IFSHC WORKSHOP 2021

6 - 9 SEPTEMBER 2021

ONLINE

PRAGUE, CZECH REPUBLIC

LOCAL WORKSHOP ORGANIZER:

INTERNATIONAL FEDERATION OF SOCIETIES FOR HISTOCHEMISTRY AND CYTOCHEMISTRY

PROF. PAVEL HOZAK, SECRETARY OF THE SOCIETY FOR HISTOCHEMISTRY

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IFSHC WORKSHOP 2021

MONDAY, SEPTEMBER 6

TURKISH SOCIETY FOR ELECTRON MICROSCOPY - SESSION CHAIR: SERAP ARBAK, PRESIDENT

10:00 - 11:00 PRAGUE TIME

Halime Kenar, Acibadem University, Istanbul, Turkey

TITLE: Developing an in vitro functional heart muscle model from human iPSC derived cardiomyocytes

11:00 - 11:30 PRAGUE TIME

Menekse Ermis Sen, Middle East Technical University, Ankara, Turkey

TITLE: Determining cell phenotype and fate at cell-material interfaces with immunocytochemistry and fluorescence microscopy

BREAK: 11:30 - 12:00 PRAGUE TIME

ITALIAN SOCIETY OF HISTOCHEMISTRY - SESSION CHAIR: CARLO PELLICCIARI

12:00 - 13:00 PRAGUE TIME

Lucio Cocco, University of Bologna, Italy

TITLE: Inositide signalling location: from bench to clinics

13:00 - 13:30 PRAGUE TIME

Carolina Simioni, University of Ferrara, Italy

TITLE: Morphological analysis and ultrastructural tissue alterations in SARS-CoV-2 positive and tested negative patients: role of receptors and vascular biomarkers and correlation with viral entry

TUESDAY, SEPTEMBER 7

THE JAPAN SOCIETY FOR HISTOCHEMISTRY AND CYTOCHEMISTRY - SESSION CHAIR: HITOSHI OZAWA, PRESIDENT

09:00 - 10:00 PRAGUE TIME

Toshiyuki Matsuzaki, Gunma University Graduate School of Medicine, Japan

TITLE: Immunohistochemistry and aquaporin research

10:00 - 10:30 PRAGUE TIME

Keiko Takanami, National Institute of Genetics, Japan

TITLE: Histochemical challenges to the itch neurotransmission and evolution

BREAK: 10:30 - 11:00 PRAGUE TIME

POLISH HISTOCHEMICAL AND CYTOCHEMICAL SOCIETY - SESSION CHAIR: ZBIGNIEW KMIEĆ, PAST PRESIDENT

11:00 - 12:00 PRAGUE TIME

Jerzy Dobrucki, Jagiellonian University, Kraków, Poland

TITLE: Microscopy studies of DNA damage and repair

12:00 - 12:30 PRAGUE TIME

Maciej Gagat, Nicolaus Copernicus University, Toruń, Poland

TITLE: F-actin dynamics play a crucial role in the regulation of the inflammatory response of endothelial cells

WEDNESDAY, SEPTEMBER 8

SOCIETY FOR HISTOCHEMISTRY – SESSION CHAIR: KLARA WEIPOLTSHAMMER, PRESIDENT

13:00 - 13:45 PRAGUE TIME

Hari Shroff, Robert Feulgen Prize 2020 awardee
National Institute of Biomedical Imaging and Bioengineering (NIBIB), USA
TITLE: Enhancing Fluorescence Microscopy with Computation

13:45 - 14:30 PRAGUE TIME

Christian Mühlfeld, Robert Feulgen Prize 2020 awardee
Institute for Functional and Applied Anatomy, MHH, Germany
TITLE: Looking at the pulmonary vasculature: Stereology and 3D reconstruction

BREAK: 14:30 - 15:00 PRAGUE TIME

THE ROYAL MICROSCOPICAL SOCIETY - SESSION CHAIR: THERESA WARD, PRESIDENT

15:00 - 16:00 PRAGUE TIME

Maddy Parsons, Randall Division of Cell and Molecular Biophysics, London, UK
TITLE: Imaging cytoskeletal contributions to cancer cell invasion

16:00 - 16:30 PRAGUE TIME

Evie Garlick, University of Birmingham, UK
TITLE: Investigating the role of actin in membrane organisation of adenosine receptors using super-resolution microscopy'

THURSDAY, SEPTEMBER 9

CZECH SOCIETY FOR HISTO-AND CYTOCHEMISTRY - SESSION CHAIR: TBD

15:00 - 16:00 PRAGUE TIME

Mária Hovořáková, Institute of Histology and Embryology, First Faculty of Medicine, Charles University, Prague, CR
TITLE: The tooth-forming capacity kept in nondental areas of oral cavity

16:00 - 16:30 PRAGUE TIME

Karolína Strnadová, Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, CR
TITLE: Biological models to study melanoma behaviour and tumour microenvironment: using chicken chorioallantoic membrane and single-cell

BREAK: 16:30 - 17:00 PRAGUE TIME

THE HISTOCHEMICAL SOCIETY - SESSION CHAIR: LILIANA SCHAEFER, PRESIDENT

17:00 - 18:00 PRAGUE TIME

John Couchman, Biotech Research & Innovation Centre, University of Copenhagen, Denmark
TITLE: Syndecan receptors in disease: identifying roles at increasing resolution

18:00 - 18:30 PRAGUE TIME

Tirthadipa Pradhan-Sundd, Heart, Lung and Blood Vascular Medicine Institute, University of Pittsburgh, USA
TITLE: Understanding the molecular mechanisms of Liver injury in sickle cell disease patients: are we there yet?

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Type of presentation: Oral

MS-O-2377 Developing an in vitro functional heart muscle model from human iPSC derived cardiomyocytes

Kenar H.[1], Aydin L.[2], Coskun K.[3]

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Development of an in vitro 3D human heart muscle model with natural micro-anatomical structure and capability of performing physiological functions is aimed for testing cardiovascular drugs. Use of microfluidic systems allows testing drugs on target human cells, but a single cell type in a 2D culture does not reflect the exact response that occurs in the natural 3D complex tissues. Today, producing the natural functional 3D tissue substitutes in vitro has become possible with precise positioning of cells by 3D bioprinting. A 3D biomimetic in vitro human heart muscle model is being developed in our study by means of bioprinting. Beating cardiomyocytes are being obtained through differentiation of human induced pluripotent stem cells (iPSCs) and are loaded in a hydrogel together with human coronary artery endothelial cells and human fibroblasts to obtain the bioink to be printed. Formation of capillary vessels within the heart muscle model is being achieved by means of natural cell motility and interaction between the endothelial cells and the fibroblasts. Fluorescence and confocal microscopy results of cell viability and function, cardiomyocyte characterization, capillary vessel formation and heart muscle tissue organization will be presented.

Acknowledgement: This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) (grant number 218S717).

Type of presentation: Oral

JS-O-2375 Determining cell phenotype and fate at cell-material interfaces with immunocytochemistry and fluorescence microscopy

Ermis M.[1], Antmen E.[1], Hasirci V.[1, 2, 3]

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Many different cell types making up tissues and organs of an organism are adherent. Therefore, cell adhesion is a fundamental process for these cell types. It is the binding process of a cell to the extracellular matrix, a surface, or another cell using some specific surface proteins. It is also one of the critical processes while developing biomaterials and tissue engineering products. Developments in surface patterning technologies at micro and nano-scale paved the road to studying cell-surface interactions from a novel point of view. For example, microfabrication techniques can be employed to create topographies with geometrical micro and nanopatterns, like channels, pillars, and pits with controlled dimensions.
Today, light microscopes allow very sophisticated analysis of cells and subcellular compartments with the help of the fluorescence principle. The fluorescence principle allows imaging subcellular structures tagged with desired fluorescence probes with a dark background and increases resolution and pattern recognition with the human eye and computers. Using a fluorescence microscope and associated techniques (widefield fluorescence, confocal laser scanning microscope, spinning disk confocal, multiphoton confocal,live-cell imaging, etc.), one can visualize cells and cellular processes both in a static or dynamic format. However, when we consider the complexity of cell biomaterials interactions, imaging complex samples like those (other than conventional histology sections), application-specific interfacial devices need to be developed. Therefore, this study combines traditional fluorescence imaging techniques with image analysis to investigate cell-biomaterials interactions. Mesenchymal stem cells and cancer cells from various origins interacted with biomaterials, and their interaction was characterized using fluorescence microscopy. Cells were characterized for their morphology, expression, and distribution of specific proteins and fate (differentiation, apoptosis, etc.).

Acknowledgement: ME, EA, and VH acknowledge the Ministry of Development of Turkey, METU BAP-01-08-2013-003 and BAP-08-11-DPT2011K120350, and METU BIOMATEN.

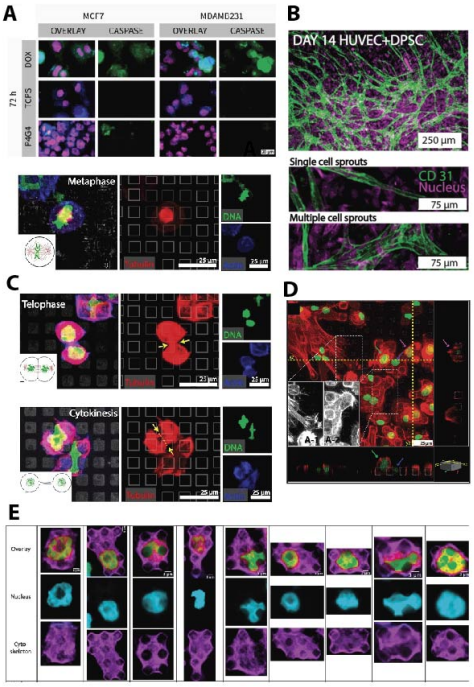

Fig. 1: Confocal microscope images of cells interacting with biomaterials. A-Breast cancer cells on micropatterns. B-Human umblical cord endothelial cells and mesenchymal stem cells (MSC). C-Human osteosarcoma cells on polymeric micropatterned substrate during cell division. D- MSC on micropatterns. E- Prostate cancer on micropatterns.

Type of presentation: Oral

MS-O-2344 Inositide signalling location: from bench to clinics

Cocco L.[1]

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Phosphoinositide-specific Phospholipases C (PI-PLCs) are involved in signaling pathways related to critical cellular functions, such as cell cycle regulation, cell differentiation, and gene expression. PI-PLCs are highly expressed in different brain areas, and nuclear PI-PLCs have been studied as key enzymes, molecular targets, and clinical prognostic/diagnostic factors in many physiopathologic processes. Here, we summarize the main studies about nuclear PI-PLCs taking into account the subcellular localisation and pathologies related to loss or gain of these enzymes. Specifically the imbalance of isozymes such as nuclear PI-PLCβ1 and nuclear PI-PLCζ, in cerebral, hematologic, neuromuscular, and fertility disorders. Nuclear PI-PLCβ1 and cytoplasmatic PI-PLCγ1 affect epilepsy, depression, and bipolar disorder. In the brain, PI-PLCβ1 is involved in endocannabinoid neuronal excitability and is a potentially novel signature gene for subtypes of high-grade glioma. An altered quality or quantity of PI-PLCζ contributes to sperm defects that result in infertility, and PI-PLCβ1 aberrant inositide signaling contributes to both hematologic and degenerative muscle diseases. Understanding the mechanisms behind PI-PLC involvement in human pathologies may help identify new strategies for personalized therapies of these conditions.

Acknowledgement: This work has been supported by Italian Ministry for Research, Intesa S. Paolo Foundation and CARISBO Foundation

Type of presentation: Oral

JS-O-2369 Morphological analysis and ultrastructural tissue alterations in SARS-CoV-2 positive and tested negative patients: role of receptors and vascular biomarkers and correlation with viral entry

Simioni C.[1,2], Varano G.[3], Conti I.[3], Brenna C.[3], Costanzi E.[3], Laface I.[3], Neri L. M.[2,3]

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Increasing interest is arousing in the analysis of a possible correlation between SARS-CoV-2, responsible for coronavirus disease (COVID-19), and multi-organ vascular damage. Several scientific reports described how the virus is able to exert its activity in organs other than lung, focusing the relevance of SARS-CoV-2 dissemination in the body associated with peripheral vascular injury.
In particular, several evidences suggest that, in addition to the respiratory tract, also the gastrointestinal tract may be a main site of SARS-CoV-2 infection, possibly associated to poor prognosis.
The structure of blood vessel wall can be strongly altered during SARS CoV-2 infection, therefore the identification of morpho-functional alterations of the vessel wall is a very important aspect to characterize the infection. A correlation between autophagy and vascular damage has been hypothesized and this process may be involved in the modulation of the vascular process during infection. Recent observations in pregnant SARS-CoV-2 infected women pointed out that also placenta vessels may be affected by viral infection, with consequences to the fetus.
We carried out analysis of tissue, vascular and placenta samples by transmission (TEM) and scanning (SEM) electron microscopy. In addition to the ultrastructural characterization of specific tissue organelles comparing non-pathological to pathological tissues, a morphological evaluation of biomarkers associated with vascular damage has been performed, as well as the analysis of the autophagic marker LC3B and the expression of the cellular transmembrane glycoprotein CD147 and ACE2 receptor, that can interact with viral proteins. The data therefore support the existence of a peculiar pathogenic process for SARS-CoV-2 infection in multiple tissues, also with an involvement of vascular damage at the endothelial level.

References

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[2] Hosier H et al. (2021). SARS-CoV-2 infection of the placenta. J Clin Invest. 2020 Sep 1;130(9):4947-4953.

Type of presentation: Oral

MS-O-2363 Immunohistochemistry and aquaporin research

Matsuzaki T.[1]

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I have focused on membrane water channel protein, aquaporins. We generated specific antibodies to each aquaporin isoform and examined tissue distribution by immunohistochemistry. In this workshop, I would like to talk about the generation of aquaporin antibodies as well as the tissue distribution of aquaporins, in which I would focus especially on aquaporin-2 (AQP2) phosphorylation and its trafficking. AQP2 is a vasopressin-sensitive water channel in the kidney collecting duct cells and plays an important role in urine concentration. AQP2 is stored in intracellular vesicles under lower vasopressin level and is acutely trafficked to the plasma membrane upon the elevation of vasopressin. AQP2 has 4 serines (S256, S261, S264, and S269) at COOH terminal region that are phosphorylated or dephosphorylated by the change of serum vasopressin level. It was previously thought that phosphorylation at S256 was necessary and sufficient for trafficking to the plasma membrane. However, importance of phosphorylation at S269 for trafficking or membrane retention after reaching plasma membrane is recently suggested by some papers including us. We generated some phosphospecific antibodies to phosphorylated AQP2 at S256 or S269, carefully confirmed the specificity, and used in immunohistochemistry to examine the change of phosphorylation of these serines and change of intracellular distributions in rats and mice kidney tissues. In short, a considerable amount of AQP2 was phosphorylated at S256 but none at S269 under lower vasopressin level, and AQP2 was acutely phosphorylated at S269 upon administration of vasopressin. We thought that S269 is critical for trafficking to the plasma membrane. Recently we generated genome-editing mice that has a mutation resulting in S269A or S269D to confirm how the phosphorylation at S269 affects AQP2 trafficking. I would like to introduce some results obtained with these mice as well.

Acknowledgement: This work was supported by JSPS KAKENHI Grant Numbers JPS0801035, JP22590230, JP22790191, JP26460267, JP18K06816, JP22790191, and JP25463068.

Type of presentation: Oral

JS-O-2379 Histochemical challenges to the itch neurotransmission and evolution

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The neural circuits of “pain” and “itch” have much in common, and itch-specific molecules have long been unknown. Gastrin-releasing peptide (GRP) receptor (GRPR) was first identified as an itch specific mediator in the spinal cord. Then we focused on the ligand of this receptor, GRP as an itch neural marker in the somatosensory system in rats. We found GRP was expressed in the small-sized primary afferent (dorsal root ganglion and trigeminal ganglion) and GRP-immunoreactive (ir) fibers projected to the superficial layers of the spinal cord and spinal trigeminal nucleus Caudalis. Expression of GRPR was observed at the GRP projection cites. Next, we focused on the synaptic cites of the GRP terminals in the spina cord. Immunoelectron microscopy showed the GRP-ir terminals contain many clear round vesicles and dense-cored vesicles, suggesting contain excitatory neurotransmitters. We also combined High Voltage Electron Microscopy and 3D-SEM with immunohistochemistry to analyze the 3-D ultrastructure of itch-mediating synapse. These study showed the GRP-ir terminals contained many vesicles and mitochondria, formed varicosity structure, and were surrounded by many postsynaptic components including glial cells, suggesting that itch neurotransmission is complexly controlled. Next, we had the question of how organism acquires the itch sensation during evolution and why we possess an unpleasant itch sensation. To address these questions, we utilized the phylogenetic and comparative analyses using medaka fish, Xenopus, primitive eutherians, rodent, and primates. We found that amino acid sequence of GRP had highly been conserved among vertebrate. Immunohistochemical analysis showed that the expression of GRP was consistent across vertebrate. These results suggest that this system may be a conserved property for itch-mediating function in vertebrate. We would like to introduce behavioral analysis of itch in fish and rodents.

Acknowledgement: 19th Paper Award of “Acta Histochemica et Cytochemica (Journal of Japan Society of Histochemistry and Cytochemistry)” Vol.49 (2016) No.6, pp.181-190

Type of presentation: Oral

MS-O-2382 Microscopy studies of DNA damage and repair

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DNA is not fully resistant to spontaneous chemical alterations and various types of damage inflicted by endogenous and exogenous factors. Cells of living organisms constantly monitor their DNA for breaks and changes of shape. When a DNA lesion is detected a cascade of signals leads to activation of a specific repair pathway. Several major repair pathways operate in human cells. Nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), homologous recombination (HR) and nonhomologous end joining (NHEJ) repair perform complex tasks of returning the damaged DNA to its original state. Recent advances in optical microscopy opened new research avenues in the field of DNA damage response. I will introduce and discuss examples of induction of DNA lesions of various types by drugs, radiation and focused beams of visible laser light. I will subsequently describe applications of advanced optical microscopy methods, including fluorescence correlation spectroscopy (FCS), fluorescence lifetime imaging (FLIM) and super-resolution imaging in studies of the mechanisms of DNA damage response, investigations into dynamics of repair factors recruited to DNA lesions, and studies of architecture of DNA repair foci.

Acknowledgement: Financial support of National Science Center, Poland (grant 2017/27/B/NZ3/01065)

Type of presentation: Oral

JS-O-2374 F-actin dynamics play a crucial role in the regulation of the inflammatory response of endothelial cells

Gagat M.[1], Zielińska W.[1], Mikołajczyk K.[1], Krajewski A.[1], Klimaszewska-Wiśniewska A.[2], Grzanka D.[2], Grzanka A.[1]

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The endothelial barrier plays a pivotal role in the regulation of the functioning of the entire circulatory system. For this reason, any disturbances in the endothelial structure relate to unfavorable health effects. Endothelial dysfunction manifests into abnormalities in the anti-coagulant and anti-inflammatory properties of cells as well as alterations in vascular growth and vascular remodeling. Tumor necrosis factor α (TNFα) is a proinflammatory cytokine, which affects many processes associated with the growth and characteristics of endothelial, smooth muscle, and immune system cells. However, there is no correlation between most in vivo and in vitro studies on its role in endothelial cell proliferation and migration.

In this study, we examined the effect of recombinant human TNFα produced in HEK293 cells on primary human coronary artery endothelial cells (pHCAECs) in the context of F-actin organization and such processes as migration and adhesion. Furthermore, we evaluated the possibility of inhibiting the endothelial inflammatory response by the CRISPR-based regulation of tropomyosin-1 (*TPM1*) gene expression.

We showed that TNFα-induced activation of pHCAECs was related to the changes in the organization of the actin cytoskeleton. The response of pHCAECs to TNFα induced its organization into parallelly arranged stress fibers running along the longer axis of pHCAECs. It allowed the cells for directed and parallel motion during coordinated migration. This change in F-actin organization promoted strong but discontinuous cell-cell contacts involved in signalization between migrating cells. Moreover, this form of intercellular connections, together with locally increased adhesion, was related to the formation of migrasomes and further migracytosis. Stabilization of the actin cytoskeleton through the CRISPR-based activation of endogenous expression of *TPM1* resulted in the inhibition of the inflammatory response of pHCAECs following treatment with rh TNFα and stabilization of cell-cell junctions through reduced cleavage of VE-cadherin and maintaining the stable levels of α- and β-catenins. We also showed that CRISPR-based activation of *TPM1* expression reduced inflammatory activation, proliferation, and migration of primary human coronary artery smooth muscle cells.

Therefore, TPM1 may be a potential therapeutic target for the treatment of proinflammatory vascular disorders. The obtained results are important not only from the mechanistic point of view but also have the potential to be translated into clinical research and adapted during the design and development of new coronary stent devices.

Acknowledgement: This study was supported by grant no. 2015/17/D/NZ7/00809 to MG from National Science Centre, Poland.

Type of presentation: Oral

MS-O-2371 Enhancing fluorescence microscopy with computation

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Biomedical optical microscopy is undergoing a renaissance in performance, with better resolution, depth penetration, and speed than ever before. While new microscopes generate terabytes of data with a click of a button, our ability to generate biological insight has not kept pace, in part because the post-processing necessary for image restoration considerably lags data acquisition. In the first part of my talk, I will discuss computational tools that accelerate deconvolution, registration, and multiview fusion of large optical microscopy datasets. In the second part of my talk, I will describe additional methods that leverage data driven inference for further enhancing contrast, resolution, and experiment duration beyond the capabilities of the base microscope.

Type of presentation: Oral

MS-O-2345 Looking at the pulmonary vasculature: Stereology and 3D reconstruction

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The main function of the lung is the exchange of gases between air and blood. The deoxygenated blood is transported via an arterial tree into a huge network of capillaries within the alveolar septa (ACN). After oxygenation, the blood is drained by the branches of a venous tree. The involvement of the pulmonary circulation in various human lung diseases emphasizes the demand for morphological tools to analyse the pulmonary vasculature. An important way of morphological analysis is quantification by design-based stereology which is generally considered to be the gold standard of morphometry. Besides the classical stereological parameters volume and surface area, the estimation of the number of alveolar capillary loops has recently been established which enhances the conclusive power of stereological analyses of the ACN. Apart from quantitative data, there is also a need to visualize the 3D characteristics of both conduction and gas-exchanging blood vessels in the lung. New electron microscopic techniques, such as serial block-face scanning electron microscopy, and radiological methods, such as micro-computed x-ray tomography, have greatly expanded the possibilities of gaining 3D data sets for segmentation and 3D analysis and visualization. In this talk, I will summarize some of the recent stereological and imaging developments in relation to the pulmonary vasculature with respect to their challenges and potential.

Type of presentation: Oral

MS-O-2343 Imaging cytoskeletal dynamics in invading cancer cells

Parsons M.[1]

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Cells have to dynamically adapt their shape to respond to changes in the extracellular environment. This is a key feature of many cells in both homeostatic situations such as embryonic development and differentiation as well as pathological scenarios such as wound healing and cancer cell invasion. Changes in cell shape require dynamic reorganisation of the F-actin cytoskeleton. Fascin is a highly conserved F-actin bundling protein that plays a key role in controlling cytoskeletal stability in migrating cells. Fascin is also highly upregulated in human cancers, correlates with poor clinical prognosis and metastasis, and is required for cancer cell invasion. However, the mechanisms governing spatial, temporal and functional regulation of fascin remains poorly understood. We have developed a range of different advanced microscopy methods to address this key question and enable the study of fascin dynamics and binding partners in live human cancer cells. Our data has revealed highly localised, rapid molecular shuttling of fascin within F-actin bundles, that correlates with unexpectedly fast fascin-actin binding kinetics. We further show that fascin function is finely tuned to adapt to changes in local cytoskeletal organisation as well as the mechanical environment of the cell. Our studies shed light on the molecular control of fascin activity within living tumour cells and may provide new routes for therapeutic development to halt cancer progression.

Acknowledgement: Medical Research Council UK

Type of presentation: Oral

JS-O-2380 Investigating the role of actin in the membrane organisation of adenosine receptors using super-resolution microscopy

Garlick E.[1,2], Faulkner E. L.[1,2], Briddon S. J.[2,3], Thomas S. G.[1,2]

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Cellular organisation of G-Protein Coupled Receptors (GPCRs) is important in determining signalling responses and associated pharmacological parameters. There is increasing evidence this is relevant in disease. However, the molecular determinants of this organisation are not fully understood. Previous work has identified a role for the actin cytoskeleton in constraining receptor movement and the creation of signalling 'hot spots, but little is known about how the dynamics and structure of the cortical actin contribute to specific receptor behaviour. Here, we use super-resolution microscopy to investigate the role of cortical actin in the organisation of the human adenosine-A_{2A} ($A_{2A}R$) and -A_{2B} receptors ($A_{2B}R$).

dSTORM imaging of SNAP tagged A2 receptors revealed disruption of cortical actin by CytoD had no significant effect on $A_{2B}R$ clusters, but significantly reduced the number of detections in $A_{2A}R$ clusters (Veh: 83.1 detections/cluster ± 9.7 SEM, CytoD: 47.5 detections/cluster ± 3.9 SEM). Stimulation and actin disruption was repeated to obtain super-resolved fixed actin images using both super-resolution radial fluctuations (SRRF) analysis and expansion microscopy techniques. Analysis of the meshwork using SRRF showed a significant increase in corral area when treated with CytoD (↑ of 0.31μm^2 ± 0.04 SEM), as well as a small but significant increase with NECA (↑ of 0.02 μm^2 ± 0.01 SEM). The analysis workflow used is being further developed for application to live cell imaging and ExM-3D SIM imaging is being applied to additional labels to investigate the effect further.

These experiments indicate a role for actin in mediating $A_{2A}R$ and $A_{2B}R$ membrane organisation, with potential for different regulatory contributions between receptors and across organisational scales. Actin organisation also appears to be affected when receptors are activated, and more detailed investigation could reveal interesting reciprocal interactions.

Acknowledgement: Authors would like to acknowledge the Imaging Suite at the University of Birmingham, and thank the British Heart Foundation and COMPARE for funding.

Type of presentation: Oral

MS-O-2373 The tooth-forming capacity retained in nondental areas of the oral cavity.

Hovořáková M.[1], Dalecká L.[1,2], Hutečková B.[3,4], Qiu T.[5], Buchtová M.[3,4], Tucker A. S.[1,5]

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The dental lamina is an epithelial structure running around both jaw arches that gives rise to tooth germs forming teeth. In humans, a vestibular lamina has been described located labially to the dental lamina, which gives rise to the oral vestibule - a free space formed between the lips or cheeks and the teeth and gingiva. The epithelium of the human vestibular lamina interacts closely with the dental lamina during development. The vestibular and dental epithelial structures arise from a common epithelial thickening in the lower incisor region in both humans and mice, providing an interesting tool to study cell fate in an experimental model. Here we will focus on the morphogenesis of the oral vestibule during human and mouse embryogenesis, as well as on its developmental relationship with the dental lamina. Furthermore, we focus on differences in gene expressions in the dental and vestibular epitheliums using RNA sequencing. Taking advantage from the available mouse experimental model we show an evidence of the retained odontogenic potential in the vestibular epithelium, and we test this potential using in vitro culturing experiments based on the RNA sequencing results. The close developmental relationships between the dental and vestibular structures with respect to our results could elucidate the formation of pathologies with dental tissue presence in the vestibular area in humans as peripheral odontoma or ameloblastoma.

Acknowledgement: This study was supported by Czech Grant Agency (Project Nr.18-04859S).

Type of presentation: Oral

JS-O-2346 Biological models to study melanoma behaviour and tumour microenvironment: using chicken chorioallantoic membrane and single-cell RNA sequencing

Strnadová K.[1,2], Novotný J.[3,4], Dvořánková B.[1,2], Kolář M.[3], Lacina L.[1,2,5], Klepáček I.[1], Smetana K.[1,2]

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Malignant melanoma is one of the most dangerous forms of skin cancer, most often caused by exposure to UV radiation. UV rays cause DNA changes that accumulate with age. The tumour cell is affected by its microenvironment. There are cellular contacts with stromal populations, especially with cancer-associated fibroblasts. Moreover, there is paracrine signalling through the exchange of growth factors, cytokines or chemokines. Standard 2D culture conditions do not allow to confidently mimic these complex multilateral interactions and therefore gradually lose their significance in the experiment. Research techniques are increasingly focusing on the 3D modelling or *in vivo* biological models.
The aim of the first presented series of experiments (1) was to define functional heterogeneity in the tumour microenvironment on the 3D spheroid melanoma model. The aim of the second series of experiments (2) was to monitor the behaviour of melanoma cells on the chicken choriolallantoic membrane *in vivo*.
(1) Heterogeneous spheroids were formed from melanoma cell line and variously photodamaged dermal fibroblasts. Normal dermal fibroblasts from the sun-protected skin of a juvenile donor and fibroblasts from the sun-exposed skin of a senior donor were used to model the heterogeneity of the stromal microenvironment. A key method to distinguish the behaviour of differently sun-exposed fibroblasts in creating a melanoma microenvironment was single-cell RNAseq. Bioinformatic analysis showed that both types of fibroblasts form clusters, defined by (a) the expression of proinflammatory factors, (b) genes for the extracellular matrix, and (c) genes for the TGFbeta signalling cascade. Photodamaged fibroblasts showed higher heterogeneity and in addition, their expression of proinflammatory factors promoting tumour progression, such as IL6 or CXCL8, was significantly stronger. Single-cell RNAseq thus reveals the effect of actinic damage on the behaviour of different fibroblasts and their interaction within the melanoma environment.
(2) The chicken chorioallantoic membrane represents an interesting and affordable model for studying the behaviour of malignant melanoma *in vivo*. It enables the study of invasion and migration, or tumour-induced angiogenesis. After the application of melanoma cells to the membrane, we observed remodelling of the bloodstream architecture. On the other hand, invasion of melanoma cells into the stroma of the chorioallantoic membrane was observed generally rare. The reason may be either the short development of the chicken *in ovo*, and thus the insufficient time for melanoma invasion, or the ability of this embryonic environment to overdrive the tumour cell malignant potential.
Both biological models represent a practically useful way to further advanced study of malignant melanoma in the context of the tumour microenvironment.

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Type of presentation: Oral

MS-O-2354 Syndecan receptors in disease: identifying roles at increasing resolution.

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Syndecans are a small family of cell surface receptors. They comprise an extracellular domain, bearing glycosaminoglycan chains, a single membrane spanning domain and a small cytoplasmic domain. The glycosaminoglycans are usually heparan sulfate, the most complex polysaccharide of mammalian cells. Heparan sulfate's importance lies in an ability to interact with a wide array of ligands, including growth factors, cytokines, chemokines, enzymes, morphogens and extracellular matrix macromolecules. These are concentrated at the cell surface where they can then interact with high affinity receptors, e.g. integrins, growth factor receptors. The short cytoplasmic domains have no intrinsic kinase activity, but can signal through interactions with cytoplasmic enzymes as well as elements of the actin cytoskeleton. We and others have mapped these pathways and interactions that frequently involve regulation of junction assembly.

Although there are no disease-causing mutations of any of the four human syndecans, there is compelling evidence of involvement in disease. For example, blocking antibodies against syndecan-1 are being trialled for treatment of myeloma. Other work implicates syndecan-4 in some forms of heart failure, while syndecan-2 promoter hypermethylation is potentially a powerful marker of early colorectal carcinoma. For these reasons, it is important to understand how syndecans, an evolutionarily ancient group of receptors, function at the molecular level. A variety of techniques including analysis of structure and signaling pathways is beginning to reveal their various roles.

Type of presentation: Oral

JS-O-2381 Molecular characterization of sickle cell Anemia induced hepatobiliary injury

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Sickle cell disease (SCD) is caused by a homozygous mutation in the β-globin gene, which leads to erythrocyte sickling, vaso-occlusion, and intense hemolysis. Vaso-occlusion and hemolysis are the two predominant pathophysiologic processes in SCD that contribute to chronic organ damage. Although progressive liver injury affects 10-40% of SCD patients, therapeutic approaches to prevent liver injury in SCD do not exist, and the etiological mechanism promoting progressive liver injury in SCD remains poorly understood. Unfortunately, the reported incidences of liver complications have increased with the growing life-expectancy of SCD patients, suggesting that therapies to halt the progression of liver injury in SCD patients are urgently needed.

In this study, we have used a transgenic, humanized mouse model of SCD that exclusively expresses sickle human hemoglobin. Our findings reveal that SCD mice developed chronic liver injury with age, which was manifested by sustained inflammation, hyperbilirubinemia and cholestasis. Using real-time in vivo imaging of the intact liver of live mice, we discovered the presence of sinusoidal ischemia and impaired bile transport across the apical membrane of hepatocytes in SCD mice. Mechanically we found that hepatobiliary injury in SCD was associated with significant liver senescence. To ameliorate liver damage in SCD mice we used a genetic model of p-selectin depletion in SCD mouse. Previously, P-selectin inhibition/deletion was shown to attenuate acute lung vaso-occlusion in transgenic humanized SCD mice administered an inflammatory stimulus. These findings were further validated by the significant reduction in VOE among SCD patients receiving the P-selectin blocking antibody. P-selectin Ab therapy is now FDA approved for prevention of VOE in SCD patients, however, the chronic effect of P-selectin inhibition in SCD remains to be determined. Using quantitative liver intravital microscopy (qLIM) in our recently generated P-selectin deficient SCD mice (SS-Selp−/−) we have shown that chronic P-selectin deficiency attenuates liver ischemia but fails to prevent hepatobiliary injury. Remarkably, we found that this failure in resolution of hepatobiliary injury in SS-Selp−/−mice was associated with impaired migration of leukocytes into the liver tissue, exacerbated cellular senescence, reduced epithelial cell proliferation, and reduced iron clearance in the liver. Moreover, we also found impaired iron trafficking in in SS-Selp−/− mice. Work is currently underway to understand how p-selectin loss promotes liver senescence and impaired iron trafficking in the liver. In summary, our results reveal a significant defect in iron homeostasis and exacerbated senescence in the liver of SS-Selp−/− mice suggesting that increased liver senescence might impair iron homeostasis which then contribute to hepatobiliary injury in SCD. Thus, the efficacy of P-selectin inhibition in preventing SCD warrants further studies to determine whether long term inhibition of P-selectin would lead to end stage complications.

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