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Poster 1:

Title:
Derivatives of the posterior arch of the Proatlas: Ligamentum condylicum posterius and related osseous structures at the human craniocervical junction

Authors:
Michael Wolf-Vollenbröker (Institut für Anatomie 1, Universitätsklinikum Düsseldorf, Düsseldorf), Andreas Prescher (Institut für Molekulare und Zelluläre Anatomie, Universitätsklinikum Aachen, Aachen); wolfvoll@hhu.de

Abstract:
A neglected ligamentous structure of the craniocervical junction (CCJ), the Ligamentum condylicum posterius (LCP), was examined in a dissection and maceration series and related to the manifestation of an osseous variation of the human occipital bone, the Processus condylicus posterior (PCP). This aimed to investigate whether the LCP is a regular part of the CCJ and whether LCP and PCP originate from tissue derived from the material of the dorsal arch of the Proatlas, a rudimentary vertebra between occipital bone and atlas.

An anatomical dissection and maceration of 50 fresh frozen human craniocervical junctions, including axis, atlas and occipital bone with subsequent measurement and photographic documentation was performed.

The examined ligament was found in 98% of all cases. Bony spurs or elongated ossicles located in the ligament were found at the insertion points on the occipital bone. In two cases, the bony formation of a PCP (4 %) was found, which enabled further investigations of the anatomy of the ligament in these cases. Additionally, various other osseous Proatlas-manifestations have been found, which served to classify and interpret the findings of the much rarer PCP.

Concluding, the LCP can be seen as (1) a part of the normal anatomy of the craniocervical junction and (2) a precursor structure of the osseous process, a common descent from dorsal Proatlas material is assumed.

As a result of this preparation series and a literature review, a typology of manifestations of the LCP, the PCP, and related structures was introduced.

Poster 2:

Title:
The Trochlea of the Superior Oblique Muscle – Anatomic Landmarks for its Localization

Authors:
Christoph Fuchssteiner (Center for Anatomy and Cell Biology, Medical University of Vienna, Vienna), Raffaela Morgenbesser (Center for Anatomy and Cell Biology, Medical University of Vienna, Vienna), Lena Hirlter (Center for Anatomy and Cell Biology, Medical University of Vienna, Vienna); christoph.fuchssteiner@meduniwien.ac.at

Abstract:
Operative approaches in the fields of neuro- and otolaryngological surgery require reliable anatomic landmarks to facilitate navigation. In general, osseous structures are among the preferable anatomic landmarks due to their easy detection. As such, the trochlea of the superior oblique muscle might be useful as an anatomical navigational aid for surgical approaches to the medial orbital wall and endoscopic approaches to the anterior cranial fossa.
However, a detailed knowledge of its exact anatomic localization is still missing. Therefore, the main objective of this study was to identify novel reliable landmarks for the precise localization of the trochlea.

25 anatomical fresh-frozen head and neck specimens were used. The orbital region was dissected bilaterally. The Glabella, the nasion and the lacrimal caruncle were defined as external landmarks, while the superior orbital fissure as well as the anterior and posterior ethmoidal foramen were defined as internal landmarks. Resulting distances were measured and analyzed statistically.

The nasion (v=0.085) and the superior orbital fissure (v=0.085) proved to be the most reliable landmarks. The distance along the medial orbital wall up to the superior orbital fissure (v=0.076) appeared to be even more reliable.

This study has identified multiple, novel and reliable landmarks for the localization of the trochlea. Furthermore, an in depth insight into the anatomical relations of the trochlea has been provided. The presented data might facilitate preoperative planning of surgical approaches in the fields of neurosurgery and ENT surgery.

Poster 3:

Title:
Propriosensors in the parametries

Authors:
Maya Friederich (Institut of Anatomy 1, Medical Faculty & University Hospital, Heinrich Heine University, Düsseldorf, Düsseldorf), Tanja Fehm (Clinic for Gynecology and Obstetrics, Medical Faculty & University Hospital, Heinrich Heine University, Düsseldorf, Düsseldorf), Timm Filler (Institute of Anatomy 1, Medical Faculty & University Hospital, Heinrich Heine University, Düsseldorf, Düsseldorf); timm.filler@hhu.de

Abstract:
Contrary to the importance of perception and control of internal processes, knowledge of the corresponding sensors is poor. There are practically no substantial publications for the female pelvis, which is, to put it mildly, astonishing because of the connection between proprioception and the pain-processing system. The presented study aims to record the proprioceptors in the area of parametria morphologically.

We examined samples from ten body donors on predetermined locations at the parametria through histo- and immunohistochemical methods. Type, distribution, morphology, and special features were determined, assessed comparatively, and statistically evaluated.

For the first time, Ruffini-like bodies could be identified in the area of the internal female genital organs. They differ from the Ruffini corpuscles of the musculoskeletal system in vascularization, association with smooth muscle cells, and distribution of elastic fibers. There is an apparent left-dominant side asymmetry.
We did not observe Vater-Pacini-, Golgi-Mazzoni-, Dogiel-bodies, or Krause's end bulbs.

The concept that the internal female genital organs may use their supplying connective tissue for mechanoreception analogous to that of the musculoskeletal system was confirmed. By this, the supporting ligaments come into question as noteworthy pain generators. We found evidence that the movements of the organs primarily explain the localization. Based on our findings, the left-sided dominance has a rationale in the vascular supply. A possible connection to the lateralization of endometriosis in the left hemipelvis is part of the ongoing work. The age of the body donations limits the significance of the results.

Poster 4:

Title:
The antebrachial interosseous membrane exhibits a gender- and function-specific distribution of mechanoreceptors

Authors:
Anne-Marie Kamp (Institut of Anatomy 1, Medical Faculty & University Hospital, Heinrich Heine University, Düsseldorf, Düsseldorf), Philipp Albrecht (Department of Neurology, Medical Faculty & University Hospital, Heinrich Heine University, Düsseldorf, Düsseldorf), Timm Filler (Institut of Anatomy 1, Medical Faculty & University Hospital, Heinrich Heine University, Düsseldorf, Düsseldorf); timm.filler@hhu.de

Abstract:
The objective of this study was to explore the presence of proprioceptors at the antebrachial interosseous membrane (IM) and muscle attachments. Here we report on the particular findings of Golgi-Mazzoni (GM) and Vater-Pacini corpuscles (VP).

Both IMs with muscle attachments from six donors, three female and three male, were dissected. Then, three one-centimeter samples were excised: proximally, from the middle portion and distally. Finally, short serial sequences of sections in different histochemical stains were scanned and analyzed.

Twelve clusters of GM corpuscles and 16 VP corpuscles were identified. GM structures were found in eight samples of four donors (3 F and 1 M). Three specimens were from the right, five from the left extremity, five were proximally, and three distally. VP corpuscles were detected in ten samples of four donors (3 F and 1 M), three specimens were from the right, six from the left extremity, four samples were proximally located, three in the middle, and three distally.

Proprioception is partially involved in pain generation. We detected a pronounced topographical distribution of GM and VP structures in the antebrachial interosseous membrane. Since the complex regional pain syndrome (CRPS) shows a clear female predominance, it is of particular interest that GM or VP were predominantly observed in women while, in contrast, corpuscles were rarely identified in men, who usually report a different CRPS pain sensation than women. However, the age and amount of the body donors limit the conclusions of the study.

Poster 5:

Title:
Usability of abattoir-acquired pig eyes for refractive excimer laser research

Authors:
Marius Topka (Institute of Functional and Clinical Anatomy, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen), Christian M. Hammer (Institute of Functional and Clinical Anatomy, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen), Antonia Bock (, WaveLight GmbH, Erlangen), Peter Riedel (, WaveLight GmbH, Erlangen), Johannes Lörner (, WaveLight GmbH, Erlangen), Alexander Hammer (Department of Neurosurgery, Paracelsus Medical University, Nürnberg), Eva Maier (Dental Clinic 1, Operative Dentistry and Periodontology, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen), Friedrich Paulsen (Institute of Functional and Clinical Anatomy, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen), Yao Zhang (, WaveLight GmbH, Erlangen);
marius.topka@fau.de

Abstract:
The purpose of this study was to elucidate, under which conditions abattoir-acquired pig eyes are suitable for refractive excimer laser experiments and how different scalding methods, including no scalding at all might influence ablation attempts.

Porcine eyes from tunnel-scalded (n = 5) and tank-scalded (n = 10) pigs were compared to unscalded eyes (n = 5) and to eyes scalded in the laboratory (n = 5). The corneal epithelium was manually removed before an excimer laser was used to perform a -8.0 D photoablation. Corneal thickness was measured by optical coherence topography before and after photoablation. The ablation depth was determined with a contour measuring station, the morphology of the ablated areas was characterized by scanning electron microscopy and white-light interferometry.

The scalded eyes showed an increase in corneal swelling which gained statistical significance in tank-scalded eyes, showing a wedge-shaped opaque stromal lesion in the nasal corneal quadrant. A measurable deterioration of photoablation was only found in tank-scalded eyes that exhibited the opaque lesion. Ablated area morphology was smooth and regular in the unscalded and tunnel-scalded eyes. The tank-scalded eyes showed conspicuous wrinkles.

While unscalded eyes should always be preferred for excimer laser laboratory experiments if avaiable, the data suggest that the use of tunnel-scalded eyes may also be acceptable and should be chosen over tank-scalded eyes.

Poster 6:

Title:
The sacral preauricular extension in modern humans and its associated soft tissues

Authors:
Barbara Maurer-Gesek (Anatomy, Medical University of Vienna, Vienna), Doris Pany-Kucera (Anthropology, Natural History Museum, Vienna), Michaela Spannagl-Steiner (Anthropology, Natural History Museum, Vienna), Wolfgang J Weninger (Anatomy, Medical University of Vienna, Vienna); barbara.maurer-gesek@meduniwien.ac.at

Abstract:
This study builds on the ERC funded VAMOS project, which aimed to analyse motherhood in prehistory. In a paper resulting from this project, a recently new anatomical feature in prehistoric pelves was described, which was termed as sacral preauricular extension. It represents an osseous protrusion of the sacrum at the level of the terminal line and is likely to be associated with pregnancy and childbirth. In this study we aimed at identifying this extension in pelves of modern humans and at examining the soft tissues associated to it.

32 pelvis specimens (50% male and 50% female) were included. All were subjected to computed tomography (CT), then carefully dissected and afterwards macerated. The dimensions of the anterior sacroiliac ligament in specimens with and without extension were measured.

In the CT-data a total of five sacral preauricular extensions were identified. Analysis of the macerated bones confirmed this diagnosis. Extensions were solely found in females (1 specimen bilateral, 3 specimens unilateral right). The mean height of the triangular part of the anterior sacroiliac ligament at the level of the terminal line was significantly larger in females than in males, regardless of whether an extension was diagnosed or not.

Our study provides first evaluations of osseous pelvic features linked with childbirth and pregnancy in modern humans. It further provides information on their relationship with soft tissues, in particular the anterior sacroiliac ligament and discusses the effect of biomechanical forces on the genesis of the sacral preauricular extension.

Poster 7:

Title:
Collecting material of the upper airways – is there a danger for the brain?

Authors:
Paata Pruidze (Division of Anatomy, Medical University of Vienna, Vienna), Plamena Mincheva (Division of Anatomy, Medical University of Vienna, Vienna), Jeremias Weninger (Division of Anatomy, Medical University of Vienna, Vienna), Lukas Reissig (Division of Anatomy, Medical University of Vienna, Vienna), Andreas Hainfellner (Division of Anatomy, Medical University of Vienna, Vienna), Wolfgang Weninger (Division of Anatomy, Medical University of Vienna, Vienna); andreas.hainfellner@meduniwien.ac.at

Abstract:
Nasopharyngeal and nasal swabs collect material for diagnosing diseases such as Covid-19 and others, which affect the respiratory system. Sceptics of Covid-19 testing quite often argue that during the collection of material, the tips of swabs might penetrate the cribriform plate of the ethmoid bone and destroy basal parts of the forebrain. We therefore set out to examine how swabs must be inserted into the nasal cavity to target the cribriform plate.

Metal probes were inserted via the nares into 94 nasal cavities of mediansagittally cut head specimens. They were pushed forward until their tips touched the cribriform plate. The insertion angles and depths were photo-documented and measured.

The angle between the probe inserted to touch the cribriform plate and the subnasale/nasion line measured 36.7° (29.5-48); the angle in respect to the subnasale/tragus line -35.4° ((-45.5)-(-25.5°)). The average distance between the posterior lower rim of the nares and the cribriform plate measured 6.1 cm (5.0-7.7).

Our data demonstrate that the danger for damaging the cribriform plate with swabs is impossible if collecting nasopharyngeal material and following the guidelines for performing nasopharyngeal swabs. When following the guidelines for collecting material from the nasal cavity, swabs might be directed towards the cribriform plate. However, if pushed moderately forwards for less than 5 cm, the tip does not touch or penetrate it.

Poster 8:

Title:
The role of cathepsin B and mechanical stress in the development of Focal Segmental Glomerulosclerosis

Authors:
Makhabbat Saudenova (Anatomy, Christian Albrechts University Kiel, Kiel), Jessica Promnitz (Anatomy, Christian Albrechts University Kiel, Kiel), Felix Kliewe (Anatomy and Cell Biology, Universitätsmedizin Greifswald, Greifswald), Nicole Endlich (Anatomy and Cell Biology, Universitätsmedizin Greifswald, Greifswald), Franziska Theilig (Anatomy, Christian Albrechts University Kiel, Kiel); m.saudenova@anat.uni-kiel.de

Abstract:
Focal Segmental Glomerulosclerosis (FSGS) is one of the most common causes of nephrotic syndrome (NS) characterized by proteinuria, edema, hyperlipidemia, hypoalbuminemia and hypertension. FSGS is characterized morphologically by sclerotic lesions in a subset of glomeruli. The mechanisms underlying glomerular damage and disease progression are not completely understood and are the aim of this work.

The FSGS mouse model combined with cathepsin B (CTSB)flox or CTSB KO were characterized at different time points. Renal function analysis, western blot (WB), histological analyses were performed. Additionally, immortalized mouse podocytes were used for RT-PCR, WB and immunohistochemistry analyses following the application of mechanical stretch.

FSGS animals with CTSB deletion in podocytes, as well as in the whole body showed significantly reduced glomerular damage as well as the appearance of NS hallmarks, such as proteinuria and hypertension compared to the respective controls. Morphological analyzes of kidney sections of FSGS and wild type groups revealed augmented number of lysosomes in podocytes of FSGS mice already at day 9 (+104%) as well as at later time points day 17 and 28 (+185% and +187%*, *$P < 0.05$) after induction. Moreover, immunohistochemistry analyses revealed increased TFEB, a master regulator of lysosomal biogenesis, and Galectin 3, lysosomal damage marker, in podocytes of FSGS group compared to control. In addition, immortalized podocytes showed an increase in lysosomal marker LAMP1 (+135%) 5 days after mechanical stress induction.

We conclude that CTSB deletion prevents proteinuria and glomerular damage during FSGS development and that lysosomal biogenesis and – damage precedes podocyte injury and loss.

Poster 9:

Title:
Proprioceptive supply of thoracolumbar fascia

Authors:
Robert Terzis (Institute of Anatomy 1, Medical Faculty & University Hospital Düsseldorf, Heinrich Heine University, Düsseldorf), Richard Bostelmann (Neurosurgery, Christliches Krankenhaus Quakenbrück gGmbH, Quakenbrück), Christoph Eichhorn (, Praxiszentrum Orthopädie-Unfallchirurgie Nordrhein, Aachen), Timm Filler (Institute of Anatomy 1, Medical Faculty & University Hospital Düsseldorf, Heinrich Heine University, Düsseldorf); timm.filler@hhu.de

Abstract:
In industrialized societies, up to 85% of the population experience low back pain at least once in their lifetime. Diagnostic and therapy focus does not include the thoracolumbar fascia, the largest aponeurosis in the human body. To clear the question of clinicians whether this complex formation could be a pain generator, we performed a systematic analysis on a histological level.

We examined samples from ten body donors on six predetermined locations on the lumbar spine region at the level of L4 and L5 through histo- and immunohistochemical methods, i.e., antibodies against pNFM, Tyrosine Hydroxylase, and PGP9.5, for qualitatively and quantitatively analysis on neuronal structures with an image processing software.

Besides apparent interindividual differences in the shaping and compactness of thoracolumbar fascia, we unmasked in all specimen Golgi tendon organs and a pronounced network of nerve fibers. We also found Ruffini bodies close to and within the fascia. Fibers of the autonomic nervous system accompanied these findings.

The described findings prove that this aponeurosis is vital to transmitting information about position and movement for a large region and inserting musculoskeletal structures. Considering that the proprioceptive system is involved in pain pathways, the thoracolumbar fascia may also work as a possible pain generator in the lower back. In addition, we suggest a nociceptive role by the detected fibers of the autonomic nervous system. Irritation of the system could also lead to wrong posture. Therefore, we think therapists should include the thoracolumbar fascia in the algorithm of low back pain.

Poster 10:

Title:
Fallopian tube morphometry based on x-ray microtomography and histology – implications for a stent-based treatment of proximal re-stenosis

Authors:
Jonas Keiler (Institute of Anatomy, Rostock University Medical Center, Rostock), Jens Runge (Institute of Anatomy, Rostock University Medical Center, Rostock), Ewelina Malanowska (Department of Gynecology, Endocrinology, Gynecologic Oncology, Pomeranian Medical University Szczecin, Szczecin), Rebekka Einenkel (Department of Gynecology and Obstetrics, University Medicine Greifswald, Greifswald), Bastian-Jesper Klussmann-Fricke (, Institute for ImplantTechnology and Biomaterials e.V., Rostock), Stefan Siewert (, Institute for ImplantTechnology and Biomaterials e.V., Rostock), Niels Grabow (Institute for Biomedical Engineering, Rostock University Medical Center, Rostock), Klaus-Peter Schmitz (, Institute for ImplantTechnology and Biomaterials e.V., Rostock), Markek Zygmunt (Department of Gynecology and Obstetrics, University Medicine Greifswald, Greifswald), Andreas Wree (Institute of Anatomy, Rostock University Medical Center, Rostock); jonas.keiler@med.uni-rostock.de

Abstract:
About 25% of cases of female infertility are assumed to be caused by an occlusion or stenosis of the fallopian tube (FT) with the proximal FT being affected most frequently. In-vitro fertilization is a common therapy but is rejected by some of the affected couples. Although recanalization with transcervical catheterization (TCR) represents a minimally invasive alternative, fibrotic re-stenosis appears in about 50% of cases. For the enhancement of treatment options, we are developing a stent-based implant for the proximal FT which has the purpose to permanently maintain FT patency after TCR. Besides biological and biomechanical aspects, it is important to define the geometric parameters for such a stent, which is a completely new application in gynecology. Therefore, our aim was to assess the 3D morphology of the proximal human FT. Given the difficulty to obtain appropriate pre-menopausal FTs, we have focused on dissected FTs from post-menopausal women.

FTs (n=26) from 16 post-menopausal women were obtained either in the course of a salpingectomy or post mortem. Morphometric measurements were done based on micro computed tomography (µCT), histological sections, or both.

For the transmural and isthmic FT portion, epithelial cell morphology, luminal feret diameter, fitting circle diameter and mucosal folding degree were assessed. 3D surface models were generated for visualization and implant customization. Geometric requirements for a stent-based implant for the FT are discussed.

µCT-based 3D reconstruction and morphometric analysis help to better understand FT morphology and provide geometric requirements for a stent-based implant for the treatment of proximal FT (re-)stenosis.

Poster 11:

Title:
Micro-CT based evaluation of a newly developed glaucoma microstent in the iridocorneal angle

Authors:
Jens Runge (Institute of Anatomy, Rostock University Medical Center, Rostock), Jonas Keiler (Institute of Anatomy, Rostock University Medical Center, Rostock), Stefan Siewert (Institute for ImplantTechnology and Biomaterials e.V., Rostock University Medical Center, Rostock), Sabine Kischkel (Institute for Biomedical Engineering, Rostock University Medical Center, Rostock), Nils Grabow (Institute for Biomedical Engineering, Rostock University Medical Center, Rostock), Klaus-Peter Schmitz (Institute for ImplantTechnology and Biomaterials e.V., Institute for Biomedical Engineering, Rostock University Medical Center, Rostock), Andreas Wree (Institute for Anatomy, Rostock University Medical Center, Rostock), Rudolf F. Guthoff (Department of Ophthalmology, Rostock University Medical Center, Rostock), Thomas Stahnke (Department of Ophthalmology, Rostock University Medical Center, Rostock); jens.runge@med.uni-rostock.de

Abstract:
Glaucoma is one of the most prevalent causes of visual impairment and irreversible blindness worldwide. Surgically placed drainage systems represent therapy options to decrease intraocular pressure and to prevent optic nerve degeneration. In open angle glaucoma, a microstent-based implant can be inserted through the trabecular meshwork in the iridocorneal angle to restore aqueous humor drainage.
Within our team, a silicone based and valve controlled microstent as glaucoma therapy option was developed (Rostock Glaucoma Stent). Surgical placement of the microstent was tested pre-clinically in rabbits. Proper surgical placement which is a prerequisite to ensure drainage functionality is crucial. Post-surgical evaluation in vivo, however, remains limited due to low resolutions of the respective imaging techniques. We therefore investigated positioning and adjacent tissues of the microstent by means of micro-computed tomography (micro-CT) ex vivo.
Microstents were surgically implanted in the eyes of healthy adult rabbits to drain into the suprachoroidal (n=4) and subconjunctival space (n=3). Postsurgically, eyes were enucleated, fixed with buffered formaldehyde, and contrasted using aqueous iodine–potassium iodide solution. Subsequently, eyes were scanned with micro-CT reaching resolutions between 8 and 21 µm.
The high-resolution 3D volumes allowed us to evaluate the morphological outcomes of the implantation and to judge the tissue-specific postsurgical position of the microstent in detail. In this context, also the condition of adjacent tissues was assessed. Divergences from aimed surgical results were specifically identified.
Micro-CT based visualization of target tissues allows an in-depth and detailed evaluation of surgical implantation and represents an indispensable addition to other imaging methods.

Poster 12:

Title:
Body painting, ultrasound, clinical investigation and peer-teaching: a student-orientated unconventional approach to enhance anatomy learning.

Authors:
Alessandro Bilella (Department of Medicine, University of Fribourg, Fribourg), Elisabeth Eppler (Department of Medicine, University of Bern, Bern), Luis Filgueira (Department of Medicine, University of Fribourg, Fribourg); alessandro.bilella@gmail.com

Abstract:
Traditional anatomy teaching to medical students consists in one-way procedure of lectures combined with practical courses, such as dissection, prosection or specimen.
We present a new approach to enhance the learning of clinical-orientated anatomy of the musculoskeletal system.
From 2016 to 2021, five cohorts of second-year medical students voluntarily experimented this new procedure at the University of Fribourg.

The new course covered the musculoskeletal anatomy combining clinical investigation and ultrasound examination of the joints and studying the physiological/anatomical conditions and pathological alterations of the musculoskeletal system.
The course aimed to enhance the acquisition of knowledge in anatomy and physiology, and to offer a maximum of student-focused and hands-on learning.
The students rotated through three activities, i.e. body painting, clinical investigation, and ultrasound, under the supervision of a faculty member or an experienced medical doctor.
The students followed an introduction and prepared a PowerPoint presentation related to the clinical anatomy.
At the end of the course, the students reported on their own learning experience through a personal journal summarizing the work and topics treated and by voluntarily answering an anonymous online questionnaire (SurveyMonkey). The latter had a special emphasis on the impact of such an optional course on the improvement of students' knowledge of the musculoskeletal system.

The analysis of the journal reports and answers given via the questionnaire revealed that the aim of the course was fully achieved. Overall, the students appreciated the course, with 78% showing very high interest and 22% showing interest. For 58% of the students, the course helped to enhance their knowledge of anatomy, while 42% of the students the course stabilized it.

All the students recommend the course to their younger peers.

Poster 13:

Title:
Sculpturing anatomy: hands-on comprehension of form and function

Authors:
Gerlinde Maria Gruber (Division of Anatomy and Developmental Biology, Karl Landsteiner University of Health Sciences, Krems), Sophie Förster-Streffleur (Division of Anatomy and Developmental Biology, Karl Landsteiner University of Health Sciences, Krems), Karin Pfeifenberger (Klinische Abteilung für Orthopädie, Universitätsklinikum Krems, Krems), Johannes Streicher (Division of Anatomy and Developmental Biology, Karl Landsteiner University of Health Sciences, Krems); johannes.streicher@kl.ac.at

Abstract:
Aiming at a time-efficient but integrated, in-depth understanding of the principles of muscle anatomy, physiology and pathology, students in small groups of six, model soft tissues of the articulatio humeri onto the skeletal elements.

Students sculpture ligaments, muscles, and bursae from plasticine and attach them to the correct sites at the skeleton. The moulding of anatomical structures follows a given procedure allocating different roles and tasks to the individual members of the team. Thin metal probes visualise the axes of motion of the joint. From the orientation of muscle fibres relative to the axes of motion, the functions of the muscles are deduced.

The stepwise corroboration of individual tasks - i.e. interpretation of the instruction (text & video), moulding the muscle, attaching it to sites of origin and insertion, deducing function and symptoms of palsy, and presenting to the group - results in an exemplary, sustainable understanding of the relationship between form and function, as well as malfunction.

The collaborative sculpturing approach knits together visual, auditory, motoric, and haptic learning pathways and strengthens interactive teamwork.

Poster 14:

Title:
Preparation of the eyeball

Authors:
Irina Anikeva (Faculty of Medicine, Saratov State Medical University named after V. I. Razumovsky, Saratov, Russia), Anastasia Soloveva (Faculty of Medicine, Saratov State Medical University named after V. I. Razumovsky, Saratov, Russia), Victoria Didenko (Faculty of Medicine, Saratov State Medical University named after V. I. Razumovsky, Saratov, Russia), Anastasia Anikienko (Faculty of Medicine, Saratov State Medical University named after V. I. Razumovsky, Saratov, Russia), Tatiana Zagorovska (Anatomy Department, Saratov State Medical University named after V. I. Razumovsky, Saratov, Russia, Saratov), Radik Khairullin (Anatomy Department, REAVIZ University, Санкт-Петербург);
ira.anikeva@yandex.ru

Abstract:
One of the basic principles in the pedagogical process in the Anatomy Department is the principle of visualisation, the basis of which is the dissection method. Dissection trains students to think for themselves, increases the motivation and clinical relevance in studying process of human anatomy, and carries with it elements of the students' learning and research work.

This method originated in the School of Alexandria as a basic method of studying anatomy and was carried out regularly. The anatomical preparation is an "open, natural book" by which the student studies the structures of the human body. Educational video-films allow students to consecutively master the training material of each module of the discipline Anatomy.

Video-film «Preparation of the eyeball» demonstrates method of preparation of eyeball. It explains coats of eyeball: structures of fibrous, vascular coats, very important anatomical structures of retina for visual function. Demonstrativeness and meaning fullness of the film increasing the effectiveness of teaching students of organ of vision, they use it during preparing exam and mastering practical skills. During the preparation, the eyeball was fixed in a 10% formalin solution for 10 days. Special anatomical instruments - scalpel and anatomical forceps, clips and holders - were used during the dissection.

We have the successful assimilation by students of anatomical knowledge, abilities and skills, confirmed by the annual increase in the average mark at intermediate certification, allows us to ascertain the optimality of the chosen methodological pedagogical approaches and modern computer technologies.

Poster 15:

Title:
Peyton's Four-Step Approach: Positive effect on student's ability to use the light microscope? - a pilot study

Authors:
Katharina Nökel (University of Oldenburg, , Oldenburg), Jana Melina Krallmann (University of Oldenburg, , Oldenburg), Nele Naß (University of Oldenburg, , Oldenburg), Veysel Ödemis (University of Oldenburg, Anatomy, , Oldenburg); katharina.noekel@uni-oldenburg.de

Abstract:
The aim of the study was to evaluate the applicability of Peyton's four-step approach to teach microscopic skills in undergraduate medical students.

The study was realised from October 2019 until July 2020 at the University of Oldenburg. 63 first semester students, who attended the regular histology course were randomized in two groups. The intervention group was taught according to the Peyton’s fourstep approach, whereas the control group was taught the conventional way ('See one, do one'). The learning progress was evaluated in two objective structured practical examinations (OSPE) within 15 weeks and by collecting error reports during the laboratory sessions. Two surveys raised the students’ perception of their competence and evaluation of the respective method.

In OSPE I the Peyton-Group achieved significantly higher results concerning the mean of total score than the control group. OSPE II showed no significant differences between means of total scores of the groups, however, the mean of total score of the control group increased significantly compared to the first examination. The control group had considerably more error reports than the Peyon-Group. In both surveys the students assessed their respective method predominantly positive relating to the introductory seminar.

The Peyton’s four-step approach has a higher impact on the short-term learning success than the conventional method. Concerning the long-term outcome both methods are equally effective.

Poster 16:

Title:
Extended evaluation of vascular normalization after anti-angiogenic therapy

Authors:
David Ascheid (Institiute of Anatomy and Cell Biology, JMU Wuerzburg, Wuerzburg), Erik Henke (Institiute of Anatomy and Cell Biology, JMU Wuerzburg, Wuerzburg), Süleyman Ergün (Institiute of Anatomy and Cell Biology, JMU Wuerzburg, Wuerzburg);
david.ascheid@uni-wuerzburg.de

Abstract:
In tumors, vascular normalization by anti-angiogenic therapy has the potential to improve outcome of subsequent treatment. Scoring the success of normalization is critical and needs to be more precise to explain contradictory outcomes of therapy after vascular normalization. The standard evaluation process focusses on maturation and patency of individual vessels. We aimed to include structural changes of the whole vasculature.

Intravital fluorescent vessel staining combined with tissue clearing allowed us to generate high-resolution images of the vasculature of various normal organs and tumors by light-sheet fluorescence microscopy. 3D reconstruction and tracing gave us access to several vessel parameters. The complex data sets were used to identify parameters useful to quantify the „abnormality“ of the vasculature in malignant versus normal tissues. These parameters were used to follow the effects of anti-angiogenic therapies.

The distilled parameters showed significant differences in the structure of vasculature of tumor versus healthy tissues. While anti-angiogenic treatment improved maturation and patency of individual vessels, it failed to improve structural parameters of the vasculature towards a normal appearance.

This data implies that the structure of the vasculature does not improve under anti-angiogenic treatment. We suggest incorporating the presented parameters into vascular normalization assessment and optimization of anti-angiogenic therapy. We are confident that upon improvement in those parameters, subsequent therapy will have higher success rates.

Poster 17:

Title:
Neuroligin-3 regulates excitatory synaptic transmission and EPSP-spike coupling in the dentate gyrus in vivo

Authors:
Julia Muellerleile (Institute of Clinical Neuroanatomy, Goethe University Frankfurt, Frankfurt), Matej Vnencak (, , Frankfurt), Angelo Ippolito (, , Frankfurt), Dilja Krueger-Burg (Department of Molecular Neurobiology, Max-Planck-Institute of Experimental Medicine, Göttingen), Tassilo Jungenitz (Institute of Clinical Neuroanatomy, Goethe University Frankfurt, Frankfurt), Stephan W. Schwarzacher (Institute of Clinical Neuroanatomy, Goethe University Frankfurt, Frankfurt), Peter Jedlicka (Faculty of Medicine, Justus-Liebig-University Giessen, Giessen); muellerleile@med.uni-frankfurt.de

Abstract:
Neuroligin-3 (Nlgn3), a neuronal adhesion protein implicated in autism spectrum disorder (ASD), is expressed at excitatory and inhibitory postsynapses in mice and hence may regulate neuronal excitation/inhibition balance. To date, most studies of Nlgn3 function have used in vitro or ex vivo preparations, which limits the conclusions that can be drawn regarding Nlgn3 function in living animals.

Here, we recorded local field potentials (fEPSPs) in the dentate gyrus of urethane-anesthetized Nlgn3-knockout (KO) and wildtype mice.

Synaptic transmission evoked by stimulation of the perforant path was reduced in KO mice, but the coupling of the fEPSP to the granule cell population spike was increased, indicating a compensatory increase in granule cell excitability. However, in contrast to mice lacking neuroligin-1 (Nlgn1), another Nlgn family member that heterodimerizes with Nlgn3 at excitatory synapses, Nlgn3 KO mice showed no impairments in long-term potentiation (LTP) of these synapses. We also observed a reduction in the expression levels of Nlgn1 and vesicular glutamate transporter 1 (VGlut1) in hippocampal synaptosomal preparations from Nlgn3 KO mice, which could partially explain the decrease in synaptic strength.

We conclude that while Nlgn1 and Nlgn3 have distinct functions, both are required for intact excitatory synaptic transmission in the mouse dentate gyrus. Therefore, ASD-related neuroligin mutations may also affect the availability of other neuroligins.

Poster 18:

Title:
Rab6A immunolabelling in mouse and human brain: Establishing an astrocyte-specific marker

Authors:
Linda Melzer (Institute of Anatomy II, University of Frankfurt, Frankfurt am Main), Thomas Freiman (Department of Neurosurgery, Rostock University Medical Center, Rostock), Amin Derouiche (Institute of Anatomy II, University of Frankfurt, Frankfurt am Main); Derouiche@em.uni-frankfurt.de

Abstract:
Astrocytes contribute to many higher brain functions. A key mechanism in glia-to-neuron signalling is vesicular exocytosis, however the identity of exocytosis organelles remains a matter of debate. Vesicles derived from the trans-Golgi-network (TGN) are not considered in this context.

We thus study the astrocyte TGN by immunocytochemistry applying anti-Rab6A and cell type specific markers, in mouse and human brain.

In mouse brain, Rab6A immunostaining is unexpectedly massive, diffuse in all regions, and detected preferentially and abundantly in the peripheral astrocyte processes, which is hardly evident without GFAP co-staining. All cells positive for the astrocytic markers glutamine synthetase (GS), GFAP, aldehyde dehydrogenase 1 family member L1 (Aldh1L1), or SOX9 are Rab6A+. Rab6A is excluded from microglia, oligodendrocytes, and NG2 cells using cell type specific markers. In human cortex, Rab6A labelling is very similar and associated with GFAP+ astrocytes. The mouse data also confirm the specific astrocytic labelling by Aldh1L1 or SOX9; the astrocyte specific labelling by GS sometimes debated is replicated again. In mouse and human brain, individual astrocytes display high variability in Rab6A+ structures, suggesting dynamic regulation of the glial TGN.

In summary, Rab6A expression is an additional, global descriptor of astrocyte identity. Rab6A might constitute an organelle system with a potential role of Rab6A in neuropathological and physiological processes.

Poster 19:

Title:
Vascularisation of epicardiac ganglionated nerve plexus (epiGP) in pig model

Authors:
Dmitrij Kvitka (Institute of Anatomy, Lithuanian University of Health Sciences, Kaunas),
Dainius H. Pauza (Institute of Anatomy, Lithuanian University of Health Sciences, Kaunas);
Dmitrij.kvitka@lsmuni.lt

Abstract:
Study is aimed to determine (1) sources of blood supply to epiGP and (2) morphologic pattern of epineurial vessels (ENVs) in epiGP.

Thirty four newborn Landrace pigs were used in the study. Seventeen hearts were extirpated from chest, washed out from blood with saline and prefixed by 4% paraformaldehyde through coronary arteries (CAs). Subsequently, CAs of 17 specimens were filled with different in color low-viscosity epoxy resins, and 12 - with ink solutions distinct in color. Then, eleven hearts were stained histochemically for acetylcholinesterase to show epiGP together with ENVs. Five piglets underwent thoracotomy, hearts were washed out from blood, the roots of CAs were clamped to avoid staining of CAs and different in color ink was injected into abdominal aorta. Heart preparations were analyzed with a stereomicroscope.

Blood supplies the pig epiGP through both CAs and non CAs that enter heart together with accessing nerves. Non-CAs supply nerves and ganglia of epiGP distributed on both atria, especially in the limits of heart hilum and at the base of right cranial (superior caval) vein. Several branches from different CAs may overlap and supply the blood for the same epicardiac nerves and ganglia. Contrarily to epicardiac nerves, epicardiac ganglia are usually supplied by a sole artery only. The branches of CAs supplying the epiGP are mostly of fifth-sixth succession.

EpiGP in pigs are supplied by blood via both CAs and non-CAs. This finding implies the better survival and functioning of epiGP compared with myocardium in a case of heart stroke.

Poster 20:

Title:
ADAM10 and ADAM17 - novel players in retinoblastoma carcinogenesis?

Authors:
Dario Fynn Van Meenen (Department of Neuroanatomy, University of Duisburg-Essen, Medical Faculty, Institute for Anatomy II, Essen), Oliver Dräger (Department of Neuroanatomy, University of Duisburg-Essen, Medical Faculty, Institute for Anatomy II, Essen), Maike Busch (Department of Neuroanatomy, University of Duisburg-Essen, Medical Faculty, Institute for Anatomy II, Essen), Nicole Dünker (Department of Neuroanatomy, University of Duisburg-Essen, Medical Faculty, Institute for Anatomy II, Essen);
dario.vanmeenen@uk-essen.de

Abstract:
Retinoblastoma (RB) is the most common primary malignant intraocular tumor in early childhood.
ADAMs, a disintegrin and metalloproteinases, are members of a family of sheddases involved in the
ectodomain cleavage of various transmembrane proteins. Most recent data by our group showed
that the cellular adhesion molecule L1CAM, cleaved by ADAMs, is crucial for retinoblastoma progression. In this study we seek to investigate the role of ADAM10 and ADAM17 in L1CAM cleavage and RB cell tumorigenicity.

Effects of lentiviral ADAM10 and ADAM17 single and double knockdown (KD) on RB cell viability, proliferation and apoptosis will be investigated by WST-1 assays, BrdU and DAPI stains and growth curve analyses. L1CAM ectodomain shedding will be detected in ADAM10/17 deficient RB cells and cell culture supernatants. Anchorage independent growth, tumor formation capacity, invasiveness and dissemination will be investigated via soft agarose and in ovo chorioallantoic membrane (CAM) assays.

All RB cell lines investigated display increased ADAM10 and ADAM17 expression compared to healthy human retinal tissue. Preliminary data show a decrease in cell viability and proliferation of RB cells as well as an increase in apoptotic levels upon ADAM10 and 17 single and ADAM10/17 double KD. First in ovo CAM assays revealed an effect on tumor growth following ADAM depletion.

Based on our preliminary data, we expect decreased tumorigenicity and impaired dissemination capability of ADAM10/17 deficient RB cells, potentially revealing a role of ADAM10 and ADAM17 as a novel therapeutic target for RB treatment.

Poster 21:

Title:
FEAR&FOOD: ANXIETY AS A RISK FACTOR FOR ANOREXIA NERVOSA

Authors:
Anna Lotte Schotten (Institute of Neuroanatomy, RWTH Aachen University, Aachen), Constanze Schwenzer (Institute of Neuroanatomy, RWTH Aachen University, Aachen), Cordian Beyer (Institute of Neuroanatomy, RWTH Aachen University, Aachen), Jochen Seitz (Department of Child and Adolescent Psychiatry, RWTH Aachen University, Aachen), Stefanie Trinh (Institute of Neuroanatomy, RWTH Aachen University, Aachen); anna.schotten@rwth-aachen.de

Abstract:
Anorexia nervosa (AN) is a severe eating disorder and common comorbidities in AN are anxiety disorders. Previously, we showed that anxiety makes rats more susceptible to weight loss in an AN animal model. Here, we additionally investigated anxiety-like behavior in rats after refeeding and analyzed the relation between starvation-related hyperactivity and anxiety.

The well-established activity-based-anorexia (ABA) model was applied to female rats. After a habituation phase, food intake was reduced by 60% until the rodents lost 25% of their body weight (acute starvation) and stabilized for two weeks (chronic starvation). Afterwards, the animals had ad libitum access to food for ten days (refeeding). Anxiety-like behavior was tested with the Elevated Plus Maze.

Anxiety-like behavior was significantly reduced in food-restricted rats during starvation and normalized with increasing body weight after refeeding. We also showed that running-wheel activity after refeeding was correlated with reduced anxiety-like behavior, similar to wild-type animals, but opposite to earlier findings in ABA animals during food restriction.

Food restriction seems to have an anxiolytic effect in rats during starvation that declines after refeeding. Hyperactivity also appears to be associated with anxiousness depending on the body weight status. These results support anxiety as an influencing factor for the course of AN and could help explain the interaction with anxiety disorders as risk factors and comorbidities. Clinicians should consider anxiety as a major risk factor for AN and the possible anxiolytic and rewarding effect of food restriction as well as hyperactivity in AN therapy.

Poster 22:

Title:
Mapping the cytoarchitectonic basis of functional variability in the human insula

Authors:
Julian Quabs (Institute for Anatomy I (Düsseldorf), C. and O. Vogt Institute for Brain Research (Düsseldorf), Institute of Neuroscience and Medicine (INM-1)(Jülich), University of Düsseldorf and Research Centre Jülich, Düsseldorf), Hartmut Mohlberg (Institute of Neuroscience and Medicine (INM-1), Research Centre Jülich, Jülich), Sebastian Bludau (Institute of Neuroscience and Medicine (INM-1), Research Centre Jülich, Jülich), Katrin Amunts (Institute of Neuroscience and Medicine (INM-1)(Jülich), C. and O. Vogt Institute for Brain Research (Düsseldorf), Research Centre Jülich and University of Düsseldorf, Jülich), Svenja Caspers (Institute for Anatomy I (Düsseldorf),Institute of Neuroscience and Medicine (INM-1)(Jülich), University of Düsseldorf and Research Centre Jülich, Düsseldorf);
julian.quabs@uni-duesseldorf.de

Abstract:
Neuroimaging studies reported that the insular cortex is a hub region for multifunctional integration in the human brain, including interoceptive, somatomotor, cognitive and social-emotional processing. The underlying cytoarchitecture on the other hand and especially its relation to the functional variability remains inconclusive. The present study was carried out to examine those microstructural areas on statistical basis and to provide a cytoarchitectonic parcellation for establishing a dedicated structure-function relationship in the human insula.

We analyzed histological sections of ten post mortem brains, using statistical image analysis for identification of cytoarchitectonic borders. The detected areas were further examined with regard to structural differences and similarities by means of cluster analysis. Additionally, 3D probability maps were computed for each discovered area within a common reference space and integrated in the Juelich-Brain Atlas and the Human Brain Project.

Seven new distinct areas were discovered in the human insula from granular (Ig3), agranular(Ia) and dysgranular (Id2-Id6) quality next to previously detected areas from our research group. The cluster analysis provided evidence for a tripartition of the insula into a granular posterior cluster, a dysgranular-agranular posterior cluster and a dysgranular, dorsal anterior cluster.

This study provides a new microstructural parcellation of the posterior and dorsal anterior insula, showing that this brain region consists of a multitude of different cytoarchitectonic areas and superordinated structural groups. Since all areas are available in a common reference space, our maps can be used as a microanatomical basis for studying the structural-functional organization in this functionally dense region of human neocortex.

Poster 23:

Title:
Choosing memory retrieval strategies: a critical role for inhibition in the dentate gyrus

Authors:
Anne Albrecht (Institute of Anatomy, Otto-von-Guericke University Magdeburg, Magdeburg), Alićе Weiglein (Institute of Anatomy, Otto-von-Guericke University Magdeburg, Magdeburg), Iris Müller (Institute of Pharmacology and Toxicology, Otto-von-Guericke University Magdeburg, Magdeburg), Gürsel Çalışkan (Institute of Biology, Otto-von-Guericke University Magdeburg, Magdeburg), Oliver Stork (Institute of Biology, Otto-von-Guericke University Magdeburg, Magdeburg); anne.albrecht@med.ovgu.de

Abstract:
Remembering the location of food is essential for survival. Rodents and humans employ mainly hippocampus-dependent spatial strategies, but when being stressed they shift to striatal stimulus-based strategies. We therefore tested whether mice with a heightened stress susceptibility due to a lack of the GABA-synthetizing enzyme GAD65 (GAD65 knock out mice) would demonstrate a lack of spatial learning preference and investigated underlying brain circuits.

The strategy preference of GAD65 knock out mice was tested in a dual solution task, where mice have to learn the location of a food reward in an open field either by spatial navigation via distal cues or by stimulus-based guidance via a proximal cue. The activation of neuronal circuits was assessed by cFos immunohistochemistry, circuits were probed by shRNA mediated knock down of GAD65 and chemogenetic interventions.

GAD65 knock out mice lacked a spatial preference during retrieval, but showed normal spatial learning. Their cFos expression patterns displayed a shift in the co-activation of the hippocampal dentate gyrus (DG) with multiple brain areas. The local knock down of GAD65 within the DG was sufficient to replicate the phenotype of the global GAD65 knock out. Similarly, disturbing DG excitability before choice retrieval, either by inhibiting granule cells or activating parvalbumin interneurons, induced a loss of spatial preference.

These data pinpoint towards the dorsal DG as a critical hub for choosing between spatial and non-spatial foraging strategies. The inhibitory/ excitatory balance in the DG appears as a crucial factor for determining cognitive flexibility under stress.

Poster 24:

Title:
The influence of 17ß-estradiol on brain size - preliminary investigations of the brain-gonadal axis in laying hens (Gallus gallus f.d)

Authors:
Julia Mehlhorn (Institute for Anatomy I, University of Düsseldorf, Düsseldorf), Beryl Eusemann (Institute of Animal Welfare and Animal Husbandry, Federal Institute of Animal Health, Celle), Stefanie Petow (Institute of Animal Welfare and Animal Husbandry, Federal Institute of Animal Health, Celle); julia.mehlhorn@hhu.de

Abstract:
In commercial laying hens the egg production has increased to about 340 eggs/year in the last decades. The consequences of this high productivity on their physiology, e.g. their reproductive system and brain morphology have been poorly investigated.

Hence, the aim of this study was to allometrically compare the brain sizes of a high performing layer line (WLA; 320 eggs/year) with a) its low performing counterpart (G11; 200 eggs/year) and b) seven breeds which are not selected for egg production. In parallel, half of G11 and WLA was given a 4.7 mg deslorelin acetate implant (Suprelorin ®) at the onset of lay (approximately 17th week) (G11 S, WLA S) to avoid follicle maturation. Additionally, we measured blood concentration of 17ß-estradiol in all laying hens.

Brains were significantly smaller in both treatment groups of WLA and G11 compared to the seven non-commercial breeds. Relative brain size of Suprelorin ® treated hens of both lines was significant larger compared to untreated hens of the same line. G11 S hens showed significantly larger brains than WLA S hens. Untreated WLA hens showed the highest 17ß-estradiol concentration in the plasma, followed by G11. WLA S and G11 S had the lowest 17ß-estradiol concentration.

Our results indicate that, next to egg production itself and the selection for laying performance, the hormone status/17ß-estradiol plasma concentration have a quantitative impact on brain size. Further investigations will determine this impact in detail and will describe the neurological basics of the hypothalamic-pituitary-gonadal axis histologically and morphometrically in the laying hen.

Poster 25:

Title:
Of mice and men – and gerbils! Unexpected NLGN4X/Y gene diversification in rodents

Authors:
Stephan Maxeiner (Anatomy and Cell Biology, Saarland University, Homburg), Gabriela Krasteva-Christ (Anatomy and Cell Biology, Saarland University, Homburg);
stephan.maxeiner@uni-saarland.de

Abstract:
As part of an ancestral mammalian gene cluster present on both sex chromosomes, the neuroligin-4 gene diverged into two different “gametologous” genes in humans and in horses, respectively. Whereas mutations of the human NLGN4X gene have been shown to be present in cases of the autism spectrum, the role of NLGN4Y remains elusive. In mice and other rodents neuroligin-4 appears to be pseudoautosomal. Fragments of two different, incomplete NLGN4-like genes in the gerbil genome hint at the first incidence of NLGN4X/Y diversification in a rodent. Are there two NLGN4 genes in gerbils, and how do they compare to those in humans and horses?

We employed sequence comparison (blastn suite, NCBI), various bioinformatical tools as well as traditional DNA cloning techniques (5’RACE and DNA sequencing) to assess and experimentally determine whether two different NLGN4 gametologs are present in gerbils.

Starting with fragments of two NLGN4-like genes, we were able to determine the presence of an X- and Y-specific neuroligin-4 gene. Sequencing gaps in the respective genes were closed and the transcriptional start as well as all exons have been determined. Phylogenetic analysis revealed that X/Y diversification has occurred independently in gerbils from those found in humans or horses. Furthermore, both genes are considered suitable applying a PCR-based sex-typing strategy.

The identification of gerbil NLGN4X/Y gametologs presents the first case in rodents embedded on a phylogenetic branch troubled by an erosion of the pseudoautosomal region. Both gametologs retain characteristics of this developmental process, such as accumulations of GC-rich and repetitive sequences.

Poster 26:

Title:
Disinhibitory Circuit Motifs in Mouse Primary Somatosensory (Barreel) Cortex

Authors:
Felix Preuß (Center for Anatomy / Institute for Neuroanatomy, University Medical Center Göttingen, Göttingen), Martin Möck (Center for Anatomy / Institute for Neuroanatomy, University Medical Center Göttingen, Göttingen), Mirko Witte (Center for Anatomy / Institute for Neuroanatomy, University Medical Center Göttingen, Göttingen), Jochen Staiger (Center for Anatomy / Institute for Neuroanatomy, University Medical Center Göttingen, Göttingen); felix.preuss@med.uni-goettingen.de

Abstract:
GABAergic interneurons (IN) play a crucial role in information processing in the rodent neocortex and are intricately integrated into the circuitry of mouse barrel cortex. Martinotti cells (MC) are a well-described IN cell type which are thought to control the activity of pyramidal neurons. However, MC are also targeted by other IN which is called disinhibiton. It has been reported that layer 2/3 (L2/3) MC are targeted by vasoactive intestinal peptide (VIP) and parvalbumin (PV) expressing IN, but little is known about disinhibitory circuit motifs involving L5 MC.

We performed intralaminar (L5 to L5) and translaminar (L2/3 to L5) VIP to L5 MC patch clamp recordings in barrel cortex of mice using acute brain slices. After experiments, slices were stained, and individual pairs were morphologically reconstructed.

Paired patch clamp recordings resulted in a connection probability of ≈30 % for L2/3 VIP to L5 MC and ≈26 % for L5 VIP to L5 MC. Single action potentials led to postsynaptic currents in >50 % of trials with similar latencies but lower amplitudes in intralaminar compared to translaminar connections. Train stimulation resulted in synaptic facilitation only at 40 Hz stimulation. In both connections, individual pairs with above-average current amplitudes were found. Morphological reconstructions revealed putative contact sites at the soma and soma proximity in one of those pairs.

We could demonstrate that L2/3 and L5 VIP neurons effectively target L5 MC, thereby confirming and extending the VIP cell to MC connection as a prominent disinhibitory circuit motif in mouse primary somatosensory cortex.

Poster 27:

Title:
The oligodendrocytic gap junction protein Connexin 29 is a downstream target of cuprizone intoxication

Authors:
Caroline Kremer (Anatomy and Cell Biology, Saarland University, Homburg / Saar), Anja Beckmann (Anatomy and Cell Biology, Saarland University, Homburg / Saar), Nadine Hainz (Anatomy and Cell Biology, Saarland University, Homburg / Saar), Alexander Grissmer (Anatomy and Cell Biology, Saarland University, Homburg / Saar), Carola Meier (Anatomy and Cell Biology, Saarland University, Homburg / Saar); Carola.Meier@uks.eu

Abstract:
The intoxication with cuprizone (CPZ), which has destructive effects on developing and mature oligodendrocytes, is considered a model of multiple sclerosis. The copper chelator CPZ affects maturation and myelination of oligodendrocytes. Its demyelinating effects in vivo were ameliorated when animals were treated with probenecid, a pannexin-1 inhibitor. Oligodendrocytes express various gap junction proteins in a cell type-specific manner, including Connexin (Cx) 29, Cx32 and Cx47, which contribute to the formation of a pan-glial syncytium. Cx29, in addition, might also be important for establishing axonal contact.

We analysed the effects of CPZ and PBN on Oli-neu cells, an oligodendrocytic precursor cell line with the potential to differentiate. The expression of myelin proteins MBP, PLP1 and connexins was determined by quantitative RT-PCR analysis and immunohistochemistry. Also, we used freeze-fracture immunogold-labeling to assess the subcellular distribution and morphology of gap junctions.

Undifferentiated Oli-neu cells already express small amounts of myelin proteins MBP and PLP1 and were immunopositive for Cx29 and, to a lesser extent, Cx47. CPZ diminished the immunopositivity of all proteins investigated. This effect was prevented by probenecid. Ultrastructurally, Cx29-formed connexons were arranged in semi-circles.

CPZ reduced expression of myelin proteins. Cx29, also expressed in Schwann cell precursors, was demonstrated in undifferentiated Oli-neu cells. Its connexons assembled in semi-circles, possibly evolving rosettes, which is the morphology observed in mature oligodendrocytes. PBN prevented the CPZ-induced protein loss. Thus, connexin and pannexin channels might both contribute to the demyelinating effects of cuprizone and therefore possibly be involved in the pathomechanisms of multiple sclerosis.

Poster 28:

Title:
Entorhinal cortex lesion induces homeostatic synaptic plasticity of CA3 pyramidal neurons

Authors:
Pia Kruse (Department of Neuroanatomy, Institute of Anatomy and Cell Biology, Faculty of Medicine, University of Freiburg, Freiburg), Andreas Vlachos (Department of Neuroanatomy, Institute of Anatomy and Cell Biology, Faculty of Medicine, University of Freiburg, Freiburg), Maximilian Lenz (Department of Neuroanatomy, Institute of Anatomy and Cell Biology, Faculty of Medicine, University of Freiburg, Freiburg); pia.kruse@anat.uni-freiburg.de

Abstract:
Denervation of brain regions is a common feature of many neurological diseases which are accompanied by demyelination or cell death. Nevertheless, the mechanisms involved in the reorganization of neural networks upon lesion warrant further investigation. Here, we aimed to elucidate the effects of a partial denervation of CA3 pyramidal neurons, which receive input from the entorhinal cortex as well as from dentate granule cells.

Using an entorhinal cortex lesion (ECL) of organotypic entorhino-hippocampal tissue cultures, resulting in a denervation of distal apical dendrites of hippocampal neurons, lesion-induced changes in excitatory neurotransmission onto both dentate granule cells and CA3 pyramidal cells were assessed with single and paired whole-cell patch-clamp recordings.

ECL leads to homeostatic adjustments of excitatory synapses of dentate granule cells and CA3 pyramidal cells. Notably, these homeostatic adaptions occur predominantly in the fraction of the strongest excitatory synapses as shown by a hierarchical analysis of spontaneous excitatory postsynaptic currents. Furthermore, paired recordings of dentate granule cells and CA3 pyramidal neurons revealed that synaptic transmission at the mossy fiber/CA3 synapse is a target of lesion-induced synaptic plasticity.

We conclude that ECL induces homeostatic synaptic plasticity of both dentate granule cells and CA3 pyramidal neurons that may lead to hetero- and transsynaptic adaptations of hippocampal networks.

Poster 29:

Title:
Microglia mediate synaptic plasticity induced by repetitive magnetic stimulation

Authors:
Amelie Eichler (Department of Neuroanatomy, Institute of Anatomy and Cell Biology, Faculty of Medicine, University of Freiburg, Freiburg), Zsolt Turi (Department of Neuroanatomy, Institute of Anatomy and Cell Biology, Faculty of Medicine, University of Freiburg, Freiburg), Dimitrios Kleidonas (Department of Neuroanatomy, Institute of Anatomy and Cell Biology, Faculty of Medicine, University of Freiburg, Freiburg), Matthias Kirsch (Department of Neuroanatomy, Institute of Anatomy and Cell Biology, Faculty of Medicine, University of Freiburg, Freiburg), Takahiro Masuda (Institute of Neuropathology, Faculty of Medicine, University of Freiburg, Freiburg), Marco Prinz (Institute of Neuropathology, Faculty of Medicine, University of Freiburg, Freiburg), Maximilian Lenz (Department of Neuroanatomy, Institute of Anatomy and Cell Biology, Faculty of Medicine, University of Freiburg, Freiburg), Andreas Vlachos (Department of Neuroanatomy, Institute of Anatomy and Cell Biology, Faculty of Medicine, University of Freiburg, Freiburg); amelie.eichler@anat.uni-freiburg.de

Abstract:
Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive tool commonly used to drive neural activity in the human brain. This is achieved by delivering short pulses (~0.5 ms) of magnetic fields over the scalp at high stimulation intensities (> 1 Tesla). Recent data from mouse models have shown that rTMS induces synaptic plasticity. Based on the suggestion that microglia, the brain's resident immune cells, modulate synaptic plasticity, we tested for the role of microglia in synaptic plasticity induced by repetitive magnetic stimulation (rMS).

3-week-old entorhinal-hippocampal tissue cultures prepared from wild type and transgenic mouse lines were stimulated with a 10 Hz stimulation protocol. The CSF1R inhibitor PLX3397 was used to deplete microglia from tissue cultures. Whole-cell patch-clamp recordings, morphological analysis and computational modeling of CA1 pyramidal neurons were used to assess the effects of rMS on neurons. The effects of rMS on microglia were studied with qPCR-analysis, protein detection assays, and live cell microscopy of tdTomato expressing microglia.

10 Hz rMS does not induce synaptic plasticity in microglia-depleted tissue cultures. Interestingly, no major changes in microglia morphology and dynamics are observed up to 3 h after rMS. However, 10 Hz rMS triggers the release of plasticity-promoting microglial factors.

These results establish a role for microglia in rMS-induced synaptic plasticity, which is of considerable relevance in the context of brain diseases treated with rTMS.

Poster 30:

Title:
Arterial hypertension change phenotype of nerve fibers in epicardiac nerves

Authors:
Dalia Ranceviene (Institute of Anatomy, Lithuanian University of Health sciences, Kaunas), Joana Azukaite (Institute of Anatomy, Lithuanian University of Health Sciences, Kaunas), Kristina Rysevaite - Kyguoliene (Institute of Anatomy, Lithuanian University of Health Sciences, Kaunas), Neringa Pauziene (Institute of Anatomy, Lithuanian University of Health Sciences, Kaunas); dalia.ranceviene@lsmuni.lt

Abstract:
To detect early changes of epicardiac nerves' due to arterial hypertension.

Transverse ventricular cryosections of 7 spontaneously hypertensive rats (SHR), 6 Wistar-Kyoto rats (WKY) were examined in juvenile (<2 months) and adult (>3 months) groups. Neural structures were stained immunohistochemically for protein gene product 9.5 (PGP95 – general neuronal marker) and calcitonin gene-related peptide (CGRP – potent vasodilator). Total epicardiac nerve cross-section area and area stained with both neuromarkers were measured and their ratio expressed as percentage. Results were described as means and compared by Student t-test, p-value<0.05.

In juvenile WKY (n=151) vs SHR (n=162) nerves comparative area of PGP9.5 (72.5% vs 73.3%), CGRP (4.7% vs 5.9%) did not differ p>0.050, but CGRP/PGP9.5 ratio (6.7% vs 8.2%) was higher in SHR, p=0.009.
In the adult WKY (n=49) vs SHR (n=111), PGP9.5 was lower in SHR by 12.1% (81.6% vs 71.7%), p<0.000, as well as CGRP was lower by 20.62% (5.5% vs 4.4%), p=0.040. CGRP/PGP9.5 ratio did not differ between adult groups (6.8% vs 6.0%), p=0.304. Comparing the SHR according to the age, PGP9.5 distribution remained similar. However, in SHR, comparative area of CGRP and CGRP/PGP9.5 ratio were lower in the adult group by 25.0% and 26.0%, respectively, p=0.001. In contrast, there was a 12.4% increase in PGP9.5 in the WKY adult group, p<0.000, although the CGRP distribution and CGRP/PGP9.5 ratio did not differ, p=0.897.

Neural and vasodilator components were more plentiful in juvenile rats however decreased in the adult spontaneously hypertensive rats; in opposite – both components increased in control group with ageing.

Poster 31:

Title:
Contamination of the angiographic work environment with textiles: Detection and characterization of particles and fibers

Authors:
Tim Clarner (Institut für Neuroanatomie, Uniklinik Aachen, Aachen), Christiane Franz (Neuroradiologie, Uniklinik Aachen, Aachen), Andreas Herrmann (Leibnitz Institut für Interaktive Materialien, RWTH Aachen, Aachen), Vanessa Kogel (Neuroanatomie, Uniklinik Aachen, Aachen), Omid Nikoubashman (Neuroradiologie, Uniklinik Aachen, Aachen); tclarner@ukaachen.de

Abstract:
Stroke is a common and devastating neurovascular disease and the second leading cause of death worldwide. Most strokes are ischemic, usually caused by the occlusion of a cerebral artery with an embolized blood clot. For diagnostic work-up and treatment of neurovascular diseases, cerebral angiography is a common tool. To prevent embolization, catheters are continuously flushed with heparinized saline solution throughout the procedure. However, embolization can be caused not only by blood clots but by foreign materials such as hair fragments, starch, cellulose, or other materials. Particles and fibers from the work environment can inadvertently be imported into blood vessels during angiography. This can result in thrombosis, inflammation and the development of granulomas. Here, we characterize contaminations in neuro-angiographic fluids, both quantitatively and qualitatively. Our aims were 1) to identify whether fibers or particles contribute to contamination, 2) to determine the origin of these contaminations and 3) to identify potential measures for their reduction.

Angiographic flushing fluids from patient and animal angiographies were used and fibers isolated using filtration under sterile conditions, vacuum centrifugation and adopted cytospin protocols.

We identified high numbers of fibers from different sources such as cellulose, cotton and woven gauze. Our results suggest that not only compresses but also surgical gowns and patient drapes contribute to fiber contamination.

In a next step, we investigate the effects of these fibers for inflammatory reactions in the brain of experimental animals. Our study will contribute to the improvement of the medical quality and safety of angiographic procedures in patients.

Poster 32:

Title:
Localisation and Functional Role of MAGI-1 in Neurons

Authors:
Lukas Einhäupl (Institute of Anatomy, Brandenburg Medical School, Neuruppin), Julia Knocks (Institute of Anatomy, Brandenburg Medical School, Neuruppin), Andreas Winkelmann (Institute of Anatomy, Brandenburg Medical School, Neuruppin), Nina Wittenmayer (Department Humanmedizin, Medical School Hamburg, Hamburg);
lukas.einhaeupl@mhb-fontane.de

Abstract:
MAGI (membrane associated guanylate kinase inverted) proteins belong to the MAGUK-family (membrane associated guanylat kinase) of synaptic scaffolding proteins. They consist of three members: MAGI-1, MAGI-2 (also known as S-SCAM) and MAGI-3 which are expressed in the brain. Though MAGI-2 function in the brain has been studied intensively, little information about MAGI-1 function in neuronal tissue is available.

In this study we analyzed the localisation and functional role of MAGI-1 by using transfections and immunocytochemical analysis in primary cultured rat hippocampal neurons.

Transfection of recombinant MAGI-1 and immunofluorescent analysis in neurons revealed diffuse distribution of MAGI-1 in the cell body and neurites at early DIV stages of the culture system followed by synaptic enrichment at DIV7 to 21. MAGI-1 is transported in axons and dendrites of neurons and shows colocalisation with excitatory and inhibitory synaptic proteins.

Recombinant MAGI-1 and S-SCAM/MAGI-2 share a similar localisation pattern in primary hippocampal neurons which could be explained by an analogous domain structure of the MAGI family of proteins. Since S-SCAM/MAGI-2 is the predominantly expressed MAGI protein in the brain, further functional investigations of MAGI-1 will reveal distinct or substitutional roles of MAGI proteins in neurons.

Poster 33:

Title:
Functional analysis of the transcription factor Bcl11a/Ctip1 in the developing hippocampus

Authors:
Verena Stelz (Institute of Molecular and Cellular Anatomy, Ulm University, Ulm), Artemis Koumoundourou (Institute of Molecular and Cellular Anatomy, Ulm University, Ulm), Stefan Britsch (Institute of Molecular and Cellular Anatomy, Ulm University, Ulm); verena.stelz@uni-ulm.de

Abstract:
The transcription factor Bcl11a/Ctip1 has been linked with neurodevelopmental and neuropsychiatric disorders that are associated with impaired cortical development. Our group has previously shown that in mice, Bcl11a/Ctip1 is important for correct neocortical development. Bcl11a/Ctip1 is also expressed in the CA area of the developing and adult hippocampus, however its cellular and molecular functions remain to be determined. In this study we characterized the role of Bcl11a/Ctip1 during hippocampal development. Forebrain specific ablation of Bcl11a/Ctip1 during development leads to severe morphological impairments and size reduction of the hippocampus, predominantly in CA3. During early embryonic development of the CA3 area (E14.5) we observed a decreased proliferation rate and impaired migration of the newborn neurons, while at later stages (E18.5) increased cell death of neurons occurred. At postnatal stages, a reduction in the number of CA3 pyramidal cells of approximately 30% and a loss of the laminar organization was observed. Mossy fiber boutons were still present, however their spatial organization was impaired in mutants, suggesting that the remaining CA3 pyramidal neurons retain part of their functionality. Together, our data suggest that Bcl11a/Ctip1 is an important regulator of hippocampal development, and involved in neurogenesis as well as survival of hippocampal neurons

Poster 34:

Title:
Ablation of scleral TGF-BETA signaling increases susceptibility to IOP-induced optic nerve damage

Authors:
Magdalena Schneider (Lehrstuhl für Humananatomie und Embryologie, Universität Regensburg, Regensburg), Ernst R. Tamm (Lehrstuhl für Humananatomie und Embryologie, Universität Regensburg, Regensburg), Andrea E. Dillinger (Lehrstuhl für Humananatomie und Embryologie, Universität Regensburg, Regensburg); magdalena.schneider@ur.de

Abstract:
The amounts of TGF-BETAs and their mRNA are increased in optic nerve (ON) heads of patients with primary open-angle glaucoma. It is unclear though if increased levels of TGF-βs are cause or consequence of ON axonal degeneration. Here we followed up on the hypothesis that increased TGF-BETA signaling is part of a repair mechanism to induce molecular changes in the extracellular matrix of lamina cribrosa/peripapillary sclera that protect from IOP-induced ON axonal degeneration.

We induced deficiency of TGF-BETA receptor type II (TGF-β RII) that is essential for TGF-BETA signaling in fibroblasts (including those of the sclera) of mutant mice. Tgfbr2fl/fl mice with a floxed allele of Tgfbr2 were crossed with Col1a2-CreER mice that express Cre-recombinase under control of a tamoxifen inducible promoter. After treatment with tamoxifen, ocular hypertension (OHT) was induced using a magnetic-microbead-model. After six weeks of OHT, ON axons were PPD-stained and quantified and retina ganglion cells were stained with RBPMS.

Following treatment with tamoxifen, Tgfbr2 mRNA in ocular RNA was decreased significantly. After six weeks of OHT a reduced number of OH axons was seen in OHT eyes in comparison to un-injected contralateral eyes. Moreover, OHT also led to a decrease of retinal ganglion cell somata as seen in RBPMS stained whole mounts. Quite intriguingly, axon loss was significantly higher in mice with a fibroblast specific deficiency of TGF-β RII in comparison to control animals.

We conclude that scleral TGF-BETA signaling plays an important part in mechanisms that induce resistance to IOP-induced damage in ON axons.

Poster 35:

Title:
Versican GAG-α domain deficiency causes rosette formation and detachment of the sensory retina in the mouse eye

Authors:
Andrea E. Dillinger (Institute of Human Anatomy and Embryology, University of Regensburg, Regensburg), Nadine Bauer (Institute of Human Anatomy and Embryology, University of Regensburg, Regensburg), Roswitha Seitz (Institute of Human Anatomy and Embryology, University of Regensburg, Regensburg), Rudolf Fuchshofer (Institute of Human Anatomy and Embryology, University of Regensburg, Regensburg), Ernst R. Tamm (Institute of Human Anatomy and Embryology, University of Regensburg, Regensburg); andrea.dillinger@ur.de

Abstract:
Versican, a large chrondroitin sulphate proteoglycan is a major component of the extracellular matrix. To learn more about its specific function in the retina, we analyzed the effects of versican deficiency in mutant mice.

VCAN(tm1Zim)-mice were investigated with a splice-variant specific gene inactivation of V0/V2 -isoforms resulting in glycosaminoglycan (GAG)-α domain deficiency of versican. We analyzed the eyes of four- and eight-week-old Versican-deficient mice in comparison to wildtype-littermates. Semithin-sections were investigated by light microscopy and retinal thickness was quantified. The distribution and expression of versican binding-partners, fibronectin and hyaluronan was investigated. In addition, glial acidic fibrillary protein (GFAP) was analyzed.

The loss of V0/V2-isoforms resulted in the formation of retinal rosettes in homozygous and heterozygous versican V0/V2 deficient mice. This frequently caused detachment of the retina. The analysis of the anterior chamber angle showed a wide-open angle with no obvious structural changes. The analysis of GFAP showed no changes in its expression in Müller-cells, even in eyes with rosette formation and retinal detachment. The immunohistochemical analysis of versican binding partners show a dramatic reduction of fibronectin in the entire retina. Analysis of hyaluronan identified an extensive downregulation in the inner plexiform layer accompanied with an increase in the ganglion cell layer and the vitreous of versican V0/V2-/- mice.

Deficiency of the versican isoforms V0/V2 causes retinal changes that indicate its important role for attachment of the sensory retina. Versican appears to play an important role in the interphotoreceptor matrix that might be affected in some forms of inherited retinal disorders.

Poster 36:

Title:
Antidepressant-like properties of intrastriatal botulinum neurotoxin-A injection in a unilateral 6-OHDA rat model of Parkinson's disease

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

Abstract:
Parkinson patients often suffer from depression and anxiety, for which there are no optimal treatments. Hemiparkinsonian (hemi-PD) rats were used to test whether intrastriatal Botulinum neurotoxin-A (BoNT-A) application could also have antidepressant-like propertiesin addition to the known improvement of motor performance.

To quantify depression- and anxiety-like behavior, the forced swim test, tail suspension test, open field test and elevated plus maze test were applied to hemi-PD rats injected with BoNT-A or vehicle. Furthermore, we correlated the results in the forced swim test, open field test and elevated plus maze test with the rotational behavior induced by apomorphine and amphetamine.

Hemi-PD rats did not show significant anxiety-like behavior as compared with Sham 6-OHDA- + Sham BoNT-A-injected as well as with non-injected rats. However, hemi-PD rats demonstrated increased depression-like behaviors compared with Sham- or non-injected rats; this was seen by increased struggling frequency and increased immobility frequency. Hemi-PD rats intrastriatally injected with BoNT-A exhibited reduced depression-like behavior compared with the respective vehicle-receiving hemi-PD animals. The significant effects of intrastriatally applied BoNT-A seen in the forced swim test are reminiscent of those found after various antidepressant drug therapies.

Our data correspond with the efficacy of BoNT-A treatment of glabellar frown lines in treating patients with major depression and suggest that also intrastriatal injected BoNT-A may have some antidepressant-like effect on hemi-PD.

Poster 37:

Title:
Bryostatin and Anacardic Acid: Dual Role in Microglia-mediated Neuroprotection

Authors:
Isabella Luisa Walther (Institute of Anatomy, CAU Kiel, Kiel), Ralph Lucius (Institute of Anatomy, CAU Kiel, Kiel), Uta Rickert (Institute of Anatomy, CAU Kiel, Kiel);
stu207837@mail.uni-kiel.de

Abstract:
Rising incidence of neurodegenerative diseases enhanced the focus on the pathophysiology of neurodegenerative disorders, which often share an underlying inflammatory mechanism. Thus the microglial cell population has been gaining importance as recent studies showed inflammatory, therefore detrimental effects as well as protective, homeostatic functions in the central nervous system by these.
We therefore investigated effects of the small molecules Bryostatin (a macrolide lacton compound) and Anacardic Acid (nonadecyl salicylic acid)- both known to have immunomodulatory and anti-cancer activity- on in-vitro grown microglia and the range of microglial activation.

This study-design was based on in-vitro grown rat microglia, simulating brain-inflammation through the application of lipopolysaccharide (LPS).
To those activated cells either one of the test-substances has been applied and the response was analyzed: NO-secretion (Griess-reagent), gene-expression (qPCR), cytokine-release (ELISA) and MAPK-pathway (Western-Blot-analysis).

Our study results indicate that both substances only have significant effects on stimulated microglia, supporting a general activation with enhanced gene-expression and cytokine-secretion of COX-2, IL-1beta, IL-6, TNF-alpha, iNOS, IL-10 and TGF-beta; combining the substance to be tested and LPS exceeded the sole LPS-induced upregulation.
This applies for both substances regarding inflammatory signals and less pronounced for antiinflammatory markers, in comparison Bryostatin accomplished greater downregulation of MAPK-signaling and anti-inflammatory effects in addition to the general activation.

Anacardic Acid and Bryostatin have shown under inflammatory conditions general microglial activation regarding pro- as well as antiinflammatory markers, indicating an immunomodulatory influence on microglial phenotype with both inflammatory and protective activity.
However, the possible benefit needs to be further evaluated.

Poster 38:

Title:
DA-CH5, a novel dual GLP-1/GIP receptor agonist, impairs microglia activation/neuroinflammation in vitro

Authors:
Timm Lasse Schöning (Anatomisches Institut, Christian-Albrechts-Universität zu Kiel, Kiel), Uta Rickert (Anatomisches Institut, Christian-Albrechts-Universität zu Kiel, Kiel), Christian Hölscher (Neurology departmen, Shanxi Medical University, Taiyuan), Ralph Lucius (Anatomisches Institut, Christian-Albrechts-Universität zu Kiel, Kiel); timm.schoening@t-online.de

Abstract:
Type 2 diabetes mellitus is a risk factor for developing Parkinson's disease (PD). In a recent clinical trial, the glucagon-like peptide-1 (GLP-1) receptor agonist exendin-4, has shown good neuroprotective effects in PD patients. Based on these results studies with a novel dual GLP-1/GIP receptor agonist (DA-CH5) have shown disease modifying effects in the MPTP mouse model of PD. As microglia mediated neuroinflammation plays an important role in neurodegenerative diseases like PD, we investigated the effect of DA-CH5 on activated microglia in vitro.

Lipopolysaccharid (LPS) stimulated microglia were used to mimic brain inflammation in vitro. Therefore primary rat microglia were treated with 5 ng/ml LPS in the presence or absence of DA-CH5 (10, 1000 nM). The effects of DA-CH5 on the mRNA-expression of inflammation-associated molecules like iNOS, MMP-3, MMP-9, TNF-alpha and IL-6 were analyzed using qPCR. Moreover, protein levels of TNF-alpha were measured using enzyme-linked immunosorbent assay (ELISA).

Compared to control treated cells, DA-CH5 significantly downregulates pro-inflammatory microglia cell functions based on the results of mRNA-expression of iNOS, MMP-3, MMP-9, TNF-alpha and IL-6. Furthermore, DA-CH5 treatment also reduced the secretion of TNF-alpha.

In summary, this study indicates that a novel dual GLP-1/GIP receptor agonist (DA-CH5) is able to downregulate pro-inflammatory microglia cell functions and suggests that part of the disease modifying effects seen in other models may be related to the suppression of microglial activation/neuroinflammation. We conclude that dual agonists like DA-CH5 may provide a novel way of treating neurodegenerative diseases by slowing down or halting disease progression.

Poster 39:

Title:
Interaction of a polyclonal antiserum to Helicobacter pylori with Astrotactin-2 correlates to reduced neurite length in SiMa human neuroblastoma cells

Authors:
Aaron David Kleine (Institute for Neuroanatomy, Universitary Medical Center Göttingen, Göttingen), Bernhard Reuss (Institute for Neuroanatomy, Universitary Medical Center Göttingen, Göttingen); breuss@gwdg.de

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

We hypothesize an immune mediated mechanism for this, and demonstrate now that an antiserum to H. pylori (α-HPy) on a human first trimester prenatal brain multiprotein array (HexSelect, Engine, Berlin, Germany), interacts with a set of 104 different proteins, including the neural migration and neurite outgrowth factor astrotactin 2 (Astn2).

Interaction of the latter protein with α-HPy was confirmed independently by Western blotting with a whole cell protein extract of HEK293 cells transiently overexpressing Astn2. We can further demonstrate expression of Astn2 in the human neuroblastoma cell line SiMa, an established in vitro model for neuronal differentiation by one- and two-dimensional Western blot analyses. On a functional level, preincubation of this cell line with 10µg/ml α-HPy resulted in a significant and persistent reduction in neurite length, and also a preincubation 10µg/ml α-Astn2 revealed a similar effect.

These results demonstrate for the first time immunological crossreactivity and also functional interference of α-HPy with the neural migration and neurite outgrowth factor Astn2 due to molecular mimicry. Although highly speculative, together with previous reports on mutations of Astn2 at least in some schizophrenic patients,* the present results could also be of importance for a better understanding of at least some of the structural and functional changes in the brains of schizophrenic patients. Arioka et al., 2018, Stem Cell Res 30:81-4.

Poster 40:

Title:
Identification and characterization of blood vessel reactive autoantibodies in encephalitis patients

Authors:
Lucie Yuanting Li (Institut für Integrative Neuroanatomie, Charité-Universitätsmedizin Berlin, Berlin), Malgorzata Burek (Klinik und Poliklinik für Anästhesiologie, Intensivmedizin, Notfallmedizin und Schmerztherapie, Universitätsklinikum Würzburg, Würzburg), Paula Barthel (Institut für Integrative Neuroanatomie, Charité-Universitätsmedizin Berlin, Berlin), Jakob Kreye (Klinik für Pädiatrie mit Schwerpunkt Neurologie, Charité-Universitätsmedizin Berlin und Deutsches Zentrum für Neurodegenerative Erkrankungen, Berlin), Momsen Reincke (Klinik für Neurologie mit Experimenteller Neurologie, Charité-Universitätsmedizin Berlin und Deutsches Zentrum für Neurodegenerative Erkrankungen, Berlin), Harald Prüß (Klinik für Neurologie mit Experimenteller Neurologie, Charité-Universitätsmedizin Berlin und Deutsches Zentrum für Neurodegenerative Erkrankungen, Berlin), Markus Höltje (Institut für Integrative Neuroanatomie, Charité-Universitätsmedizin Berlin, Berlin);
markus.hoeltje@charite.de

Abstract:
Autoantibodies against neuronal receptors and synaptic proteins play a key role in the pathogenesis of autoimmune encephalitis, which can lead to certain forms of neuropsychiatric disorders. In order to reach their targets in the central nervous system and to exert their pathological effects on neural circuits, they have to overcome the blood-brain barrier (BBB. Antibodies directed against blood vessels play a putative role in this mechanism. Our goal is to analyze the antibody repertoire of patients with autoimmune encephalitis specifically directed against blood vessels. The identification of target antigens and functional analyis of BBB models therefore will help to decipher the involved pathomechanisms.

Human recombinant monoclonal autoantibodies from patients diagnosed with autoimmune encephalitis are tested for binding to brain blood vessels using indirect immunofluorescence. They are characterized more precisely with further immunohistochemical and biochemical methods such as immunoprecipitation, mass spectrometry and Western blotting. Functional measurements are carried out in an in vitro BBB model.

In a repertoire of 159 monoclonal autoantibodies from 5 patients, we were able to detect 8 antibodies with a pronounced binding pattern on brain blood vessels. The immunohistochemical characterization revealed specific patterns, such as preferential binding to certain vessel sizes or simultaneous reactivity in individual brain regions. Antigen identification as well as results from the BBB in vitro cell culture model have already provided indications of alterations in permeability.

Autoantibodies from autoimmune encephalitis patients show distinct binding patterns to brain blood vessels. Experiments carried out on the BBB in vitro model have provided clues for an antibody effect on the transendothelial electrical resistance (TEER) and permeability. Further systematic testing on the model together with the identification of antigens are under way to elucidate the role of blood vessel-reactive autoantibodies in encephalitis.

Poster 41:

Title:
Knockdown of the mitochondrial calcium uniporter protects against activated glia-induced impairment of the neuronal mitochondrial membrane potential

Authors:
Angela Casaril (Department of Medical Cell Biology, Institute for Anatomy and Cell Biology, Heidelberg University, Heidelberg), Athanasios Katsalifis (Department of Medical Cell Biology, Institute for Anatomy and Cell Biology, Heidelberg University, Heidelberg), Rolf Schmidt (Department of Medical Cell Biology, Institute for Anatomy and Cell Biology, Heidelberg University, Heidelberg), Carlos Bas-Orth (Department of Medical Cell Biology, Institute for Anatomy and Cell Biology, Heidelberg University, Heidelberg);
angela.casaril@gmail.com

Abstract:
Introduction: Inflammation-associated mitochondrial dysfunction in neurons is a hallmark of neurodegenerative and neuroinflammatory diseases. Mitochondrial malfunction is often associated with mitochondrial Ca2+ uniporter (MCU)-mediated overload of matrix Ca2+, that decreases the mitochondrial membrane potential (MMP) and reduces mitochondrial ATP synthesis. Therefore, sustained mitochondrial Ca2+ homeostasis might protect mitochondrial function during inflammation. Objective: We aimed to elucidate whether glia-conditioned medium (GCM) collected under low-grade inflammation conditions impairs neuronal MMP and whether this effect is MCU-dependent.

Methods: Primary mixed glial cultures were either left untreated or were stimulated with a low dose of lipopolysaccharide (LPS) followed by ATP (10ng/ml LPS for 3 hrs + 1mM ATP for 21 hrs) before GCM was collected. Primary cortical neurons were then exposed to 50% GCM for 24 hrs and subsequently assessed for their viability or MMP. Viability assessment was based on nuclear morphology, MMP was probed with tetramethylrhodamine ethyl ester (TMRE), and MCU knockdown was mediated by AAV infection. As a control, the mitochondrial pyruvate carrier-inhibitor UK5099 was used to disrupt MMP pharmacologically.

Results: GCM had no effect on neuronal viability. The MMP of neurons was not affected by GCM of unstimulated glia but was reduced upon treatment with UK5099 or GCM of stimulated glia. MCU knockdown specifically mitigated the GCM-induced drop in neuronal MMP, but not that induced by UK5099.

Conclusions: Our findings support the idea that impairment of the neuronal mitochondrial membrane potential during low-grade inflammation is caused by MCU-dependent mitochondrial Ca2+ dysregulation.

Poster 42:

Title:
CD44, ADAM10 and MMP14 form a complex regulatory network in cellular homeostasis.

Authors:
Birte Wöhner (Department of Anatomy, Kiel University, Kiel), Wenjia Li (Institute of Functional and Clinical Anatomy, FAU Erlangen, Erlangen), Alice Drobny (Molecular Neurology, FAU Erlangen, Erlangen), Ralph Lucius (Department of Anatomy, Kiel University, Kiel), Friederike Zunke (Molecular Neurology, FAU Erlangen, Erlangen), Philipp Arnold (Institute of Functional and Clinical Anatomy, FAU Erlangen, Erlangen);
b.woehner@anat.uni-kiel.de

Abstract:
CD44 is a multifunctional, ubiquitously expressed transmembrane glycoprotein, involved in various physiological and pathological processes, including cancer progession and metastasis. Here, we show the interaction between CD44, ADAM10 and MMP14 on multiple levels. We demonstrate transcriptional and protein level interdependencies as well as an impact on cell proliferation, adhesion and cellular motility.

For our experiments, different CRISPR/Cas9-mediated HeLa knockout cell lines were generated. With these knockout cell lines cell viability, cell migration, adhesion and spheroid formation assays were performed, as well as qRT PCRs.

Our results show, that knockout of CD44 or MMP14 results in similar affection of cellular migration, adhesion and spheroid formation, as well as mutual downregulation on mRNA and protein level, suggesting a complex interaction between these two proteins. Interestingly, loss of ADAM10 did not impair adhesion, migration and proliferation, but resulted in accumulation of CD44 at the cell surface, suggesting ADAM10 to be the constitutive sheddase of CD44. Analysing transcriptional data of control tissue derived from the TCGA (The Cancer Genome Atlas) directly links the expression of MMP14 to the expression of ADAM10 and CD44.

Taken together, our results show that cleavage of CD44 and mediated cellular processes are differently influenced by various proteases. We suggest, that CD44, ADAM10 and MMP14 form a regulatory network in cellular homeostasis and it is crucial to gain more insight into this complex network to understand how CD44 itself or cleavage of CD44 is altered during cancer cell initiation and thereby identify new therapeutic targets.

Poster 43:

Title:
Suppressive Impact of Resveratrol on Tumor Microenvironment Cross-Talk in a Novel Multicellular in vitro Model

Authors:
Constanze Buhrmann (Institute of Anatomy and Cell Biology, Faculty of Medicine, University of Augsburg, Augsburg), Parviz Shayan (Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran), Aranka Brockmueller (Musculoskeletal Research Group and Tumor Biology, Chair of Vegetative Anatomy, Institute of Anatomy, Faculty of Medicine, Ludwig-Maximilian-University Munich, Munich), Mehdi Shakibaei (Musculoskeletal Research Group and Tumor Biology, Chair of Vegetative Anatomy, Institute of Anatomy, Faculty of Medicine, Ludwig-Maximilian-University Munich, Munich);
constanze.buhrmann@med.uni-augsburg.de

Abstract:
Cross-talk in the tumor microenvironment is essential for enhancing tumor malignancy and metastasis. The natural Sirt1- targeted subcellular protein of resveratrol has been investigated before for its anti-tumor potential. In this in vitro inflammatory tumor micorenvironment model, we investigated the potential of resveratrol in suppressing cross-talk between the tumor and its microenvironment as a novel strategy for targeting in tumor therapy.

Colorectal cancer cell line (HCT116) in 3-dimensial alginate beads were cultured in a multicellular inflammatory tumor microenvironment (TME model with fibroblasts and T-lymphocytes) with/without TNF-β, Sirt1-ASO and/or resveratrol to better understand the correlation and impact of resveratrol/Sirt1-activation on members of the TME.

In vitro multicellular TME- promoted tumor cell proliferation, colony formation and invasion, similar to TNF-β stimulation, was markedly suppressed by resveratrol. Additionally, resveratrol blocked multicellular TME-stimulated NF-κB activation and expression of NF-κB-promoting genes associated with proliferation, invasion, survival and promoted cancer stem cells activation by inducing cancer stem cells markers (ADH1, CD44 and CD133) in CRC cells. However, transient knockdown of Sirt1 activity by Sirt1-ASO blocked positive effects of resveratrol in multicellular TME-stimulated CRCs, pointing to a central role of Sirt1- NF-κB-axis in the resveratrol- suppressed TME pathway.

Our results point out that targeting inflamamtory cross-talk in TME is a promising strategy for resveratrol/Sirt1-mediated anti-tumor effect in future therapies.

Poster 44:

Title:
Role of protein tyrosine phosphatase receptor type E (PTPRE) in chemoresistant retinoblastoma cells

Authors:
Lars Mohren (Institute of Anatomy II, Department of Neroanatomy, University hospital Essen, Essen), Oliver Dräger (Institute of Anatomy II, Department of Neroanatomy, University hospital Essen, Essen), Natalia Miroschnikov (Institute of Anatomy II, Department of Neroanatomy, University hospital Essen, Essen), Maike Busch (Institute of Anatomy II, Department of Neroanatomy, University hospital Essen, Essen), Nicole Dünker (Institute of Anatomy II, Department of Neroanatomy, University hospital Essen, Essen);
Lars.Mohren@uk-essen.de

Abstract:
Protein tyrosine phosphatase receptor type E (PTPRE) is a member of the “classical” protein tyrosine phosphatase subfamily and regulates a variety of cellular processes. PTPRE plays a tumorigenic role in different human cancer cells, including hepatocellular carcinoma and breast cancer. Etoposide resistant retinoblastoma (RB) cell lines show a higher expression of PTPRE than their chemosensitive counterparts. This study aims at investigating the role of PTPRE in chemoresistance of retinoblastoma, the most common malignant intraocular childhood cancer.

Lentiviral knockdown (KD) of PTPRE in etoposide resistant RB cells was followed by proliferation and cell viability studies via BrdU immunostaining, growth curve analysis and WST-1 assays. Apoptosis was investigated by DAPI cell counts after caspase inhibition, caspase 3/7 assays and caspase immunostaining. Soft agarose assay and in ovo chorioallantoic membrane (CAM) assays were used to study colony and tumor formation capacity of PTPRE deficient cells in vitro and in vivo.

Depletion of PTPRE leads to significantly decreased growth kinetics and cell viability in etoposide resistant Y79 and WERI-Rb1 RB cells. Fittingly, caspase-dependent apoptosis rates are significantly increased. As revealed by soft agarose assay, anchorage independent growth of PTPRE KD cells is significantly decreased. In ovo CAM assays reveal a slightly decreased tumor formation capacity and reduced tumor size following PTPRE KD.

Our results indicate that PTPRE depletion diminishes the tumorigenic potential of etoposide resistant RB cells by decreasing cell growth and survival. Thus, PTPRE may have the potential of a novel target for treating chemoresistant retinoblastoma.

Poster 45:

Title:
Interaction between Megalin and FcRn promoting proximal tubular function

Authors:
Eileen Dahlke (Anatomisches Institut, CAU Kiel, Kiel), Yaman Anan (Anatomisches Institut, CAU Kiel, Kiel), Lea Klie (Anatomisches Institut, CAU Kiel, Kiel), Franziska Theilig (Anatomisches Institut, CAU Kiel, Kiel): e.dahlke@anat.uni-kiel.de

Abstract:
Renal proximal tubules (PTs) are responsible for rescuing small proteins including albumin from urinary excretion. Albuminuria is a risk factor for cardiovascular diseases underlining the importance of renal endocytosis. The principal scavanger receptor of PTs is megalin acting via receptor-mediated endocytosis (micropinocytosis). The neonatale Fc-receptor (FcRn) is known to transcytose albumin. Currently, it remains unknown whether megalin/FcRn interact and whether they play an additional role in proximal tubular macropinocytosis.

We analyzed interaction of megalin/FcRn using bimolecular fluorescence complementation, co-immunoprecipitation as well as super-resolution microscopy.

Using venus-constructs and biochemically, we show that megalin/FcRn interact. Interaction was localized to Golgi apparatus, suggesting that both proteins accommodate similar sorting signals in their sequence. In addition, co-localization was observed in the endosomal compartment. Using lactoglobulin (ligand specific for megalin) or albumin (bind to megalin and FcRn), upon induction of endocytosis super-resolution microscopy revealed an enhanced co-localization of megalin/FcRn in clathrin-vesicles and early endosomes (only for albumin). No change in recycling endosomes and a reduction in co-localization in LAMP1-positive late endosomes/lysosomes was encountered. No interaction was detected between FcRn and other receptors for micropinocytosis such as amnionless, the ‘membrane anchor’ of cubilin, demonstrating the specificity of megalin/FcRn interaction. Interestingly in contrary to megalin, FcRn colocalizes with sorting nexin 5 - a marker for macropinocytosis.

In conclusion, megalin/FcRn interact and may share functional similarities since both proteins traffic together. In comparison to megalin, which uses micropinocytosis, FcRn uses macropinocytosis as an additional source to transcytose albumin. The functional role of interaction need to be further explored.

Poster 46:

Title:
Desmosomal hyper-adhesion ameliorates effects of direct inhibition of Dsg3 interactions in pemphigus

Authors:
Letyfee Steinert (Institute of Anatomy and Cell Biology, Department I, Faculty of Medicine, Ludwig-Maximilians-Universität, München), Jens Waschke (Institute of Anatomy and Cell Biology, Department I, Faculty of Medicine, Ludwig-Maximilians-Universität, München), Franziska Vielmuth (Institute of Anatomy and Cell Biology, Department I, Faculty of Medicine, Ludwig-Maximilians-Universität, München); franziska.vielmuth@med.uni-muenchen.de

Abstract:
Keratinocytes, during differentiation, acquire a strong adhesive state, in which desmosomal cadherins become independent of Ca2+. This state is referred to as hyper-adhesive and was proposed to be present in all desmosomes of human epidermis. In pemphigus, autoantibodies against the desmosomal cadherins desmoglein (Dsg) 1 and 3 cause blistering in the epidermis and mucous membranes. As hyper-adhesion was suggested to diminish loss of keratinocyte cohesion caused by pemphigus autoantibodies (PV-IgG), we here investigated the effect of hyper-adhesion on loss of intercellular adhesion and particularly altered Dsg binding properties in pemphigus.

Keratinocyte dissociation assay, Immunofluorescence (IF), Western blot, Atomic force microscopy (AFM)

Murine keratinocytes acquire a Ca2+-independent, hyper-adhesive state during 96h of differentiation, whereas they reveal a strong Ca2+-dependency after 24h. In accordance, Dsg protein expression was significantly altered during differentiation and hyper-adhesive keratinocytes are less susceptible to PV-IgG-induced loss of intercellular adhesion. Additionally, PV-IgG-induced depletion of Dsg1 and 3 was diminished in hyper-adhesive keratinocytes.
Interestingly, a pathogenic Dsg3 antibody directly inhibited Dsg3 interactions under non-hyper-adhesive as well as hyper-adhesive conditions, indicating that other mechanisms than reduced direct inhibition are crucial for ameliorated effects of PV-IgG in hyper-adhesive keratinocytes.
In contrast, no direct inhibition for Dsg1 was observed under non-hyper-adhesive and hyper-adhesive conditions. Further, increase in Dsg1 motility accompanied by impaired cluster size of Dsg1 was comparable under both conditions.

Taken together, these data indicate that changes in Dsg1 and 3 single molecule binding properties do not account for reduced susceptibility of hyper-adhesive keratinocytes to PV-IgG-induced loss of intercellular adhesion.

Poster 47:

Title:
C1q-TNF-related-peptides 1, 6 and 8 in the ocular surface and their impact on corneal wound healing

Authors:
Hagen Nicolaus (Department of Functional and Clinical Anatomy, Friedrich Alexander University Erlangen-Nürnberg, Erlangen), Friedrich Paulsen (Department of Functional and Clinical Anatomy, Friedrich Alexander University Erlangen-Nürnberg, Erlangen), Thomas Klonisch (Department of Human Anatomy and Cell Science, University of Manitoba, Winnipeg), Fabian Garreis (Department of Functional and Clinical Anatomy, Friedrich Alexander University Erlangen-Nürnberg, Erlangen); hagen.nicolaus@web.de

Abstract:
Corneal wound healing is partly mediated via Relaxin/Insulin-like family peptide receptor 1 (RXFP1) pathway. Previous studies have shown that C1q/tumor necrose factor related peptides (CTRPs) 1, 6 and 8 are able to interact with RXFP1. In this study, we analyze the expression of CTRP1, CTRP6 and CTRP8 at the ocular surface and their effect on ocular surface wound healing.

RT-PCR and Immunohistochemistry were performed to analyze CTRP1, CTRP6 and CTRP8 expression in human tissue of the ocular surface, lacrimal apparatus, and human corneal (HCE), conjunctival (HCjE) and meibomian gland (HMGEC) epithelial cell lines. Furthermore, using a scratch assay, we analyzed the effects of human recombinant CTRP1 and CTRP6 on cell proliferation and migration in HCE and HCjE cell lines.

CTRP1 and CTRP6 transcripts and proteins are present in human cornea, conjunctiva, meibomian gland, lacrimal ducts, lacrimal gland, and all investigated cell lines. Immunohistochemistry shows CTRP8 in cornea, basal cells of meibomian gland, lacrimal ducts, lacrimal gland, and all three cell lines but not in human conjunctiva. The application of 100 ng/ml recombinant human CTRP1 and CTRP6 resulted in significantly increased surface defect healing rate in HCE cell lines by a factor of 1,34 (p=0,018) and 1,31 (p=0,031) respectively, but not in HCjE cell lines.

Our results suggest a novel role for CTRP1 and CTRP6 at the ocular surface and in the lacrimal apparatus possibly targeting the RXFP1 receptor pathway. This may provide future opportunities for drug discovery in the treatment of corneal wound healing.

Poster 48:

Title:
Establishment of a co-culture model for investigating interactions between 3D tumor spheroids and macrophages

Authors:
Rosanna Huchzermeier (Department of Anatomy and Cell Biology, Uniklinik RWTH Aachen, Aachen), Nicole Schröder (Department of Anatomy and Cell Biology, Uniklinik RWTH Aachen, Aachen), Gunnar Böttcher (nstitute for Automotive Engineering, RWTH Aachen University, Aachen), Thomas Pufe (Department of Anatomy and Cell Biology, Uniklinik RWTH Aachen, Aachen), Athanassios Fragoulis (Department of Anatomy and Cell Biology, Uniklinik RWTH Aachen, Aachen); rosanna.huchzermeier@rwth-aachen.de

Abstract:
Tumor-associated macrophages can be pro-tumorigenic, supporting invasion and metastasis. Nuclear factor-erythroid 2 related factor 2 (Nrf2) may contribute to this macrophage phenotype. How exactly the Nrf2 pathway affects the interaction of macrophages and tumor cells is still unknown. Therefore, reliable co-culture models are needed to address these kinds of research questions.

We established a novel transwell-based co-culture procedure by which two cell types are grown on both sides of the membrane. Bone-marrow derived macrophages were seeded on the bottom side. An adaptor system was 3D-printed to overcome associated issues. 3D spheroids generated in advance from non-alcoholic steatohepatitis-derived hepatocellular carcinoma cells were then seeded on the inside of the transwell. Thereby, direct contact and substrate exchange was ensured while avoiding mixing up both cell types. RNA from 3D spheroids were used for qRT-PCR – supernatants of both cell types were analyzed by ELISA.

We were able to develop a 3D-printed construction that allows the co-cultivation of 3D tumor spheroids with macrophages to investigate cell-cell interactions. A great advantage of this procedure was the possibility to obtain RNA and supernatant samples of both cell types separately. The pilot study with different Nrf2-dependent KO macrophages showed no differences with respect to selected parameters of tumor behavior. However, this could be due to too short incubation periods, an issue that will be addressed in upcoming studies.

Our experimental setup offers a great opportunity to study the interaction between two different cell types. Subtleties have to be adjusted to the needs of each research question.

Poster 49:

Title:
Desmoglein-2 identified as a potential interaction partner of CD109

Authors:
Wiebke Lückstädt (Anatomical Institute, Christian-Albrechts-University Kiel, Kiel), Simon Bub (Department of Molecular Neurology, University Hospital Erlangen, Erlangen), Tomas Koudelka (Systematic Proteomics and Bioanalytics, Institute for Experimental Medicine, Christian-Albrechts-University Kiel, Kiel), Ralph Lucius (Anatomical Institute, Christian-Albrechts-University Kiel, Kiel), Philipp Arnold (Institute of Functional and Clinical Anatomy, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen); w.lueckstaedt@anat.uni-kiel.de

Abstract:
Cancer is one of the world's leading causes of death and finding markers to predict cancer at an early stage or to understand metastasis and target it are main topics in current medical research. Cluster of differentiation 109 (CD109) is discussed as a potential bio- and clinical marker for patient survival for different tumor entities such as lung cancer, glioblastoma, squamous cell carcinoma and pancreatic cancer.

Using biochemical approaches such as co-immunoprecipitation, Western blot analysis, DNA sequencing and mass spectrometry combined with immunofluorescent and electron microscopy imaging technics we were able to identify desmoglein-2 as a possible interaction partner of CD109.

Desmoglein-2 plays an important role in cell-cell contact formation and is therefore vital for tissue integrity and functionality. Using a CRISPR-Cas9 derived CD109 knock-out model of H460 cells obtained from a patient with large cell lung cancer we could observe different cell morphology and function.

Here, we propose CD109 influences cell-cell connections provided by DSG-2 in lung cancer and hence may be a possible future target to prevent tumorigenesis and formation of (pre-)metastatic niches.

Poster 50:

Title:
Expression and function of the ATP binding cassette transporter (ABC transporter) in human meibocytes

Authors:
Anna-Lena Meister (Department of Anatomy II, Friedrich Alexander University Erlangen-Nürnberg, Erlangen), Fabian Garreis (Department of Anatomy II, Friedrich Alexander University Erlangen-Nürnberg, Erlangen), Ingrid Zahn (Department of Anatomy II, Friedrich Alexander University Erlangen-Nürnberg, Erlangen), Friedrich Paulsen (Department of Anatomy II, Friedrich Alexander University Erlangen-Nürnberg, Erlangen), Martin Schicht (Department of Anatomy II, Friedrich Alexander University Erlangen-Nürnberg, Erlangen); annameister51@googlemail.com

Abstract:
Meibomian glands are critical to the health and integrity of the ocular surface. Furthermore, meibomian gland dysfunction (MGD) is the main cause of dry eye disease. Critical to the normal function of the meibomian glands is the efficient production and efflux of lipids. The ABC transporters are responsible for the successful formation and transport of lipids across the cell membrane. The expression and physiological significance of most ABC transporters in the ocular system are unknown in detail.

The expression of all 48 ABC transporters was analysed in human meibomian gland epithelial celllines (hMGEC) by PCR. The gene expression of peroxisomal fatty acid (PFA) transporters (ABCD-1, 2, 3, 4) was analyzed in hMGECs after stimulation (□-MSH) of lipid synthesis by qPCR.

Our PCR results revealed heterogeneous expression pattern of the transporters in the HMGECs and human meibocytes examined. The qPCR shows increased expression of PFA transporter (ABCD-1, 2, 3, 4) after stimulation of lipid synthesis by □-MSH. The ABC transporters (ABCA1, ABCG1, ABCA3) for lipid transport across the cell membrane show no differences.

For the first time, we show that all ABC transporters are expressed in the meibomian glands. The significant increase in PFA transporters is required for the increased lipid synthesis under □-MSH influence. No effect for ABC transporters across the cell membrane, implying that cells store lipids intracellularly. Further studies are needed to investigate the role of ABC transporters in MGD and their interaction with commonly used ocular surface drugs.

Poster 51:

Title:
Combining AFM-based functional imaging with fluorescence reporters to outline adhesion-dependent signaling

Authors:
Marie-Therès Wanuske (Department of Biomedicine, Institute of Anatomy, University of Basel, Basel), Paul Marteau (Department of Biomedicine, Institute of Anatomy, University of Basel, Basel), Volker Spindler (Department of Biomedicine, Institute of Anatomy, University of Basel, Basel); m.wanuske@unibas.ch

Abstract:
Desmosomes are cell junctions prominent in tissues exposed to high degrees of mechanical stress. In addition to their adhesive properties, they act as signaling hubs by yet unknown mechanisms. Here, we established a method to study the modulation of ERK signaling in response to altered desmosomal adhesion using a fluorescence-based kinase translocation reporter (KTR).

ERK-KTR, which allows the visualization of ERK activity through its distribution in the cell nucleus and cytoplasm, was stably expressed in normal HaCaT keratinocytes and keratinocytes lacking desmosomes [desmoplakin knockout (DP ko)] and investigated by combined live cell imaging (LCI) and atomic force microscopy (AFM) measurements.

Loss of DP led to absence of desmosomes, altered membrane localization of the desmosomal adhesion molecules desmoglein 2 (DSG2) and desmocollin 3 (DSC3) and reduced ERK activity. We thus used this model to validate ERK-KTR by LCI, which confirmed diminished ERK activity in DP ko and demonstrated pronounced ERK activation upon addition of FCS. AFM with DSC3-coated tipless cantilevers was used to mimic establishment or disruption of desmosomal junctions, which is hypothesized to trigger changes in signaling. However, ERK activity appeared to be independent of such manipulations. Furthermore, inhibition of ERK signaling in DP ctrl cells did not visibly alter DSG2 localization along the membrane, suggesting that the altered distribution in DP ko is not caused by reduced ERK activity.

The combination of single cell force spectroscopy with fluorescence activity reporters represents a promising tool to investigate the relation between the interaction of adhesion molecules and changes in intracellular signaling.

Poster 52:

Title:
Altered WNK1 isoforms expression in diabetic nephropathy and VHL conditional knockout

Absage, das Poster wird nicht präsentiert

Poster 53:

Title:
Of mice and men – and guinea pigs! Assessing expression and localization of enigmatic MXRA5

Authors:
Frederick Schweizer (Anatomy and Cell Biology, Saarland University, Homburg), Gabriela Krasteva-Christ (Anatomy and Cell Biology, Saarland University, Homburg), Stephan Maxeiner (Anatomy and Cell Biology, Saarland University, Homburg); s8ffschw@stud.uni-saarland.de

Abstract:
The human MXRA5 gene is located on the X-chromosome immediately adjacent to the pseudoautosomal region present on both sex chromosomes. Bioinformatical analysis assigns MXRA5 a role as a potential extracellular matrix protein. Sparse information is available about its function due to its absence in laboratory rodents (mice/rats). We have previously shown that this is owed to its peculiar genomic localization. Genomic association studies suggest its involvement in several pathologies, e.g., tumor metastasis. MXRA5 is present in guinea pigs. We aim at determining its tissue and cellular expression pattern as a first step to further elucidate its role underlying clinical pathologies.

We employed a wide range of DNA cloning and RNA quantification techniques to identify all MXRA5 exons and its genomic organization. Transcript levels in over twenty selected tissues have been assessed by qRT-PCR. Means of immunodetection (IB/IF) were applied comparing the antigenicity of human and guinea pig MXRA5 in cell culture using commercial MXRA5 antibodies prior to IF analyses on frozen tissue sections.

The transcriptional start of MXRA5 and the sequence of its seven exons were experimentally determined. MXRA5 transcripts were present in a range of tissues peaking highest in the kidney. Immunofluorescent staining demonstrates extracellular localization, prominently around distinct layers of pseudo-/stratified epithelia.

Absence of MXRA5 in favored rodent research models has hampered elucidating its function as well as its role in clinical pathologies. Using guinea pigs, we have successfully established a toolkit in a rodent research model to further assess its function and potential role in human pathologies.

Poster 54:

Title:
C-terminal modification of Connexin 43 and its impact on gap junction structure and function

Authors:
Alice Ferdinand (Anatomy and Cell Biology/ Prof. Dr. Carola Meier, Saarland University, Homburg), Johanna Recktenwald (, , Homburg), Alexander Grißmer (, , Homburg), Carola Meier (, , Homburg), Anja Beckmann (, , Homburg); anja.beckmann@uks.eu

Abstract:
Gap junctions (GJ) are communication elements, built by dimers of connexons, which in turn consist of connexin (Cx) proteins arranged in hexamers. Channel assembly and regulation of channel opening is regulated by various protein modifications, in particular phosphorylation of the C terminal amino acids. Cx43 is the most abundant GJ protein. The freeze-fracture immunogold labelling (FRIL) technique combines the identification of specific proteins with a high resolution of channel ultrastructure. We here analysed how C-terminal connexin phosphorylation / dephosphorylation or the deletion of the C-terminus affect channel morphology and function.

We compared three models for C-terminal modification: I. Impairing the homeostasis of cell cultures by stimulation with sucrose of high osmolarity. II Expression of a C-terminal deletion mutant of Cx43 via transient transfection. III Inhibition of connexin phosphorylation using protein kinase inhibition. Cell cultures of all models were analysed for expression of Cx43 by immunoblotting. GJ function was analysed by dye transfer assays. FRIL experiments were performed for analyses of channel morphology (e.g. particle density, nearest neighbour distance) at the ultrastructural level.

Single steps of GJ particle transport into the membrane were visualized by analysis of freeze-fractured membranes. Sucrose induced phosphorylation of Cx43 coinciding with a loss of GJ function, comparable to C terminal deletion. Dephosphorylation sustained GJ function under hyperosmolar conditions but could not prevent the sucrose-induced disaggregation of particles.

C-terminal protein modification impacts the morphology and function of Cx43 gap junctions. This could be an approach for regulating cellular communication in terms of treatment e.g. after ischemia or brain trauma.

Poster 55:

Title:
Of mice and men – and guinea pigs! A new strategy to study X-linked Kallmann syndrome

Authors:
Agnes Venghaus (Anatomy and Cell Biology, Saarland University, Homburg), Gabriela Krasteva-Christ (Anatomy and Cell Biology, Saarland University, Homburg), Stephan Maxeiner (Anatomy and Cell Biology, Saarland University, Homburg); s8agveng@stud.uni-saarland.de

Abstract:
The human ANOS1 gene, anosmia-1, is localized on the X-chromosome at the border to the pseudoautosomal region present on both sex chromosomes. Its gene product participates in neuronal migration of olfactory, hypothalamic, and cerebellar neurons during brain development. Its absence results in X-linked Kallmann syndrome. Generally, Kallmann syndrome is characterized by the inability to smell, i.e., anosmia, and GnRH hormone deficiency-induced hypogonadotropic hypogonadism (HH). Despite its clinical importance, its biological function remains elusive due to its absence in mouse and rats. We identified ANOS1 in guinea pigs reminiscent of its human orthologue. Now, this enables us to scrutinize ANOS1 protein function, tissue distribution and cellular localization in an alternative rodent research model.

We employed bioinformatical tools, e.g., genomic sequence comparison and phylogenetic tree modelling, to determine its presence in rodents. The identification of ANOS1 in the guinea pig genome prompted us to further assess transcript levels in a collection of over twenty-five tissue samples by means of qRT-PCR.

Phylogenetic analyses revealed that ANOS1 is absent in all eumuroida (26.2% of all mammals). This indicates that popular research animals (mouse/rat/hamster) are not available as research models for ANOS1 studies. However, we were able to determine its presence in guinea pigs. ANOS1 transcript levels were analyzed in a wide range of tissues and indicated abundant presence in neuronal, respiratory, and cardiovascular tissues.

Analyses of ANOS1 tissue distribution, cellular localization and physiological function in guinea pigs are eventually a novel avenue in addressing the genetic causes underlying the etiology of X-linked Kallmann syndrome.

Poster 56:

Title:
Recombinant production and structural characterization of human CD109

Authors:
Simon Bub (Department of Molecular Neurology, University Hospital Erlangen, Erlangen), Wiebke Lückstädt (Anatomical Institute, Christian-Albrechts-University Kiel, Kiel), Jan-Philipp Dobert (Department of Molecular Neurology, University Hospital Erlangen, Erlangen), Ralph Lucius (Anatomical Institute, Christian-Albrechts-University Kiel, Kiel), Friederike Zunke (Department of Molecular Neurology, University Hospital Erlangen, Erlangen), Philipp Arnold (Institute of Anatomy, Functional and Clinical Anatomy, Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Erlangen); philipp.arnold@fau.de

Abstract:
In recent years, CD109 was a focus of numerous studies because of its co-receptor character and the role as a pro-oncogenic factor in the progression of various cancers. A protein structure of CD109 that could answer many open questions about the functionality and protein-protein interactions is missing. To approach this question, large-scale production of recombinant CD109 had to be established first.

Accordingly, the expression construct for a human CD109 as a soluble variant of CD109 (sCD109) without the glycosylphosphatidylinositol anchor, which connects CD109 to the cell surface, was generated using site-directed mutagenesis. Large-scale production of sCD109, was performed in Hek293F cells and purified by affinity and size exclusion chromatography. The structural characterization of sCD109 was based on an imaging approach of negative stain single-particle electron microscopy (EM). The determined three-dimensional EM map was verified with the structural models based on homology modeling using SwissModel.

The recombinant sCD109 showed a molecular weight of approximately 190 kDa. The yield of the purified protein reached a concentration of up to 1 mg/ml. The EM-map after 3D reconstruction resulted in a low-resolution 3D EM-model with 15 Å. Verification of homology modeling via QMEAN Z-score and Ramachandran plot analysis revealed the highest sequence identity for thioester binding protein 1 from Drosophila, an ortholog to CD109.

In this study, we demonstrate a method for large-scale production of recombinant CD109 and present the first low-resolution structure. Further investigations are pending to solve the structure at atomic resolution to determine potential therapeutic target sites for interventional studies.

Poster 57:

Title:
Purification of full-length meprin β for structural analysis

Authors:
Wenjia Li (, Anatomie Lehrstuhl II Friedrich-Alexander Universität Erlangen, Erlangen), Philipp Arnold (, Anatomie Lehrstuhl II Friedrich-Alexander Universität Erlangen, Erlangen); philipp.arnold@fau.de

Abstract:
The metalloprotease meprin β is involved in various physiological and pathological conditions due to its wide range of proteolytic substrates, such as the amyloid precursor protein (APP), pro-collagen-I and the IL-6 receptor (IL-6R). Although structures for the ectodomain of meprin β were reported, the structure of the full-length protein is unknown. Ectodomain structures suggest a conformational switch upon activation of meprin β and this switch prevents shedding of meprin β from the cell surface by ADAM proteases. Hence, unraveling the full-length structure of pro- and active meprin β will be an important step towards a detailed understanding of conformational variability during meprin β activation.

Here, recombinant full-length meprin β with C-terminal His-tag was expressed in HEK 293T cell. Purified full-length meprin β carrying the transmembrane helices was then reconstituted into SMALPs (Styrene Maleic Acid Lipid Particles), an artificial cell-membrane-like polymer, to simulate the milieu of cell membrane.

With pro-meprin β and active meprin β in SMALP, TEM images were taken and particles were selected for single-particle reconstruction.

Here we present the protocol for successful expression and purification of full-length meprin β, its activation in SMAPLs and first results from structural analysis.

Poster 58:

Title:
The release of the metalloproteinase meprin beta onto extracellular vesicles

Authors:
Simon Schomburg (Functional and Clinical Anatomy, Institute of Anatomy II, Erlangen), Wenjia Li (Functional and Clinical Anatomy, Institute of Anatomy II, Erlangen), Philipp Arnold (Functional and Clinical Anatomy, Institute of Anatomy II, Erlangen); philipp.arnold@fau.de

Abstract:
The idiopathic inflammatory bowel disease (IBD) has been increasing in its incidence over the last decades and comes along with an enormous social and economic impact especially for younger patients. The full understanding of the role that the metalloproteinase Meprin beta plays is unclear, but it was found transcriptionally downregulated in IBD patients. It is important for mucus release in the small intestine as a shed soluble version. We aim to identify whether meprin beta can also be sorted to extracellular vesicles and how this changes proteolytic power.

Purification of extracellular vesicles from stimulated cell culture samples, murine intestinal tissue and chyme combined with biochemical analysis, immunofluorescent and electron microscopy imaging technics, we aim to identify conditions and factors that play a role for the release of meprin beta onto exosomes and if this is compartment specific for the intestinal tract.

We successfully showed in cell culture experiments, that loss of meprin beta sheddases ADAM10 and ADAM17 induces the release of meprin on extracellular vesicles. Stable overexpression of meprin beta in ADAM10/17 deficient and control wild type cells allows to test pro-inflammatory stimuli for their ability to induce meprin beta release on extracellular vesicles. Isolation of extracellular vesicles from the intestine and chyme, will help to identify a meprin beta specific release signature.

Here, we propose that the loss of ADAM10 and ADAM17 induces exosomal release of meprin beta and hence contribute to a changed proteolytic cleavage pattern of the intestinal mucosa, which might influence IBD development.

Poster 59:

Title:
Neuro-mesenchymal organoids as in vitro model system for peripheral nervous system development.

Authors:
Anna Rockel (Anatomy and cell biology, University of Würzburg, Würzburg), Süleyman Ergün (Anatomy and cell biology, University of Würzburg, Würzburg), Philipp Wörsdörfer (Anatomy and cell biology Uni Wuerzburg, University of Würzburg, Würzburg);
anna.rockel@stud-mail.uni-wuerzburg.de

Abstract:
Human induced pluripotent stem cell (iPSC) derived organoids allow the study of human developmental biology in vitro. The aim of this study was the generation of a novel organoid model recapitulating neural crest cell migration and peripheral nervous system (PNS) development.

The organoid model was generated by co-culturing a neural differentiated and a mesenchymal differentiated cell aggregate, inducing the formation of a neuro-mesenchymal interface. Resulting organoids were grown for up to 60 days in suspension culture and analyzed using paraffin sections, qPCR as well as tissue clearing.

We observed the appearance and delamination of neural crest cells (NCCs) in the neural compartment of the organoid and their migration into the mesenchymal part. In the mesenchyme, NCCs give rise to cell types of the PNS such as sensory neurons and Schwann cells. Immunofluorescence analyses revealed the formation of ganglion-like structures at the neuro-mesenchymal interface. The ganglion cells co-expressed Peripherin, Brn3a and Isl1, indicating sensory neurons. Axons grow into the mesenchymal part, forming a neural network accompanied by Sox10/Gap43 double-positive Schwann cells. Simultaneously, a primitive vascular plexus develops within the mesenchymal part, that interacts closely with the sensory nerve fibers. Treatment of organoids with retinoic acid resulted in a reduced amount of Sox10-positive migrating NCCs, mimicking fetal retinoid syndrome (FRS).

This study describes a novel in vitro tissue model for NCC delamination, migration and peripheral nervous system development. This organoid model represents an attractive platform to test defects in NCC delamination and migration such as FRS.

Poster 60:

Title:
Influence of PU.1, Fra2, TBX4 and meprin BETA expression in HEK293T cells, on the expression of extracellular matrix coding genes; stu210504@mail.uni-kiel.de

Authors:
Antonia Schulz (Anatomisches Institut, Christian-Albrechts-Universität Kiel, Kiel), Wenjia Li (Institute of Functional and Clinical Anatomy, Friedrich-Alexander-Universität Erlangen, Erlangen), Ralph Lucius (Anatomisches Institut, Christian-Albrechts-Universität Kiel, Kiel), Philipp Arnold (Institute of Functional and Clinical Anatomy, Friedrich-Alexander-Universität Erlangen, Erlangen)

Abstract:
PU.1/SPI1 is a transcription factor belonging to the ETS-family and upregulation is associated with a pro-fibrotic phenotype. The AP-1-family transcription factor Fra2 is upregulated in Systemic sclerosis patients and the mesenchymal-specific transcription factor T-box-gene 4 (TBX4) is a leading factor in myo-fibroblast accumulation. Meprin BETA is a zinc-endopeptidases, which is a contributor in chronic inflammations, certain cancers and fibrosis. Specifically, it maturates pro-collagen I and thus induces collagen fiber assembly. Through its shedding activity at the cell surface, it can also trigger changes in cell signaling.

To investigate the influence of these factors on the genetic expression of genes associated with the collagen turnover machinery we expressed these in HEK293T cells and measured associated changes by qRT-PCR. In spheroid assays we also measured changes in size and used histology to determine extra-cellular matrix deposition. All data was analyzed using one-way or two-way ANOVA.

We found a significant increase for aSMA, Meprin BETA, TBX4, Fra2, BMP1 and MMP9 in PU.1 transfected cells. In Fra2 transfected cells we revealed a significant upregulation for meprin BETA, TBX4, PU.1, aSMA, BMP1 and MMP9. For TBX4 transfected cells we measured a significant increase of aSMA, PU.1, BMP1 and MMP9. We identified a significant upregulation of PU.1, aSMA, Fra2 and TBX4 in meprin BETA transfected cells.

Here we show that aSMA, BMP1 and MMP9 are commonly upregulated by TBX4, Fra2 and PU.1. Furthermore, we found, that the transcription factors are upregulating each other and that meprin BETA influences transcriptional upregulation of the fibrosis associated transcription factors.

Poster 61:

Title:
Characterization of SP-G, SP-H in the human larynx

Authors:
Aurelius Scheer (Department of Anatomy II, Friedrich Alexander University Erlangen-Nürnberg, Erlangen), Martin Schicht (Department of Anatomy II, Friedrich Alexander University Erlangen-Nürnberg, Erlangen), Friedrich Paulsen (Department of Anatomy II, Friedrich Alexander University Erlangen-Nürnberg, Erlangen), Antoniu-Oreste Gostian (Department of Otolaryngology - Head & Neck Surgery, University Hospital Erlangen, Erlangen), Lars Bräuer (Department of Anatomy II, Friedrich Alexander University Erlangen-Nürnberg, Erlangen); aurelius.scheer@fau.de

Abstract:
Surfactant proteins (SP) were first described in the lung and moreover in other parts of the human body, e.g. in the human larynx. Especially SP-A, -D, -G and -H have been shown to play an important role in the innate immune system. The objective of this study was to detect and characterize the expression and distribution of SP-G, SP-H in the human larynx.

Mucus of vocal-folds was obtained from patients during panendoscopy. Expression and distribution of SP's in healthy human tissue of vocal-folds and in laryngeal carcinoma was analyzed using immunofluorescence (IF), the expression of SP-mRNA in an immortalized human laryngopharynx cell line (FaDu HTB-43TM) was characterized by RT-PCR. The possible role of the SP-G and -H during wound healing was investigated by means of a scratch assay and stimulation with cortisol, serotonin and bombesin. After assay and stimulation the mRNA-expression was quantified using qRT-PCR.

The presence of SP-G and SP-H were demonstrated in laryngeal mucus and squamous epithelium of the vocal fold. IF revealed an increased expression of SP-G in case of laryngeal carcinoma. Mechanical stress (scratch assay) and stimulation induced the expression of SP-G in the laryngeal cell line.

The results demonstrated that SP-G, SP-H are present within the epithelium and mucus of the vocal fold and therefore part of the human larynx. Based on our results, we assume that the proteins may play an important role during wound healing and tumor pathogenesis in the human larynx respectively the vocal fold.

Poster 62:

Title:
Nuclear factor erythroid 2–related factor 2 (Nrf2) deficiency causes age-dependent progression of osteoporosis in female mice

Authors:
Yusuke Kubo (Anatomy and Cell Biology, RWTH Aachen University, , Aachen), Rainer Beckmann (Anatomy and Cell Biology, RWTH Aachen University, , Aachen), Abraham Herrera (Anatomy and Cell Biology, RWTH Aachen University, , Aachen), Katharina Szymanski (Anatomy and Cell Biology, RWTH Aachen University, , Aachen), Stefanie Rosenhain (Institute for Experimental Molecular Imaging, Helmholtz Institute for Biomedical Engineering, RWTH Aachen University, , Aachen), Marek Weiler (Institute for Experimental Molecular Imaging, Helmholtz Institute for Biomedical Engineering, RWTH Aachen University, , Aachen), Athanassios Fragoulis (Anatomy and Cell Biology, RWTH Aachen University, , Aachen), Alexander Slowik (Anatomy and Cell Biology, RWTH Aachen University, , Aachen), Felix Gremse (Institute for Experimental Molecular Imaging, Helmholtz Institute for Biomedical Engineering, RWTH Aachen University, , Aachen), Christoph Jan Wruck (Anatomy and Cell Biology, RWTH Aachen University, , Aachen), Holger Jahr (Anatomy and Cell Biology, RWTH Aachen University, , Aachen), Thomas Pufe (Anatomy and Cell Biology, RWTH Aachen University, , Aachen), Mersedeh Tohidnezhad (Anatomy and Cell Biology, RWTH Aachen University, , Aachen); ykubo@ukaachen.de

Abstract:
Nrf2 is crucial for maintaining bone metabolism, but its association with osteoporosis in elderly female has yet to be fully described. The aim was to investigate age-dependent role of Nrf2 on long bone in female mice focused on osteoporosis.

Eighteen female wild type (WT) and 12 Nrf2-knockout (KO) mice was used at different time points; “young adult=12-week-old" and “old=90-week-old”. Trabecular parameters in condyle and cortical parameters in mid-shaft were measured by scanning extracted femurs on µCT. Aromatase expression in osteocytes in cortical bone was also evaluated on immunohistochemistry. Furthermore, osteoblasts were isolated from calvaria of female adult WT and KO mice to investigate their mineralization function.

Trabecular bone mineral density (BMD) and cortical thickness (Ct.Th) and area (Ct.Ar) in old KO mice were significantly lower than old WT mice, while there was no significant difference between young adult WT and KO mice. Osteoblasts from KO mice showed lower mineralization compared to WT mice by Alizarin-red staining. Significantly lower aromatase-positive osteocytes of old KO mice were observed compared to WT mice.

The µCT data showed lower trabecular/cortical bone quantity in old female KO mice, and lower aromatase expression in osteocytes in old KO mice may indicate the state of a lack of estrogen in cortical bone in old KO mice. Lower mineralization in osteoblasts from KO mice indicates the possibility that loss of Nrf2 may reduce bone acquisition in females. These results suggest that chronic Nrf2 deficiency can lead to age-dependent progression of osteoporosis in female.

Poster 63:

Title:
Protective effect of Methysticin on osteoblast function under oxidative stress through Nrf2 signaling pathway: in vitro study

Authors:
Yusuke Kubo (Anatomy and Cell Biology, RWTH Aachen University, , Aachen), Heinrich Dick (Anatomy and Cell Biology, RWTH Aachen University, , Aachen), Athanassios Fragoulis (Anatomy and Cell Biology, RWTH Aachen University, , Aachen), Mohammad Nour Al Asmi (Anatomy and Cell Biology, RWTH Aachen University, , Aachen), Holger Jahr (Anatomy and Cell Biology, RWTH Aachen University, , Aachen), Mersedeh Tohidnezhad (Anatomy and Cell Biology, RWTH Aachen University, , Aachen), Christoph Jan Wruck (Anatomy and Cell Biology, RWTH Aachen University, , Aachen), Thomas Pufe (Anatomy and Cell Biology, RWTH Aachen University, , Aachen); ykubo@ukaachen.de

Abstract:
Nrf2 is responsible for regulating fracture healing, but the effect of pharmacological Nrf2 induction on bone healing is still unknown. We investigated the effect of Nrf2-inducer on osteoblasts under oxidative stress.

Murine MC3T3-E1 preosteoblasts were used. Oxidative stress was induced by hydrogen peroxide (H2O2) injection. Methysticin was used as a Nrf2-inducer. Nrf2-target genes (Ho-1,Nqo1) were evaluated by PCR after methysticin injection. Cell Proliferation was evaluated by CyQUANT® Assay. Osteoblast mineralization was evaluated 28 days after the conversion to differentiation medium by Alizarin-red staining. The protein levels of vascular endothelial growth factor (VEGF) and osteocalcin on day 28 were measured by ELISA.

H2O2 (50,100,200,400μM) reduced cell proliferation. High amount of methysticin (25,50,100μM) reduced cell proliferation, while a low amount (5μM) did not reduce. Ho-1 and Nqo1 mRNA expressions were significantly increased after methysticin injection. Cell proliferation with 5μM methysticin treatment was significantly higher under H2O2 (50μM) than without treatment. High amount of H2O2 (100,200,400μM) significantly decreased mineralization, while 5μM methysticin significantly increased mineralization under these H2O2 conditions compared to without treatment. VEGF expression with methysticin treatment was significantly higher than without treatment under H2O2 (50,100μM), and osteocalcin expression with methysticin treatment was significantly higher than without treatment under H2O2 (200μM).

Excessive H2O2 inhibited osteoblast proliferation and mineralization, while methysticin treatment elevated these osteoblast functions and markers such as VEGF and osteocalcin through enhanced Nrf2-target genes expression. These results indicate that pharmacological Nrf2 induction can have a potential role to protect osteoblast function from oxidative stress.

Poster 64:

Title:
TAM regulate susceptibility to PD1 inhibitors

Absage, das Poster wird nicht präsentiert

Poster 65:

Title:
PD-1 inhibition in adenocarcinoma of gastric cancer

Authors:
Ngoc Anh Hoang (University Cancer Center Leipzig, University of Leipzig, Leipzig), Marlon Hußtegge (Institute for Anatomy, University of Leipzig, Leipzig), Jakob Rebstock (Institute for Anatomy, University of Leipzig, Leipzig), Astrid Monecke (Institute for Pathology, University Hospital Leipzig, Leipzig), Ines Gockel (Department of Visceral, Transplantation, Thoracic and Vascular Surgery, University Hospital Leipzig, Leipzig), Arved Weimann (Department for General and Visceral Surgery, Hospital St. Georf, Leipzig), Guido Schumacher (Department for General and Visceral Surgery, Hospital Braunschweig, Braunschweig), Florian Lordick (University Cancer Center Leipzig, University Hospital Leipzig, Leipzig), Justus Körfer (University Cancer Center Leipzig, University Hospital Leipzig, Leipzig), Sonja Kallendrusch (Institute for Anatomy, University of Leipzig, Leipzig); Ngoc.Hoang@medizin.uni-leipzig.de

Abstract:
Moderate response rates of PD-1 inhibition in gastric and esophagogastric junction cancers urge for meaningful human model systems that allow for investigating immune responses ex vivo.

The standardized patient derived tissue culture (PDTC) model was utilized to investigate the tumor response to the PD-1 inhibitor Nivolumab and the CD3/CD28 t-lymphocyte activator ImmunoCult™. Resident t-lymphocytes, tumor proliferation and apoptosis, as well as bulk gene expression data were analysed after 72 hours of PD-1 inhibition either as monotherapy or combined with Oxaliplatin or ImmunoCult™. Additionally, the supernatants of the last 48 hours were collected and cytokine concentrations are measured based on Luminex ® technology.

Individual responses to PD-1 inhibition were found ex vivo and combination with chemotherapy or t-lymphocyte activation led to enhanced antitumoral effects in PDTCs. T-lymphocyte activation. Gene signatures for Interferon γ-mediated inflammation and immune cell localization were identified and found to be relevant indicators for tissue response upon immune checkpoint inhibition. We observed alterations in cytokine concentrations like interferon γ, Interleukin 10, Granzyme A and B.

Within this complex tissue set-up, yielding an organotypic cell composition, we were able to demonstrate individual response patterns and immunological reactivity. PDTCs are potent to define biological marker sets ex vivo and to differentiate and predict clinical patient response as well as their inherent immune cell capacity.

Poster 66:

Title:
The effect of IL-17 and combined mechanical injury on meniscal tissue integrity in vitro

Authors:
Greta Ahrens (Department of Anatomy, University of Kiel, CAU, Kiel), Bodo Kurz (Department of Anatomy, University of Kiel, CAU, Kiel), Florian Gellhaus (Department of Anatomy, University of Kiel, CAU, Kiel), Peter Behrendt (Department of Orthopedics and Trauma Surgery, University Medical Center Schleswig-Holstein Campus Kiel, Kiel), Jan-Tobias Weitkamp (Department of med. oral and maxillofacial surgery, University Medical Center Schleswig-Holstein Campus Kiel, Kiel), Bernd Rolauffs (G.E.R.N. Research Center for Tissue Replacement, Regeneration & Neogenesis, Department of Orthopedicsand Trauma Surgery, Faculty of Medicine, Medical Center—Albert-Ludwigs-University of Freiburg,, Freiburg im Breisgau), Alan Grodzinksky (Departments of Biological Engineering, Electrical Engineering and Computer Science and Mechanical Engineering, Massachusetts Institute of Technology, Cambridge); g.ahrens@anat.uni-kiel.de

Abstract:
Inflammation in a joint as well as mechanical injury can cause meniscus degradation and further lead to osteoarthritis (OA). Elevated synovial levels of IL-17 have been measured in OA patients. Therefore, the aim of this study was to investigate the effect of IL-17 on meniscal tissue with and without combined mechanical injury.

Bovine meniscus explants were stimulated with IL-17A (0-100 ng/ml) and mechanical injury (single 50% compression, strain rate 1mm/sec). After 72 hours sGAG (DMMB assay), aggrecanase-specific neoepitope NITEGE (immunohistochemistry), NO release (Griess reagent), cell death (histomorphological analysis of nuclear blebbing: apoptosis), and mRNA levels of MMP-13, -3, ADAMTS-4 and collagen I, II (qRT-PCR) were measured. Statistics: one-way ANOVA with Kruskal-Wallis-post hoc test.

IL-17 increased sGAG release in a dose-dependent manner (significant effect at 100 ng/ml; 10 ng/ml p=0.076). Apoptosis increased with a significant peak at 1 ng/ml only ($p = 0.0488$). MMP-13, -3, ADAMTS-4, and COL2 mRNA levels increased non-significantly (n=5). There was no influence on the NO release.
Increased sGAG release and apoptosis by mechanical injury increased further significantly by addition of 10 ng/ml IL-17. The combination of stimuli also increased MMP-13- ($p = 0.0046$) and ADAMTS-4-levels ($p = 0.0092$) as well as NITEGE-signals (breakdown of aggrecan).

IL-17 induces matrix degeneration and cell death in meniscal tissue which in combination with a trauma intensifies. This might create a post-traumatic environment that promotes tissue degeneration and OA.

Poster 67:

Title:
Cyclic stretch causes a strong spheroid interaction for enthesis formation in vitro

Authors:
Clemens Leo Gögele (Institute of Anatomy and Cell Biology, Nuremberg, Paracelsus Medical University, Nuremberg), Julia Vogt (Institute of Anatomy and Cell Biology, Nuremberg, Paracelsus Medical University, Nuremberg), Jens Konrad (Institute of Biological Information Processing: IBI-2, Forschungszentrum Jülich GmbH, Jülich), Rudolf Merkel (Institute of Biological Information Processing: IBI-2, Forschungszentrum Jülich GmbH, Jülich), Kerstin Schäfer-Eckart (Bone marrow Transplantation Unit, Medizinische Klinik 5, Klinikum Nürnberg, Nuremberg), Bernd Hoffmann (Institute of Biological Information Processing: IBI-2, Forschungszentrum Jülich GmbH, Jülich), Gundula Schulze-Tanzil (Institute of Anatomy and Cell Biology, Nuremberg, Paracelsus Medical University, Nuremberg);
clemens.goegele@pmu.ac.at

Abstract:
The anterior cruciate ligament (ACL) is attached to bone via two entheses consisting of ligament, non- and mineralized fibrocartilage zones. The effects of mechanical loading on the different cell types are largely unknown. Therefore, the aim of this study was to gain a better understanding of the interaction of ligamentocytes with chondrogenically or osteogenically differentiated mesenchymal stromal cells (MSCs) under the influence of uniaxial cyclic strain.

Spheroids (consisting of 2.5x10^4 cells) were prepared from lapine cruciate ligamentocytes (LCL) and from 35-day chondrogenically or osteogenically differentiated human (h) MSCs. Spheroids were placed in GelTrex(R) coated crosslinked polydimethyl siloxane (PDMS) elastomeric chambers. After a 48 h adherence phase cultures underwent cyclic uniaxial stimulation (14% and 0.3 Hz) for 48 h. Cell viability, distribution, cytoskeletal stress fibers orientation and especially their tissue-specific protein and gene expression (for ligamentocytes: decorin, type I collagen and Mohawk; for cartilage: type II collagen, aggrecan and SOX9; for bone: type I and type X collagen and RUNX2 were analyzed.

During the 35-day differentiation phase cell vitality was maintained, but there was a decrease in spheroid diameter compared to the undifferentiated spheroids. The cells emigrating from the stimulated spheroids on the elastomeric chamber and also their F-actin stress fibers covered a higher area, were oriented against the stretch direction and interacted more strongly compared to the unstretched controls. Mechanostimulation induced not only maintenance but also increased expression of tissue-specific matrix proteins.

In the future, further stretching programs will be carried out in order to increase matrix formation.

Poster 68:

Title:
Modeling kidney disease in directly reprogrammed and bioprinted renal cells.

Authors:
Roman Pichler (Department of Medicine, Renal Division, University Medical Center Freiburg, Freiburg), Ludovica Rizzo (Institute of Anatomy, University of Zurich, Zürich), Kevin Tröndle (Laboratory for MEMS Applications, IMTEK - Department of Microsystems Engineering, University of Freiburg, Freiburg), Soeren Lienkamp (Institute of Anatomy, University of Zurich, Zürich); soeren.lienkamp@uzh.ch

Abstract:
Conventional cellular models of renal tubular origin only partially maintain their functional properties. We previously developed a method to directly reprogram fibroblasts into induced renal epithelial-like cells (iRECs) using four transcription factors. The objective of this study is to establish in vitro models for genetic renal diseases, and determine what microenvironmental factors enhances the physiological state of bioprinted tubules.

Mouse embryonic fibroblasts carrying a floxed allele of the Pkd1 locus were reprogrammed with the factors Emx2, Hnf1b, Hnf4a and Pax8 to iRECs. Knockout clones were induced using Cre-Recombinase and cells were embedded in Fibrinogen, Collagen, Matrigel, or bioprinted using a drop-on-demand technology. Viability, expression profile and morphology was analyzed at various time-points and in the wild-type or Pkd1 knockout conditions.

All biomaterials were compatible with embedding iRECs and bioprinting. However, the microenvironment had a strong effect on the morphology and transcriptome, including segment specific expression signatures of reprogrammed cells. Pkd1 knockout cells revealed a phenotype of spiked side-branching in bioprinted tubules.

The choice of embedding materials influences cellular identity and can be tailored to fit desired experimental conditions. Bioprinting elicits cellular phenotypes in an in vitro model of polycystic kidney disease not present in conventional culture conditions. This may facilitate scalable fabrication of tubule arrays for screening and drug development.

Poster 69:

Title:
Temporal development of nociceptive tracts in chick embryos and foetuses

Authors:
Longfei Cheng (Department of Neuroanatomy,, Institute of Anatomy, University of Bonn, Germany, Bonn), Inga Tiemann (, Institute of Animal Science, University of Bonn, Germany, Bonn), Ruijin Huang (, , Bonn); cheng110055@gmail.com

Abstract:
The common practice of killing male day-old chicks from laying lines of the domestic chicken due to a lack of economic viability has been increasingly discussed socially and above all ethically. In this context, politicians in Germany decided in early 2021 to be the first country worldwide to ban this practice. Up to date, there is no reliable experimental data to demonstrate on which incubation day the chick embryos and foetus acquire the ability to feel pain. In this project, we will determine the developing time course of nociceptive tracts.

To trace the lateral spinothalamic tract, eGFP was electroporated into the dorsolateral quadrant of the neural tube at the lumbosacral level in chick embryos. After different reincubation periods, eGFP labelled tracts were identified in the isolated spinal cord and brain.

The lateral spinothalamic tract marked by green fluorescent gradually ascended from the eGFP labelled spinal cord segments. At the embryonic day 6, the eGFP-tract ascended for only few segments. Just at day 9, it approached the hindbrain.

The formation of the nociceptive tract in chick embryos and foetuses requires a much longer time than 6 embryonic days, as supposed in references. This suggests that the nociceptive development extends at least to the second half of the fetal period.

Poster 70:

Title:
Guidance function of sensory axons on motor axons during the cranial nerve formation in chick embryos

Authors:
Peng Zhou (Department of Neuroanatomy, , Bonn), Ruijin Huang (Department of Neuroanatomy, , Bonn); zhou19931001@gmail.com

Abstract:
Some cranial nerves, such as trigeminal, facial, glossopharyngeal and vagal nerves, are made up of motor and sensory axons. Up to date, it has to be clarified whether and how these components interact during cranial nerve formation. Several lines of evidence suggested that sensory ganglia are required for motor axon formation, including axonal guidance and growth promotion. We have previously shown in vitro that sensory ganglia exert neurotrophic factors to promote motor axon growth in the head. In this study, we will further investigate whether and how sensory ganglia guide motor axons.

Motor and sensory axons were labelled by electroporation of RFP and GFP constructs into the ventral neural tube and nodose/glossopharyngeal placode, respectively. Ganglion anlagen were extirpated at early developing stages of chick embryos.

Our results showed that without their ganglia, motor axons of the vagus and glossopharyngeus nerve did not project ventrally. Moreover, the in- and outgrowth of their sensory and motor axons occurred synchronously.

Sensory axons guide the ventral projection of motor axons during cranial nerve formation in chick embryos

Poster 71:

Title:
Role of Plexin-A2 during the hypoglossal nerve formation in chick embryos

Authors:
Hengxun Tao (Department of Neuroanatomy, University of Bonn, Bonn), Ruijin Huang (, University of Bonn, Bonn); s6hetaoo@uni-bonn.de

Abstract:
Plexin and Semaphorin signaling acts as mediators of repulsive signals in cell migration and axon guidance. Plexin-A2 has been shown to regulate the axonal guidance of the somatomotor axons at the spinal cord level. But it is still unknown whether and how plexin-A2 regulates the somatomotor axon guidance at the hindbrain level.

The plexin-A2 gene expression in hypoglossal neurons was selectively silenced by electroporation of the shRNA construct into the ventral neural tube at the post-otic hindbrain level.

We showed that loss of function of plexin-A2 led to the reduction of hypoglossal motor neurons and seriously defasciculation of their axons.

Plexin-A2 regulates axonal guidance during hypoglossal nerve formation.