SCHWEIZERISCHE GESELLSCHAFT FÜR ANATOMIE, HISTOLOGIE UND EMBRYOLOGIE SGAHE

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80. Jahresversammlung SGAHE

80ème réunion annuelle SSAHE

80th Annual Meeting SSAHE

Freiburg, den 7. September 2018

Fribourg le 7 septembre 2018

Fribourg, September 7, 2018

UNI FR
UNIVERSITÉ DE FRIBOURG
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Venue: Anatomy Per03 Building, Perolles Campus

Anatomy, Faculty of Science and Medicine, University of Fribourg

Route Albert Gockel 1, CH – 1700 Fribourg

Programm/Programme/Program

09h00 – 09h30	Welcome and coffee / Empfang mit Kaffee /Accueil avec café (Per03)
09h30 – 09h40	Opening of meeting / Eröffnung der Tagung / Ouverture de la réunion (R16)
09h45 – 10h30	Professor Carla Stecco (Italy): Fascial Anatomy
10h30 – 11h15	Professor Bernat Soria (Spain): Regenerative Medicine
11h15 – 12h00	Professor Marc Vorstenbosch (The Netherlands): Anatomy Education
12h00 – 13h30	Lunch buffet (1st floor), Poster Session (2nd floor) and Sponsor exhibition (ground floor)
13h30 – 14h30	Members' meeting / Geschäftssitzung / Session administrative
14h30-15h45	Awards, Prizes and corresponding presentations
15h45-16h00	Closing remarks / Schlussbemerkungen / Clôture (Prof. B. Schwaller)
16h00	End of annual meeting / Ende der Jahresversammlung / Fin de la réunion

Stecco Carla, MD
Orthopedic Surgeon, Professor of Human Anatomy and Movement Sciences at the University of Padova, Italy. Member of the Italian Society of Anatomy and Histology. Scientific activity devoted to the study of the anatomy of the human fasciae from a macroscopical, histological and physiopathological point of view.
carla.stecco@unipd.it

Bernat Soria Escoms, MD, PhD
Extraordinary Professor of Regenerative Medicine at the Universidad Pablo Olavide (Seville) and Director of the Department of Cell Therapy and Regeneration of the Andalusian Center for Molecular Biology and Regenerative Medicine (CABIMER) in Seville, Spain. He pioneered generaton of insulin-producing pancreatic cells from mouse stem cells.
bernat.soria@cabimer.es

Marc ATM Vorstenbosch, PhD
Department of Anatomy, Radboud University Medical Centre, Nijmegen, The Netherlands. Marc does research in Curriculum Theory, Didactics and Educational Assessment. Their current project is 'Cognitive load theory and the design of education'.
Marc.Vorstenbosch@radboudumc.nl

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Notes:

Poster Abstracts

1. Anatomical, Histological and Ultrasound Topography of the Tibialis Posterior Muscle: a Focus on its Innervation and Neuromuscular Junctions

Valerie **Aurore**[1], Peter Yotovski[1], Serdar Koçer[2], Luis Filgueira[1]
[1]University of Fribourg, [2]Jura Hospital in Porrentruy

Cerebrovascular diseases including strokes are one of the leading causes of death and morbidity worldwide, affecting a variety of people including younger adults and having devastating consequences such as increased muscle tone in as much as 40% of patients. This results in limb spasticity and impairment of correct walking gait. Botulinum toxin is a well-known treatment against muscle spasticity, but although dosage and concentration has been extensively researched, location of ultrasound assisted injections, in particular in the tibialis posterior muscle remains unclear. This study focuses on the gross anatomy and histology of the tibialis posterior muscle. Ultrasound imaging was compared with dissection appearance of the muscle. In addition, its external and internal innervation leading to the neuromuscular junctions was investigated. Four Thiel preserved lower limbs underwent ultrasound imaging of which two were macroscopically dissected. Histology and immune histochemistry was performed on one formalin sample to identify nerves and neuromuscular junctions. We found a good correlation between ultrasound imaging and anatomical findings. A branch of the tibialis nerve innervates the tibialis posterior muscle. As it reaches the proximal third it divides into various branches, one of which courses in both cases alongside the fibular edge of the tendon. The highest density of nerves branches was found at the border between the proximal first and second third of the tibialis posterior muscle. Neuromuscular junctions were found at the centre of the short muscle fibres.

2. The orbitofrontal cortex projects to the parvafox nucleus of the ventrolateral hypothalamus and to its targets in the ventromedial periaqueductal grey matter

Alexandre **Babalian**[1], Simone Eichenberger[1], Alessandro Bilella[1], Franck Girard[1], Viktoria Szabolcsi[1], Diana Roccaro[1], Gonzalo Alvarez-Bolado[2], Chun Xu[3], and Marco R. Celio[1]*
[1]Anatomy and Programme in Neuroscience, University of Fribourg, Rte. A. Gockel 1, CH-1700 Fribourg (Switzerland), [2]Institute of Anatomy and Cell Biology, University of Heidelberg, im Neuenheimer Feld 307, D-69120 Heidelberg (Germany), [3]Friedrich Miescher Institute, Maulbeerstrasse 66, CH-4058 Basel (Switzerland)

Although connections between the orbitofrontal cortex and the lateral hypothalamus have been recognized in the past, the precise targets of the descending fibres have not been identified.
By means of viral tracer-transport experiments in rodents, we demonstrate neurons of the orbitofrontal cortex (OFC) to project collateral axons to a distinct, horizontally oriented, cylindrical nucleus in the ventrolateral hypothalamus, namely, the parvalbumin- and Foxb1-immunoreactive ("parvafox") nucleus.
The loose bundle of fine, descending collaterals arises at the level of the tuberal hypothalamus, from thick OFC-derived axons coursing in the internal capsule. The collaterals contact the neurons of the parvafox nucleus through VGlut2-immunoreactive "boutons". In its further caudal course, the contingent of OFC-axons gives out collaterals that terminate in two elongated neuronal columns – the SU3 – and the PV2 nuclei –, which lie ventral to the aqueduct at the border of the periaqueductal grey matter (PAG). Here, the OFC-endings overlap with terminals of the VGlut2-immunoreactive "boutons" deriving from the hypothalamic parvafox nucleus itself.
The targeting of the parvafox nucleus by the OFC-projection, and the overlapping of their terminal fields within the PAG, suggest that the two cerebral sites are closely interacting. An involvement of this OFC-driven circuit in the autonomic manifestation of a behavioural event is conceivable.

3. Malaria derived extracellular vesicles inhibit neutrophils ROS production and NETs formation.

Kehinde Adebayo **Babatunde** [1], Michael Walch [1], Isabelle Fellay [1], Solange Kharoubi-Hess [1], Luis Filgueira [1], Ionita Ghiran [2], Pierre-Yves Mantel [1].
[1] Anatomy, University of Fribourg, Fribourg, Switzerland, [2] Allergy and Infection, Beth Israel Deaconess Medical Center, Boston, MA

A dysfunctional innate immune response is believed to provide immune evasion of the malaria parasites, but also to cause increased susceptibility to bacterial infections. Neutrophils are the most abundant cells found in

the blood circulation in direct contact with parasite infected red blood cells (iRBCs). However neutrophils population with reduced oxidative burst activities are present during malaria infection. These observations suggest that neutrophil responses are fundamentally defective in malaria patients. In this present work we investigated, how extracellular vesicles (EVs) derived from iRBCs and containing both parasite and host materials, including microRNAs, modulate neutrophil response by transferring regulatory micro-RNAs.

4. The ultimate near-peer teaching approach

Luis **Filgueira** [1], Elisabeth Eppler [2]

[1] University of Fribourg, [2] University of Basel

Anatomy education has an old tradition in embracing near-peer teaching where more advanced students contribute to practical courses and teach their younger peers under the supervision of expert teacher in anatomy. However, students have been seldom included in the development of new courses. In the context of this educational project, a student initiative to introduce a new abdominal ultrasound course of the normal anatomy by 3rd year medical students for their 2nd year peers, has been followed and evaluated.
A new abdominal ultrasound course of the normal anatomy was designed by 2nd and 3rd year medical students as an optional (elective) course for 20 2nd year participants taught by 8 3rd year peer-teachers, supported by an experienced anatomy teacher and by experienced experts in abdominal ultrasound. The course was approved by the Curriculum Committee. The peer-teachers were prepared and coached for their role by expert ultrasound teachers. The course was delivered in 6 x 3 hours blocks, including the following topics: (1) Major abdominal blood vessels, (2) biliary ways and pancreas, (3) liver, (4) spleen and abdominal urogenital organs, (5) FAST (Focused Assessment with Sonography for Trauma) and (6) Revision and practical exam. Each group and topic was taught in small groups of 5 students by one near-peer teacher. The course was evaluated anonymously through a standard questionnaire for student, as well as an online questionnaire for the students and another one for the peer-teachers. In addition, written feedback was evaluated.
The course got excellent feedback for content and delivery from the students, as well as from the near-peer teachers. At the end, all students were able to handle various models and brands of ultrasound machines and knew well how to do the basic ultrasound investigation of the abdomen and recognized all relevant anatomical structures. The peer-teachers also improved their ultrasound skills, as well as their teaching skills. Therefore, this course will continue to be offered in future semesters, organized and delivered by near-peer teachers.
We conclude from our very positive experience that student participation in the design and delivery of courses should be more often considered in the future.

5. 17-β estradiol increases parvalbumin levels in Pvalb heterozygous mice and attenuates behavioral phenotypes with relevance to autism core symptoms

Federica **Filice** [1]*, Emanuel Lauber [1]*, Karl Jakob Vörckel [2], Markus Wöhr [2], Beat Schwaller [1]

[1] Anatomy Unit, Department of Medicine, University of Fribourg, Route Albert-Gockel 1, CH-1700 Fribourg, Switzerland

[2] Behavioral Neuroscience, Faculty of Psychology, Philipps-University of Marburg, Gutenbergstraße 18, D-35032 Marburg, Germany

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders characterized by two core symptoms: impaired social interaction and communication, and restricted, repetitive behaviors and interests. The pathophysiology of ASD is not yet fully understood, due to a plethora of genetic and environmental risk factors that might be associated with or causal for ASD. Recent findings suggest that one putative convergent pathway for some forms of ASD might be the down-regulation of the calcium-binding protein parvalbumin (PV). PV-deficient mice (PV-/-, PV+/-), as well as Shank1-/-, Shank3-/- and VPA mice, which show behavioral deficits relevant to all human ASD core symptoms, are all characterized by lower PV expression levels.
Based on the hypothesis that PV expression might be increased by 17-β estradiol (E2), PV+/- mice were treated with E2 from postnatal days 5-15 and ASD-related behavior was tested between postnatal days 25-31. PV expression levels were significantly increased after E2 treatment and, concomitantly, sociability deficits in PV+/- mice in the direct reciprocal social interaction and the 3-chamber social approach assay, as well as repetitive behaviors, were attenuated. E2 treatment of PV+/+ mice did not increase PV levels and had detrimental effects on sociability and repetitive behavior. In PV-/- mice, E2 obviously did not affect PV levels; tested behaviors were not different from the ones in vehicle-treated PV-/- mice.

Our results suggest that the E2-linked amelioration of ASD-like behaviors is specifically occurring in PV+/- mice, indicating that PV upregulation is required for the E2-mediated rescue of ASD-relevant behavioral impairments.

6. Tarsal tunnel pressure increase after lateral sliding calcaneus osteotomies

Halm Sebastian, Tschanz Stefan1, Schneiter Martin, Djonov Valentin, Krause Fabian
University of Bern

BACKGROUND: Lateral sliding calcaneus osteotomies are common procedures to realign hindfoot varus deformities. Shifting the calcaneal tuberosity laterally (lateralization), can lead to tarsal tunnel pressure increase and tibial nerve palsy. The purpose of this cadaveric biomechanic study was to investigate the correlation of lateralization and pressure increase underneath the flexor retinaculum.
METHODS: Twelve Thiel-fixated human cadaveric lower legs were used. The pressure underneath the flexor retinaculum was measured in proximity to the tibial nerve, with and without lateralization, in neutral position, dorsiflexion und plantarflexion.
RESULTS: Generally, mean pressure values in neutral position were higher than in plantarflexion, and significantly higher in dorsiflexion than in neutral position ($p \leq 0.02$).
The mean pressure was, without calcaneal tuberosity lateralization, in plantarflexion (PF) 2.4 mmHg, in neutral position (NP) 9.3 mmHg, in dorsiflexion (DF) 25.4 mmHg. With the tuberosity lateralization, the pressure increased in every position: 3.9 mmHg in PF, 12 mmHg in NP, and 37.5 mmHg in DF for 4 mm lateralization. 5.4 mmHg in PF, 18.8 mmHg in NP, and 45.1 mmHg in DF for 8 mm lateralization. 8.9 mmHg in PF, 28.6 mmHg in NP, and 62.4 mmHg in DF for 12 mm lateralization. The correlation for lateralization and pressure increase was linear and significant (range, $r = 0.77 - 0.86$, $p < 0.001$)
CONCLUSION: There is a close correlation of lateralization and pressure increase underneath the flexor retinaculum. The pressure increase is significantly higher with hindfoot dorsiflexion ($p \leq 0.02$), followed by neutral position, and plantarflexion.
CLINICAL RELEVANCE: Based on the findings of this study, a pre-emptive release of the flexor retinaculum for a lateralization of the calcaneal tuberosity of 12 mm is recommended. With an 8 mm lateralization, individual patient's risk factors must be taken into consideration to indicate a release. No tibial nerve palsy is expected with a 4 mm lateralization.

7. An anatomical connection between vagus and phrenic nerves at the esophagogastric junction in humans

Kati **Haenssgen**, Valentin Djonov
Institute of Anatomy, University of Bern

Background: The antireflux barrier at the esophagogastric junction (EGJ) consists of the crural diaphragm (CrD), the lower esophageal sphincter (LES) and the upper stomach. These muscles are innervated by the phrenic nerves (CrD) and by autonomous branches of the vagus nerves and coeliac plexus (LES and stomach). The left phrenic plexus (coeliac and phrenic nerve fibres) provides a thin branch to the cardia of the stomach. The muscles maintain the functional competence of the antireflux barrier thereby preventing gastroesophageal reflux. Knowledge about their neural coordination is inevitable. In this study, we found distinctive phrenic branches connecting to vagal branches at the EGJ in humans.
Methods: Macroscopic dissection of 18 human adult bodies (body donation program, Institute of Anatomy). Nerve samples were analyzed using light and electron microscopy.
Results: A distinctive phrenic branch, often connected to the left phrenic plexus, was seen in 8 samples. It connected to a branch of the anterior vagal trunk thereby forming a nerve loop in 5 samples. The course of these nerve loops showed a great variability but was always accompanied by a branch of the left inferior phrenic artery. Phrenic branches without distinct loop formation were present in 6 samples and were absent in 4 samples.
Conclusion: A nerve connection between phrenic and vagus nerve, both known to innervate the antireflux barrier, might contribute to the neural pathway that prevents gastroesophageal reflux. The nerve loop might have ancillary functions for sensing distension or contraction of the EGJ and/or provide sympathetic nerve fibres to the LES. The co-occurrence of nerve loops with arterial branches points to a common developmental background.

8. γδ T cells efficiently kill intracellular Plasmodium falciparum in a granzyme-dependent mechanism during the Malaria blood stage

Hernández-Castañeda MA1, Happ K1, Cattalani F1, Walliman A1, Blanchard M1, Fellay I1, Scolari, B1, Khaoubi Hess S1, Fellay B2, Filgueira L1, Mantel PY1, Walch M1

1 Faculty of Science and Medicine, Department of oncology, Microbiology and Immunology, Anatomy unit, University of Fribourg, Rte A. Gockel 1, CH 1700 Fribourg, Switzerland
2 Cantonal Hospital of Fribourg, CH 1708 Fribourg, Switzerland

Malaria remains one of the major health problems that challenge developing countries, with enormous social and economic implications. Plasmodium spp., the cause of malaria, have a complex life cycle, alternating between the mosquito vector and the human host. The exponential growth of the parasites during the blood stage is responsible for almost all the clinical symptoms of malaria and the associated morbidity and mortality. We recently discovered that cytotoxic lymphocytes kill intracellular bacteria and certain unicellular parasites, such as Trypanosoma cruzi, by the delivery of their cytotoxic effector proteases, the granzymes, into the pathogens. The delivery of the granzymes into intracellular pathogen is provided by the concerted action of the pore forming proteins, perforin and granulysin, that are released simultaneously with the proteases from the killer immune cells (Walch et al. Cell, 2014; Dotiwala et al. Nature Medicine, 2016).
In this study, we translated these novel insights gained in bacteria and Trypanosoma into a yet broader antimicrobial immunological concept to answer the intriguing question of how cytotoxic lymphocytes restrict the growth of blood-residing Plasmodium falciparum. We found that the particular innate lymphocyte subset of gd T cells expands massively and gains high cytolytic potential by up-regulating their cytotoxic effector proteins upon activation with Plasmodium culture supernatant. These activated killer cells specifically recognize, bind and kill Plasmodium in red blood cells via the transfer of the granzymes that is mediated by perforin and granulysin in a stage-specific manner.
Overall, these data reveal a novel innate immune mechanism to inhibit the growth of blood residing Plasmodium falciparum, and with that dissect an evolutionary shaped host-pathogen interaction. The identification of the protease substrates in the parasites will potentially reveal novel vital protein pathways in Plasmodium falciparum that can ease the rational development of immune-based interventions for the future prevention or cure of severe malaria.

9. New inter-cellular mechanism for the transmission of Japanese encephalitis virus by human microglia cells

Nils **Lannes** [1], Obdullio Garcia-Nicolas [2], Thomas Demoulins [2], Artur Summerfield [2, 3] and Luis Filgueira [1]
[1] Institute of Anatomy, Department of Medicine, University of Fribourg, Switzerland
[2] Institute of Virology and Immunology, Mittelhäusern, Switzerland
[3] Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Switzerland

Japanese encephalitis virus (JEV) is a neurotropic flavivirus and a major cause of mortality and morbidity in humans. JEV accumulates of viral particles in the hypothalamus and hippocampus and leads to Japanese encephalitis, an uncontrolled inflammatory disease of the central nervous system. Microglial cells, the brain-resident macrophages, represent the first line of defence during brain insults including viral infection. However, microglia may serve as viral reservoir and are able to transmit JEV infectivity to neighbouring cells in a cell-cell contact-dependent manner. Using JEV-treated human blood monocyte-derived microglia, the present study investigates molecular mechanisms behind cell-to-cell virus transmission by human microglia. To this end, JEV-associated human microglia were co-cultured with BHK-21 cells, a highly susceptible cell line to JEV. Here, we show that JEV-associated microglia transmitted virus to susceptible cells from JEV-associated microglia up to 10 days after virus exposure. Interestingly, neutralizing anti-JEV antibodies did not abrogate cell-to-cell virus transmission. Hence, intracellular viral RNA could be a contributing source of infectious virus material upon intercellular interactions. Importantly, the CX3CL1-CX3CR1 axis was a key regulator of cell-to-cell virus transmission from JEV-associated human microglia. Our findings suggest that human microglia may be a source of infection for neuronal populations and sustain JEV brain pathogenesis in long-term infection. Moreover, the present work emphasizes on the critical role of the CX3CR1-CX3CL1 axis in JEV pathogenesis mediating transmission of infectious genomic JEV RNA.

10. Dysregulation of Parvalbumin Expression in the Cntnap2–/– Mouse Model of Autism Spectrum Disorder

Emanuel **Lauber**, Federica Filice, Beat Schwaller
Anatomy Unit, Section of Medicine, University of Fribourg, Fribourg, Switzerland

Due to the complex and heterogeneous etiology of autism spectrum disorder (ASD), identification of convergent pathways and/or common molecular endpoints in the pathophysiological processes of ASD development are highly needed in order to facilitate treatment approaches targeted at the core symptoms. We recently reported on decreased expression of the Ca2+-binding protein parvalbumin (PV) in three well-

characterized ASD mouse models, Shank1−/−, Shank3B−/− and in utero VPA-exposed mice. Moreover, PV-deficient mice (PV+/− and PV−/−) were found to show behavioral impairments and neuroanatomical changes closely resembling those frequently found in human ASD individuals. Here, we combined a stereology-based approach with molecular biology methods to assess changes in the subpopulation of PV-expressing (Pvalb) interneurons in the recently characterized contactin-associated protein-like 2 (Cntnap2−/−) knockout mouse model of ASD. The CNTNAP2 gene codes for a synaptic cell adhesion molecule involved in neurodevelopmental processes; mutations affecting the human CNTNAP2 locus are associated with human ASD core symptoms, in particular speech and language problems. We demonstrate that in Cntnap2−/− mice, no loss of Pvalb neurons is evident in ASD-associated brain regions including the striatum, somatosensory cortex (SSC) and medial prefrontal cortex (mPFC), shown by the unaltered number of Pvalb neurons ensheathed by VVA-positive perineuronal nets. However, the number of PV-immunoreactive (PV+) neurons and also PV protein levels were decreased in the striatum of Cntnap2−/− mice indicating that PV expression levels in some striatal Pvalb neurons dropped below the detection limit, yet without a loss of Pvalb neurons. No changes in PV+ neuron numbers were detected in the cortical regions investigated and also cortical PV expression levels were unaltered. Considering that Cntnap2 shows high expression levels in the striatum during human and mouse embryonic development and that the cortico-striato-thalamic circuitry is important for speech and language development, alterations in striatal PV expression and associated (homeostatic) adaptations are likely to play an important role in Cntnap2−/− mice and, assumingly, in human ASD patients with known Cntnap2 mutations.

11. The PV2-nucleus of the brainstem

Siri **Leemann**[1], Alexandre Babalian[1], Franck Girard[1], Fred Davis[2], Alexey Larionov[1], Marco R. Celio[1]*
[1]Anatomy and Program in Neuroscience, Faculty of Science and Medicine, University of Fribourg, CH-1700 Fribourg. [2]Janelia-Farm Howard Hughes Medical Institute, Ashburn, USA

The periaqueductal gray (PAG), is known to play a key role in the integration and modulation of autonomic responses (Depaulis & Bandler, 1991). It harbors two of the main terminal fields of the hypothalamic parvafox nucleus, namely the Su3- and PV2-nuclei. The latter nucleus was yet unknown, leading us to perform diverse studies to characterize its extent, connections, gene expression and function.
The PV2-nucleus is an elongated cluster composed of 475 parvalbumin-expressing neurons, located in the ventromedial region of the distal periaqueductal gray. Using anterograde-tracing methods, the main projections of the PV2-nucleus were found to innervate the Su3-nucleus of the PAG, the parvafox nucleus of the lateral hypothalamus, the gemini nuclei of the posterior hypothalamus, the septal regions and the diagonal band in the forebrain, as well as various nuclei within the reticular formation in the midbrain and brainstem. Within the brainstem, projections were discrete, but involved areas implicated in autonomic control. The PV2- nucleus expressed various peptides and receptors, including the receptor for Adciap, a peptide secreted by one of its main affernces, namely the parvafox nucleus. Expression of Vgat-1 in a subpopulation of PV2-neurons indicate an inhibitory nature of a fraction of the nucleus. Furthermore, we conducted an experiment to determine the possible function of the PV2 in a circuitry involving the orbitofrontal cortex, the parvafox and the Su3-nucleu. While these studies suggest an involvement of the circuitry in cardiovascular control, the precise functions await further studies.

12. Parvalbumin facilitates selective removal of mitochondria through autophagy

Lucia **LICHVAROVA** [1], Thomas HENZI [1], Dzhamilja SAFIULINA [2], Allen KAASIK [2], Beat SCHWALLER [1]
[1] University of Fribourg, Department of Medicine, Unit of Anatomy, Fribourg, Switzerland
[2] Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia

Parvalbumin (PV) is a cytosolic Ca2+-binding protein, playing an important role in Ca2+-signal modulation. Antagonistic regulation of PV expression levels and mitochondrial volume occurs in several in vivo and in vitro model systems. The involvement of mitochondrial dynamics, controlling mitochondrial volume and morphology, was not yet reported. The aim of this study was to examine mitochondrial dynamics (fusion, fission, mitophagy) in MDCK cell lines characterized by either PV overexpression or PV downregulation. Cell morphology was assessed by live-cell confocal imaging and 3D-reconstruction using Imaris software. Mitochondrial fusion was analyzed as fluorescence change of the photo-convertible protein mEOS2. Selective removal of damaged mitochondria by the process of autophagy (mitophagy) was quantified from TEM images, as well as from confocal images showing fluorescence of Parkin, GFP-LC3-C and mKeima

proteins. Gene and protein expression levels of selected genes were evaluated by RT-qPCR and Western blot, respectively.
Volumetric analyses revealed the antagonistic regulation of PV and mitochondrial volume in MDCK cells. Control cells showed normal morphology with elongated mitochondria and frequent fusion-fission events. PV-overexpression resulted in smaller, roundish cells and shorter mitochondria related to reduced fusion rates and decreased expression of genes involved in mitochondrial fusion. They exhibited increased mitophagy, a likely cause for the decreased mitochondrial volumes and the smaller overall cell size. PV down-regulation reverted mitochondrial morphology to the situation in parental MDCK cells, resulting from faster mitochondrial movement and augmented fusion rates.
Our results provide novel insights into the complex crosstalk between PV content and mitochondrial volume involving mitochondrial dynamics.

13. A comparative approach to measure sex and age-related differences in shoulder morphology and body size

Sandra **Mathews** [1], Karl Link [2], Nabil Serrano [1,2], Steffen Serowy [3], Dominic Gascho [4], Michael Thali [4], Florian M. Buck [5], Oliver Ullrich [2], Magdalena Müller-Gerbl [6], Frank-Jakobus Rühli [1], Elisabeth Eppler [6], Martin Häusler [1,2]

[1] Institute of Evolutionary Medicine (IEM), University of Zurich, Zurich, Switzerland
[2] Institute of Anatomy, University of Zurich, Zurich, Switzerland
[3] Clinic of Neuroradiology, University Hospital of Magdeburg, Magdeburg, Germany
[4] Institute of Forensic Medicine, University of Zurich, Zurich, Switzerland
[5] Medical Radiology Institute, Schulthess Clinic, Zurich, Switzerland
[6] Department of Biomedicine, University of Basel, Switzerland

In the human scapula, a significant sexual dimorphism has been recently described by our group (Mathews et al., BMC Musculoskelet Disord. 2017;18:9), which was in line with the scarce data in similar populations. However, when it comes to correlation with body size, the database is still weak, particularly for women and smaller individuals. For instance, previous studies from our group (e.g. Häusler and co-workers, University of Zurich 2001; J Hum Evol 2004;46:433-65; 2007;53:383-405) in 100 skeletons with emphasis on small-bodied individuals (65 men, 35 women) originating from Europe, Asia and Africa, have revealed contradictory results for the ratio of glenoid to body size depending on the calculation method. Furthermore, in the elderly, potential osseous shrinking processes have to be considered. Thus, there is a demand to compare the individual scapula size with body size in a larger data set with special emphasis on aged and female individuals. In this multimodality study, we systematically explore the glenohumeral joint using morphological and CT-based measurements, and compare the data with donor body size using different methodological approaches. CT scans including the shoulder girdle and arms were performed prior to dissection. Glenoid size was determined on subsequently isolated scapulae and on 3D-CT reconstructions of the glenohumeral joint according to the method of Friedman as described (Mathews et al. 2017). Body length was measured by CT and additionally, body size determined from femur length and femur head diameter, respectively, and both methodological approaches were compared and correlated with the glenoid size to establish a data set to extrapolate glenoid and body size in the elderly. This study is one of the first to combine dissection with anatomical measurements and radiological CT data to systematically correlate the scapula and glenoid size with the body size as a basis for future studies in physiology and pathological state.

14. Differential pattern of expression for endothelial cell markers in the microvessels of different brain regions

Smart **Mbagwu**, Pierre-Yves Mantel, Luis Filgueira
University of Fribourg, Switzerland

Background: The role of microvascular endothelial cells in the brain is of importance in both healthy and diseased conditions. Various studies have reported heterogeneity in the phenotypical, morphological and molecular characteristics of endothelial cells in the different organs of the body. There is still not much known about the heterogeneity of the endothelial cells of the human brain microcirculation. This study was aimed at characterizing qualitatively the pattern of expression for endothelial cell markers for the microvessels within different regions of the brain.
Methods: Paraffin sections from different anatomical regions (precentral and postcentral gyrus, hippocampus, rhinal and visual cortex) of human formalin fixed brains (n=3) were stained immunohistochemically for endothelial cell markers, including CD31, claudin 5,von Willebrand Factor,E-

selectin and P-selectin. Six different regions (the visual cortex, the hippocampus, the pre and postcentral cortices and the rhinal cortex) of the human brain(n=3) were obtained and processed using routine histological methods. The samples were paraffin-sectioned and stained for immunohistochemical demonstration of the expression of brain specific endothelial cell biomarkers such as CD31 and vWF. We observed variations in the pattern of expression of the biomarkers which suggests the nature of vascularization in the regions studied.
Results: We observed different patterns of expression for both biomarkers studied indicating uniqueness of these markers in their specificity for microvascular endothelial cells. The expression of the biomarkers investigated was highest in the visual cortex in comparison to other regions of the brain studied. The expression pattern was heterogeneous between the white matter and the gray matter with the visual, rhinal and hippocampal regions showing high densities of the microvessels. The the expression pattern was alson varied among the microvessels of the brain with the capillaries showing very high expression of both biomarkers. These variations could reflect variation in the functional dynamics in differentbrain regions.
Conclusion and significance:
The expression pattern of endothelial markers is heterogenous for the microvasculature within of the brain and could result in functional differences in different regions of the brain. The above observation should be considered in studies involving invitro models of the microvascular endothelial cells from different regions of the brain.

15. A virtual microscopy web-application for interactive histology courseware

Felix **Meyenhofer**, Marlène Sanchez, Marco R Celio and Franck Girard
Histology, Faculty of Science and Medicine, University of Fribourg, CH-1700 Fribourg

Three complementary pedagogical approaches are used at the University of Fribourg for teaching / learning histology during the first and second years of studies in medicine and biomedicine:

- Learning with image projection during the theoretical courses (ppt presentation).
- Guided learning during the practical sessions (each student is provided with slides and microscope).
- Virtual microscope web-application (independent learning).

Our university provides its students since 2015 with virtual microscopy courseware in French and German language. It contains 366 sections, mostly from human organs, covering the following topics: epithelial and glandular tissues, conjunctive tissues, muscular tissues, nervous tissue, blood, skin, male and female genital systems (first year); digestive, vision, auditory, genital, respiratory, urinary, lymphatic, nervous, vascular and endocrine systems as well as the skin (second year).
Starting with the autumn semester 2018 we launch our second version of this electronic courseware, that is improved on the technological side and provides a revised course content. Furthermore, the new release will be open to the world wide web.

16. Characterization of novel subtypes of macrophages during zebrafish fin and heart regeneration

Andres **Sanz-Morejon** [1], Ana B. García-Redondo [2], Fabian Häberli [1], Inês Marques [1], Maria Galardi-Castilla [3], Hanna Reuter [4], Christoph Englert [4], Nadia Mercader [1]
[1] Institute of Anatomy, University of Bern, CH
[2] Department of Pharmacology, Universidad Autonoma de Madrid, ES
[3] National Center for Cardiovascular Research, ES
[4] Leibniz Institute for Age Research-Fritz, DE

Organ regeneration is preceded by an inflammatory response and recruited macrophages play an active role during repair and regrowth. Macrophage subtypes are well characterized in mammals, but not in the zebrafish, an animal model with high regenerative capacity. Using a wilms tumor 1b (wt1b) reporter line, we identified a subpopulation of macrophage-like cells, which accumulate at the site of injury in a model of cardiac cryoinjury and fin amputation. This population revealed a different gene expression profile and migratory behavior compared to the rest of mpeg1+ macrophages. Interestingly, adult wt1bΔ5/Δ5 animals show decreased cardiomyocyte proliferation upon cardiac injury. Furthermore, non-resident wt1b macrophages show the capacity to infiltrate the injury area in response to ventricular cryoinjury. In embryos upon caudal fin amputation, a subset of mpeg1+ cells start to express wt1b, and mpeg1+/wt1b+ macrophages are retained at the injury site. Functional inhibition of Wt1b in macrophages promoted their motility, suggesting that Wt1b is involved in the retention of macrophages at the site of injury thus allowing them to promote regeneration of the missing body part. This is the first description of a pro-regenerative macrophage subtype in the zebrafish and expands our understanding on the molecular mechanisms through which macrophages contribute to organ regeneration.

17. Time-resolved synaptic cytomatrix and exocytosis architecture

Raphaela Isabelle Marianne **Seeger** [1], Julika Radecke [2], Benoît Zuber [1]
[1]Institute for Anatomy, Switzerland; [2]University of Copenhagen, Denmark

Understanding how our brain works requires a comprehensive description of the molecular mechanism of vesicle exocytosis which allows inter-neural communication through neurotransmitter release.
The proteins involved in the vesicle exocytosis have been identified and the ultrastructure of synapses is resolved, however the protein structures have not yet been mapped in the images of the synapses and their roles in the mechanisms are often unclear. The aim is to acquire near atomic maps of synapses using Tomography and locating the components involved in vesicle exocytosis in order to get insights into their influences on the exocytosis mechanism. One main component is the network of filaments interconnecting the synaptic vesicles. They not only regulate the synaptic vesicle release activity but also is subject to rapid modification in the event of sustained activity.
An example for a component which is believed to be associated to the filaments connecting the vesicles with the plasma membrane, that would benefit from such a mapping is synaptotagmin. It is debated whether synaptotagmin is involved in vesicle insertion into the membrane as a calcium sensor, a map identifying its position would shine light onto this situation (O'Connor et al, 2002).
An effective method to visualize vesicle exocytosis is Cryo-electron Tomography (cryo-ET). Our established method of sample preparation enables us to not only resolve the environment of the synapse structurally, but also temporal with an accuracy of a few miliseconds. To analyze the obtained data, the new template matching that allows to localize a known 3D structure in a crowded molecular environment (Rickgauer et al, 2017) and the growing implementation of machine-learning frameworks into image reconstruction in cryo ET will be used (Waller et al, 2015).

18. Calretinin promotes EMT and invasiveness in malignant mesothelioma cells involving the activation of the FAK signaling pathway

Janine **Wörthmüller** Rodríguez, Walter Blum, Laszlo Pecze, Valérie Salicio & Beat Schwaller
University of Fribourg, Fribourg, Switzerland.

Background: Calretinin (CR) is a Ca2+-binding protein currently used as a positive marker for human malignant mesothelioma (MM). This protein is essential for mesothelioma cell growth/survival, since its downregulation decreases cell proliferation and viability in vitro. Yet, the putative role(s) of CR during MM formation in vivo, putative binding partners or CR's influence on specific signaling pathways remain unknown.
Methods: We assessed the effect of CR overexpression in two human MM cell lines by investigating migration, invasion and epithelial-to-mesenchymal transition (EMT). We also evaluated tumor formation after CR downregulation via lentiviral shRNA against CR in vivo in an orthotopic xenograft mouse model.
Results: CR overexpression in the MM cell lines MSTO-211H and SPC111 augmented migration and invasion in vitro. These effects involved the activation of the focal adhesion kinase (FAK) signaling pathway, since up-regulated levels of total FAK and p-FAK (Tyr397) were found in CR-overexpressing MM cells. CR was also implicated in controlling EMT, evidenced by changes of the cell morphology and upregulation of typical EMT markers. Co-IP experiments revealed FAK as a new binding partner of CR. In addition, we observed that CR co-localized with FAK at focal adhesion sites in MM cells, and moreover, that CR-overexpressing cells displayed enhanced nuclear FAK accumulation and an increased resistance towards the FAK inhibitor VS-6063, currently used in MM pre-clinical trials. Finally, in an orthotopic xenograft mouse model based on peritoneal MM cell injection, CR downregulation via a lentiviral shRNA against CR (CALB2) resulted in a significantly reduced tumor formation in vivo.
Conclusions: Our data provide a new role for CR as a protein promoting migration, invasion and EMT in MM cells. As invasion is one of the characteristic features of MM, downregulation of CR might be considered a promising strategy to impair MM progression at early stages of this disease.

List of Participants

Babatunde	Adebayo	University of Fribourg
Bulliard	Manon	University of Fribourg
Celio	Marco	University of Fribourg
De Coninck	Stien	University of Fribourg
Djonov	Valentin	University of Bern
Draeger	Annette	University of Bern
Eppler	Elisabeth	University of Basel
Fiedler	Klaus	University of Fribourg
Filgueira	Luis	University of Fribourg
Filice	Federica	University of Fribourg
Gagliano	Marco	University of Fribourg
Gehr	Peter	University of Bern
Girard	Franck	University of Fribourg
Haenssgen	Kati	University of Bern
Halm	Sebastian	University of Bern
Hernandez Castañeda	Maria Andrea	University of Fribourg
Hlushchuk	Ruslan	University of Bern
Höchel	Sebastian	University of Basel
Huber	Katrin	University of Fribourg
Kapfhammer	Josef	University of Basel
Lannes	Nils	University of Fribourg
Lauber	Emanuel	University of Fribourg
Lichvarova	Lucia	University of Fribourg
Link	Karl	University of Zurich
Loffing	Dominique	University of Zurich
Mantel	Pierre-Yves	University of Fribourg
Marques	Ines	University of Bern
Marthe	Cyril	University of Lausanne
Mbagwu	Smart	University of Fribourg
Mercader	Nadia	University of Bern
Meyenhofer	Felix	University of Fribourg
Mori	Nozomu	Nagasaki University School of Medicine, Japan
Müller-Gerbl	Magdalena	University of Basel
Riederer	Beat	University of Lausanne
Scherer	Thomas	University of Basel
Schwaller	Beat	University of Fribourg
Seeger	Raphaela	University of Bern
Stoffel	Michael	University of Bern
Tschanz	Stefan	University of Bern
Walch	Michael	University of Fribourg
Wörthmüller	Janine	University of Fribourg
Yang	Zhihong	University of Fribourg
Yotovski	Peter	University of Fribourg
Zoia	Matteo	University of Fribourg
Zuber	Benoît	University of Bern
Zubler	Cedric	University of Bern