); patricia.panther@med.ovgu.de

Abstract:
Prepulse Inhibition (PPI) is a measurement of sensorimotor gating, which is important to filter out unnecessary environmental information. PPI is known to be modulated by the dopaminergic system. Congruently, PPI deficits are detected in neuropsychiatric disorders showing disturbances within the dopaminergic system like schizophrenia, Parkinson's and Alzheimer´s disease. A target specific influence of deep brain stimulation (DBS) on PPI has been shown in animal models of schizophrenia. Conceivably, stimulation of the medial forebrain bundle or in the ventral tegmental area could influence PPI via modulation of dopamine release.

Three Patients with Alzheimer´s disease underwent DBS of the medial forebrain bundle (MFB) (age 72±2.5, duration of symptoms 5.2±0.7 years) to maintain cognitive abilities. The experimental design was approved by the local ethical committee (University of Magdeburg, Germany; reference numbers 07/12 and 131/13). PPI was tested under four conditions: no stimulation, 20Hz, 60Hz, 130Hz and with an amplitude set below occurrence of side effects and a pulse width of 90µs. Active contacts were selected using tractography of the MFB.

All three patients showed a frequency dependent pattern of PPI with a reduction with reduced or unchanged 20Hz and 130 Hz and an increase of PPI at DBS with 60Hz as compared to sham stimulation.

Our data demonstrate that PPI is modulated by electrical stimulation of the MFB in a frequency-dependent manner. PPI could serve as a potential marker for optimization of DBS settings independent of the patient or the examiner.

Poster 142:

Titel: Pro-inflammatory stimuli induce an NRF2-mediated resolution of inflammation in macrophages

Autoren/Adressen: Athanassios Fragoulis (Uniklinik RWTH Aachen), Johanna Fuchs (Uniklinik RWTH Aachen), Tim Clarner (Uniklinik RWTH Aachen), Thomas Pufe (Uniklinik RWTH Aachen), Christoph Jan Wruck (Uniklinik RWTH Aachen); afragoulis@ukaachen.de

Abstract:
Oxidative stress has been implicated in a variety of inflammatory diseases. Nuclear factor-erythroid 2 (NF-E2)-related-factor 2 (Nrf2) is a transcription factor that maintains the cellular defence against oxidative stress. This study investigates the effects of pro-inflammatory stimuli on the Nrf2/ARE signalling cascade in macrophages.

Nrf2 activation in response to different pro- and anti-inflammatory stimuli was studied via promoter studies using RAW 264.7 cells and primary murine macrophages. Therefore we used LPS, peptidoglykan, zymosan, TNF, IL-1beta and IL-6 as pro-inflammatory, and IL-4 and IL-10 as anti-inflammatory stimuli. Kinase inhibitors, antioxidants and NADPH-oxidase inhibitors were used to elucidate signal transduction. Expression of the Nrf2 target genes HO-1 and NQO1 was studied by qRT-PCR. Western Blot analysis for HO-1 was performed. ROS production in stimulated cells was investigated using the H2DCF-DA and lucigenin reagents. LPS tolerance experiments were conducted.

Treatment of macrophages with pro-inflammatory stimuli but not anti-inflammatory cytokines such as IL-4 and IL-10 showed increased Nrf2 activity. Nrf2 induction was dependent on NADPH-oxidase, ERK and p38 kinase activity. Treatment mediated an increase of HO-1, which is known to act anti-inflammatory due to carbon monoxide production and NF-κB inhibition. LPS tolerance experiments revealed an extensive anti-inflammatory potential of Nrf2 activity.

These data demonstrate an anti-oxidative and especially anti-inflammatory role of Nrf2 during inflammatory processes. Pro-inflammatory stimuli seem to promote the resolution of inflammation at an early stage of acute inflammation by Nrf2 induction. The inhibitor studies revealed ROS as a potential signalling molecule in this Nrf2 activation cascade.

Poster 143:

Titel: IL-6 promotes alternative activation and local proliferation of adipose tissue macrophages in obesity

Autoren/Adressen: Julia Braune (University of Leipzig), Ulrike Weyer (Leipzig University), Constance Hobusch (Leipzig University), Jan Mauer (Weill Cornell Medical College, Cornell University), Jens Brüning (Max Planck Institute for Metabolism Research), Ingo Bechmann (Leipzig University), Martin Gericke (Leipzig University); julia.braune@medizin.uni-leipzig.de

Abstract:
In this study, we aimed at identifying drivers of alternative activation and local proliferation of adipose tissue macrophages (ATMs) to positively alter the chronic low-grade inflammation of white adipose tissue (AT) in obesity.

Therefore, we established organotypic cell cultures of murine AT explants to study the impact of cytokine treatment and neutralizing antibodies on local ATM proliferation without the bias of early monocyte recruitment. Furthermore, gene expression analysis was used to identify cytokines and their putative receptors as potential micro-environmental cues regulating ATM proliferation in obesity.

We showed that Th2 cytokines enhance local proliferation of ATMs and polarize them toward the M2 phenotype. In contrast, stimulation with pro-inflammatory cytokines promotes M1 polarization and decreases ATM proliferation. Furthermore, IL-4 and IL-13 seem to be the potential drivers of local M2 proliferation in obese mice in vivo. Interestingly, we identified IL-6 as important stimulus for ATM proliferation in obesity, presumably due to upregulation of IL-4Rα.

Because IL-6 has been shown previously to increase systemic glucose tolerance and insulin sensitivity, the role of IL-6 as a pro-inflammatory cytokine and its prognostic value in obesity need to be reconsidered. Therefore, IL-6 or the IL-4 receptor could be potential targets to sustain insulin sensitivity in obesity.

Poster 144:

Titel: Hedgehog signaling in myeloid cells impacts on body weight, adipose tissue inflammation and glucose metabolism

Autoren/Adressen: Julia Braune (Leipzig University), Ulrike Weyer (Leipzig University), Madlen Matz-Soja (Leipzig University), Constance Hobusch (Leipzig University), Matthias Kern (Leipzig University), Anne Kunath (Leipzig University), Nora Klöting (Leipzig University), Susann Kralisch (Leipzig University), Matthias Blüher (Leipzig University), Rolf Gebhardt (Leipzig University), Yana Zavros (University of Cincinnati), Ingo Bechmann (Leipzig University), Martin Gericke (Leipzig University); martin.gericke@medizin.uni-leipzig.de

Abstract:
Recently, hedgehog (Hh) was identified as a crucial player in adipose tissue (AT) development and energy expenditure. Therefore, we tested whether Hh ligands are regulated in obesity. Further, we aimed at identifying potential target cells of Hh signaling and studied the functional impact of Hh signaling on AT inflammation and glucose metabolism.

Hh ligands and receptors were analyzed in AT or serum from lean and obese mice as well as in humans. To study the impact on AT inflammation and glucose metabolism, Hh signaling was specifically blocked in myeloid cells using a conditional knockout approach (LysMCre/SmoKO).

Desert Hh and Indian Hh are local Hh ligands, whereas Sonic Hh is not expressed in AT from mice and men. In mice, obesity leads to a preferential up-regulation of Hh ligands and signaling components in subcutaneous AT. Further, AT macrophages are Hh target cells due to expression of Hh receptors. Conditional knockout of SMO (a mandatory Hh signaling component) in myeloid cells increases body weight, AT inflammation and impairs glucose tolerance, suggesting an anti-inflammatory effect of Hh signaling. In humans, Hh ligands decrease with obesity and type 2 diabetes, which might be explained by the intake of metformin.

Hh signaling in myeloid cells affects AT inflammation and glucose metabolism and may be a potential target to treat type 2 diabetes.

Poster 145:

Titel: Immunolocalization of DMRTB1 in human testis showing normal and impaired spermatogenesis

Autoren/Adressen: Erika Hilbold (University of Veterinary Medicine Hannover, Foundation), Martin Bergmann (Justus Liebig University), Sabine Kliesch (University Hospital Münster), Wolfgang Weidner (Justus Liebig University), Marion Langeheine (University of Veterinary Medicine Hannover, Foundation), Kristina Rode (University of Veterinary Medicine Hannover, Foundation), Ralph Brehm (University of Veterinary Medicine Hannover, Foundation); ralph.brehm@tiho-hannover.de

Abstract:
The transcription factor DMRTB1 plays a decisive role in coordinating transition between mitosis and meiosis in murine germ cells (GC). Thus, the present study aimed to examine the testicular expression pattern of DMRTB1 in men showing normal spermatogenesis (nsp) and different testicular disorders, such as germ cell neoplasia in situ (GCNIS).

Immunohistochemistry was performed using 37 testicular biopsy specimens and a rabbit polyclonal Anti-DMRTB1 primary antibody (1:100, HPA036490, Sigma-Aldrich).

In patients with nsp, a strong nuclear immunostaining was detectable in a subset of spermatogonia (predominantly type B spermatogonia), adjacent Sertoli cells (SC) were found immunonegative. In GCNIS-tubules, pre-invasive tumor cells showed no immunostaining, and seminoma cells were also immunonegative for DMRTB1. In patients with a SC-only (SCO) syndrome, no immunoreactivity was detected in SC.

Our data indicate DMRTB1 expression in some spermatogonia in men showing nsp. According to previous findings in mice it seems reasonable that DMRTB1 is only expressed in these GC and that DMRTB1 plays a similar role in men. Furthermore, absence of DMRTB1 in GCNIS cells and tumor cells might be associated with neoplastic cell proliferation and progression into invasive GC tumors. This in turn supports the assumption of impaired expression profiles of pro-meiotic and anti-meiotic factors in GCNIS cells and is consistent with data that preinvasive cells and tumor cells do not complete meiosis and seem to be incapable of progressing through meiotic prophase I.

Poster 146:

Titel: Digital holographic microscopy as a potential method for fertility diagnostic

Autoren/Adressen: Caroline Wenders (University Clinic Essen), Michael Muschol (University Clinic Essen), Prof. Dr. med. Gunther Wennemuth (University Clinic Essen); Caroline.Wenders@uk-essen.de

Abstract:
The commonly used method to analyze sperm swimming behavior and flagellar waveform is CASA (Computer assisted sperm analysis). We performed comparative measurements between 2D CASA and 4D digital holographic microscopy (DHM) to demonstrate the benefit of high speed holographic imaging.
Sperm chemotaxis in mammals has been identified towards several female sources as the cumulus oophorus (CO) or the oocyte. Initially, high speed holographic imaging demonstrates the influence of oocyte and CO to sperm movement in 4D.

Murine sperm from NMRI mice and human sperm from 3 healthy donors were purified and incubated into physiological buffer at 37°C. CASA and DHM were used to record sperm swimming paths in 4 dimensions. Murine oocytes from NMRI mice were obtained after superovulation.

DHM allows 4D (XYZ-plane and t) tracking of the head of free-swimming sperm. The comparison between CASA and DHM demonstrates the benefit of high speed holographic imaging allowing the identification of sperm with minimal XY- movement and a high Z-excursion as motile sperm. Additionally, different motility parameters of sperm are measured in the presence of the oocyte and/or the CO addict to the capacitation status. Capacitated sperm showed a higher amplitude, asymmetrical flagellar bending, which was compared with higher rates in their curvilinear velocity. Also larger XY-excursions of the tracked head were detected.

The comparative measurements of 2D CASA and 4D DHM demonstrate DHM as a powerful extension with clinical relevance. 4D movement analysis identifies sperm with the opportunity to change their swimming behavior, which enables it to fertilize the oocyte.

Poster 147:

Titel: Effect of ovarian steroid hormones and human chorionic gonadotropin on human endometrial epithelial cells in a 3D confrontation system

Autoren/Adressen: Matthias Thomas Koczy (RWTH Aachen University), Volker Uwe Buck (RWTH Aachen University), Florian Streuter (RWTH Aachen University), Benjamin Rösing (RWTH Aachen University), Joseph Neulen (RWTH Aachen University), Irmgard Classen-Linke (RWTH Aachen University); iclassen-linke@ukaachen.de

Abstract:
Throughout the menstrual cycle human endometrial epithelial cells (EECs) undergo hormonally regulated differentiation which leads to a short receptive period called window of implantation (WOI). In this time frame the trophoblast can invade the endometrium. Since functional studies on human implantation are not possible, we use an in vitro model to generate gland-like endometrial structures. Aim of this study is to analyse the influence of hormonal stimulation on EEC spheroids to facilitate trophoblast invasion.

To elucidate basic mechanisms of early human implantation we studied trophoblast-endometrial interaction by confronting gland-like spheroids of an endometrial adenocarcinoma cell line (Ishikawa) with an extravillous trophoblast cell line (AC-1M88). We further studied the effect of ovarian steroid hormones and human chorionic gonadotropin (hCG) on primary EECs from scratch biopsies of women undergoing assisted reproductive technology (ART).

As we could show previously, EEC cell line spheroids can be used as a model for EECs during the WOI in vivo. Using confocal microscopy we could visualise EEC junction redistribution and penetration of the extravillous trophoblast. Supplementation of our 3D confrontation culture with ovarian steroid hormones and hCG changes junctions distribution. This hormone supplementation may have an effect on EEC differentiation in our in vitro test system facilitating trophoblast invasion.

Results from this study provide us with useful information to optimise our 3D invasion assay and may possibly lead to adjustment of clinical stimulation protocols. We expect to develop a potential clinical test system for endometrial receptivity and hormonal treatment of ART patients.

Poster 148:

Titel: Segments of the epididymis show differences in the sialylation status of spermatozoa and in the contractile pattern of the epididymal duct

Autoren/Adressen: Vera Elfgen (Justus-Liebig-Universität), Thorben Hau (Justus-Liebig-Universität), Christina Galuska (Leibniz-Institut für Nutztierbiologie), Andrea Mietens (Justus-Liebig-Universität), Sebastian Galuska (Leibniz-Institut für Nutztierbiologie), Ralf Middendorff (Justus-Liebig-Universität);
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Abstract:
Objective:
Additionally to the classical blood-epididymal barrier, which is located in the epididymal epithelium, we found a compartmentation of the interstitial and the luminal space that goes beyond the established concepts.
During transport in the epididymal duct spermatozoa pass unique segments, separated by connective tissue septa (CTS). In rat, for example, 19 segments could be defined. Spermatozoa are exposed to varying microenvironments along the length of the duct to ensure maturation. In the present study we investigated potential differences between segments of the epididymal duct (i) in sialylation status of spermatozoa which is necessary for sperm survival within the female genital tract (ii) and in contractility.
Methods:
Borders between segments of the rat epididymis were visualized by CLARITY. Segmental contractile patterns were analysed by time-lapse imaging. Sialylation status of spermatozoa in each segment was quantified by high-performance-liquid-chromatography of samples collected by laser-capture-microdissection.
Results:
The three-dimensional arrangement of CTS between segments could be revealed by CLARITY. Sialylation of spermatozoa in the lumen of the epididymal duct showed differences between the majorities (1vs.2, 3vs.4, 4vs.5, 5vs.6, 7vs.8, 10vs.11, 12d vs.13, 13vs.14, 14vs.15, 15vs.16) of neighbouring segments, but not between all neighbouring segments. Differences of these molecular characteristics of spermatozoa in the single segments were shown to be often accompanied by differences in the contractile pattern of the duct.
Conclusion:
Data suggest that segmentation of the epididymis also includes differences in the molecular characteristics of spermatozoa and the contractile pattern of the epididymal duct.

Poster 149:

Titel: Visualization of PDE5 inhibitor effects in prostate tissue: no evidence for disturbances of ejaculation

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Abstract:
Benign prostate hyperplasia (BPH) is characterized by an enlargement of prostate tissue associated with increased tone of smooth muscle cells (SMCs). Inhibitors of phosphodiesterase type 5 (PDE5) such as sildenafil relax SMCs and have emerged as alternative treatment options. The prostate consists of single glands which produce the prostatic fluid. Excretory ducts transport the fluid to the urethra during the emission phase of ejaculation. Structural and functional data of glands and ducts as well as information on the response to drugs are sparse. Analyses of PDE5 inhibitors on contractility of prostatic ducts have never been performed.

The combination of time-lapse imaging, to examine the effects of PDE5 inhibitors on prostatic ducts, with immunohistochemistry allowed the characterization and functional assessment of SMCs in intact isolated prostate ducts.

PDE5 was localized in SMCs associated with prostate ducts. Effects of PDE5 inhibition on contractile function were directly visualized by time-lapse imaging. Excretory ducts, in contrast to glands, do not contract spontaneously. Imitating the contribution to ejaculation by application of noradrenaline, we found that predominantly ductal SMCs ensure expulsion of secretions. Noradrenaline-induced contractions of the isolated duct segments occurred irrespective of sildenafil pre-treatment and in both cases, the effect of noradrenaline was the same.

Data suggest that PDE5 inhibitors do not disturb prostate secretion during ejaculation.

Poster 150:

Titel: Assessment of human endometrial receptivity for assisted reproductive technology - combining functional in vitro studies with biomarkers on human biopsies

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Abstract:
The window of implantation (WOI) is a short phase during the menstrual cycle in which the human endometrium is receptive to the embryo. As shown previously, the distribution of adhering junction proteins of endometrial epithelial cells (EECs) was altered during the WOI possibly facilitating the penetration of EECs by trophoblast cells (TCs). The aim of this study was to combine functional in vitro experiments with detecting biomarkers for receptivity to individually assess tissue samples from patients undergoing assisted reproductive technology (ART).

Functional characterisation of EEC receptivity was performed using a 3D trophoblast-endometrial confrontation culture system (TC-EEC assay). Adenocarcinoma EEC line spheroids and primary EECs from endometrial scratch biopsies of ART patients were co-cultured with invasive extravillous TCs (AC-1M88). TC-EEC interaction was evaluated by confocal and light sheet microscopy. To identify biomarkers for personalised diagnostics in ART the expression of different cycle depending proteins was immunohistochemically examined on ART scratch biopsies and archival specimens throughout the menstrual cycle.

In TC-EEC assays the strongest invasion occurred on EEC spheroids that showed a junction distribution similar to EECs during the WOI in vivo. Under the given culture conditions close interaction of TCs with primary EECs from WOI samples, characterised by our biomarkers, was observed.

By combining a functional TC-EEC in vitro assay with a set of biomarkers on patient samples we expect to get new insights into embryo-endometrial interaction in humans in order to improve the outcome of ART in the future.

Poster 151:

Titel: Spontaneous contractions of the immature epididymal duct: they drive directional movement of exfoliated epithelial cells

Autoren/Adressen: Daniela Beyer (Justus-Liebig-University Gießen), Andrea Mietens (Justus-Liebig-University Gießen), Dieter Müller (Justus-Liebig-University Gießen), Davor Ježek (Medical Faculty University of Zagreb), Gerhard Schuler (Justus-Liebig-University Gießen), Ralf Middendorff (Justus-Liebig-University Gießen); daniela.beyer@anatomie.med.uni-giessen.de

Abstract:
Contractions of the adult epididymal duct are well known in the context of sperm transport. Some reports also described contractions of the epididymal duct during development, but data about their character, regulation and function are sparse.

Luminal content of the immature epididymal duct was characterized by classical morphological methods and live microscopy of isolated tissue ex vivo. Movement of the luminal content and contractility of the wall of the duct were evaluated by Time-lapse imaging (Mietens et al. 2014).

Cellular structures in the lumen of the human prenatal epididymal duct were identified as exfoliated epithelial cells originating from the epididymis but not from the testis. These cells were also found in the rat epididymis after birth. Time-lapse imaging revealed directional movement of the luminal cells. The smooth muscle cell contracting agent noradrenaline accelerated the directional movement, while the relaxing drug sildenafil decelerated it. These effects on the transport correlated with contractions of the ductal smooth muscle cell layer. As shown above for the transport of cells, contractile frequency was also increased by noradrenaline and decreased by sildenafil. Systematic analyses revealed comparable contraction patterns in immature and adult epididymal duct isolated from caput, corpus and cauda.

Our data suggest organized waste disposal in the epididymal duct which might be important during development to avoid infertility by luminal obstruction as seen in cystic fibrosis patients.

Poster 152:

Titel: NK cell dysfunction is associated with an increased colon cancer incidence in obesity

Autoren/Adressen: Ina Bähr (Martin Luther University Halle-Wittenberg, Faculty of Medicine), Julia Spielmann (Martin Luther University Halle-Wittenberg, Faculty of Medicine), Heike Kielstein (Martin Luther University Halle-Wittenberg, Faculty of Medicine); ina.baehr@medizin.uni-halle.de

Abstract:
Natural killer (NK) immune cells control tumor progression and metastases. Previous studies showed altered NK cell functions in obese individuals. Obesity is associated with a higher colon cancer incidence, but underlying mechanisms remained unclear. We investigated the impact of an altered NK cell functionality on the increased colon cancer risk in obesity.

Cytotoxicity, expression of activating NK cell receptors and cytokine secretion of human NK cells co-incubated with human colon tumor cells was determined with or without the influence of the adipocytokine leptin. Moreover, in vivo experiments in normal weight and obese mice and rats were performed. In rats, colon cancer growth was induced in half of the animals by injection of azoxymethane (AOM). NK cell parameters in peripheral blood, spleen and liver as well as in colon tumor tissue were analyzed by real-time RT-PCR, FACS analyses and immunohistochemnistry.

In vitro investigations demonstrated a decreased IFN-GAMMA secretion and cytotoxicity of human NK cells against colon tumor cells after NK cell preincubation with leptin. In addition, leptin incubation decreased the expression of activating NK cell receptors. In obese animals, the number of NK cells as well as the expression of activating NK cell receptors in peripheral blood, spleen and liver was lower compared to the normal weight animals. This correlated with an increased quantity, size and weight of colon tumors after AOM-treatment in obese rats.

The results showed that the decreased NK cell function may be one reason for the higher colon cancer risk in obesity.