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EDITORIAL

In focus in HCB

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In this first Editorial for 2021, and our 50th overall (!), we highlight four Original Articles describing (1) the intracellular localization of the guanine nucleotide exchange factor EGFP-fused DENND1B in several different cell lines by live-cell confocal microscopy); (2) the phenotypic and functional characterization of cardiac macrophages during heart development; (3) the increased level of the redox protein thioredoxin 1 (Trx1) in the bone and bone marrow following ischemi-reperfusion injury; and (4) description of a new protocol for double whole-mount ISH combined with immunoperoxidase staining for analyzing cellular gene expression patterns during forelimb development in avian embryos. We wish you good reading!

A connecdenn family member forms gathered line structure

The DENND1 family is comprised of three members, DENND1A-C, which function as guanine-nucleotide exchanging factors for the small G protein Rab35. This function is located in the N-terminus of the highly conserved DENN domain, whereas the clathrin-binding and the adaptor protein-2-interaction motif are part of the C-terminus (Marat et al. 2011). DENND1B/connecdenn 2 is known to be involved in clathrin-mediated endocytosis and fast recycling of megalin (Shah et al. 2013). In the current work, Park et al. (2021) investigated the intracellular localization of EGFP-fused DENND1B in several different cell lines by live-cell confocal microscopy. In the renal epithelial cell line BS-C-1, DENND1B exhibited two patterns: straight-line stress fiber-like structures, and a hitherto unknown gathered line structure. The gathered line structures were located at the bottom of spreading lamellipodia and as shown by optogenetic experiments, local activation of Rac1 may be important for their formation in this subcellular locale. They disappeared at the retracting site during cell movement in EGF-stimulated BS-C-1 cells indicating a relationship to cell migration. A complex relationship of the gathered line structures with cytoskeletal elements was revealed. F-actin bundles were observed to surround clusters of gathered lines but were not in direct contact with them (Fig. 1).

However, cytochalasin D treatment resulted in the disappearance of the gathered line structures indicating that the surrounding F-actin bundles may be indirectly important for their stability. The gathered lines were also partially associated with microtubules. When microtubules were depolymerized by nocodazole, gathered lines were disrupted indicating an involvement of microtubules in their formation and/or maintenance. Since the DENND1B-localized gathered line structures could not be observed in cells coexpressing DENND1B and Rab35, it was specualated the formation and/or the recruitment of DENND1B to the gathered line structures was hampered by Rab35. Last but not least, by the expression of DENND1B truncation mutants, the localization of DENND1B to gathered line structures was shown

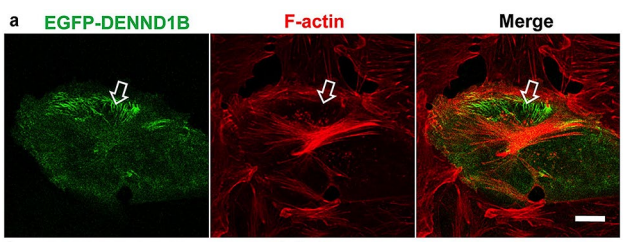

Fig. 1 **a** BS-C-1 cells transfected with pEGFP-DENND1B (green) and processed for F-actin staining with Alexa Fluor 594-phalloidin (red). The fluorescence signals of Alexa Fluor 594-phalloidin overlapped with those of EGFP-DENND1B in the straight-line structure (arrowheads), but not in gathered line structures (open arrows). From Park et al. (2021)

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to be dependent on the aa 279–692 region located in the C-terminus of DENND1B protein.

Getting to the heart of embryonic cardiac macrophages

Macrophages have often been considered as immune system-derived monocytes, circulating throughout the body and directed towards specific areas of need when required. However, more recently, mounting evidence indicates that macrophage phenotypes are tissue-specific, and the origin of tissue macrophages has become a topic of wide-spread interest in cell biology (Epelman et al. 2014). Gula et al. (2021) have now performed a series of experiments designed to characterize the phenotypes and functions of cardiac macrophages during development of the murine heart. They utilized a variety of experimental techniques including multi-label immunofluorescence for cell phenotype determination on frozen sections and tissue whole-mount preparations imaged by both conventional wide-field and confocal microscopy, flow cytometry on isolated embryonic cardiac cells, and RT-PCR on sorted single cell suspensions. For the immunofluorescence studies, fetal hearts from days E11–E18 were used, and for the flow cytometry and RT-PCR fetal hearts from E14 and E17. The phenotype of cardiac macrophages was determined by staining with antibodies for CD45, CD68, CD64, F4/80, CD11b, CD206 and Lyve-1 (Fig. 2).

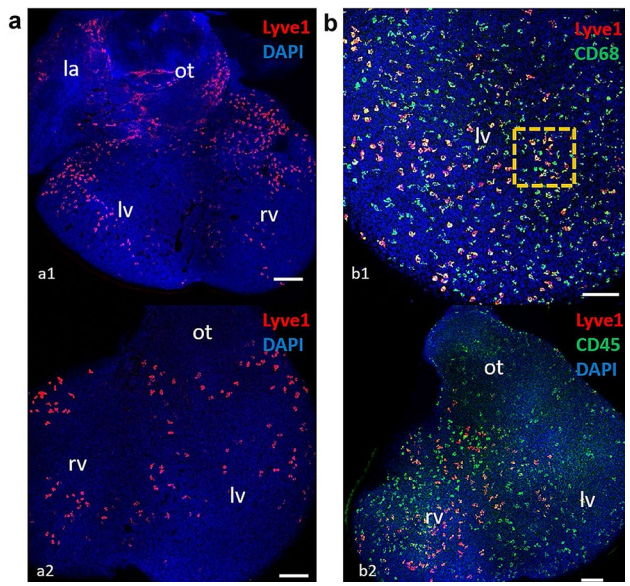

Fig. 2 Confocal images from whole-mount preparations of embryonic rat heart. **a** Lyve-1 + single stained cells located at myocardial surface. **b** Merged confocal images showing cells co-staining with Lyve-1 and CD68 or CD45. la, left atrium; lv left ventricle; rv, right ventricle; ot, outflow track. From Gula et al. (2021)

Taken together, the results provided the following details concerning embryonic cardiac macrophages: (1) by immunofluorescence they were founded in greatest abundance in the subepicardial space, as opposed to adult animals where they are located throughout the entire myocardial wall; and (2) their migration into the heart followed pathways of developing blood vessels and lymphatic vessels; (3) by flow cytometry, their heterogeneity was highlighted, and three newly defined subpopulations were identified: $CD64^{low}$, $CD64^{high}CD206^{-}$, and $CD64^{high}CD206^{+}$; and (4) by RT-PCR analysis differences were observed between the various macrophage phenotypes and embryonic stage regarding genes involved in angiogenesis, extracellular matrix remodeling, and lymphangiogenesis. Given this characterization, the authors suggest that cardiac tissue macrophages may be involved in diverse functions during cardiac development, as well as in pathological processes.

Reactive oxygen species in a remote organ response to myocardial infarction

Oxidative stress, associated with production of reactive oxygen species (ROS) is known to be involved in a myriad of disease and pathological situations via the stimulation of inflammatory processes (van der Vliet and Janssen-Heininger 2014). Indeed, ROS have been shown to be generated by subsequent reperfusion following myocardial ischemic events. Hydrogen peroxide as a diffusible ROS is known to effect redox signaling in sites distant from their tissue origin (Lismont et al. 2019). Moreover, leukocytes originating in the bone marrow appear to be the main cell types infiltrating the myocardium during reperfusion, and themselves are sensitive to alterations in redox status. This has led Godoy et al. (2021) to investigate the redox regulatory system proteins of the thioredoxin (Trxs; regulate protein thiol groups) and peroxiredoxins (Prxs; regulate hydrogen peroxide) families in myocardium, kidney, bone and bone marrow from rats following myocardial infarction-induced reperfusion. For their immunohistochemistry, Western blotting and ELISA experiments they used very carefully validated antibodies, with the data available in the "Redox Atlas of the Mouse" webpage (https://www.lillig.de/redoxatlas/).

Their results showed that following reperfusion and compared to sham-operated animals, (1) Trx1 levels were increased in the heart and femur; (2) in the femur and lumbar vertebrae, the Trx1 elevation was found to be associated with bone-lining cells, osteoblasts, and megakaryocytes (Fig. 3); (3) the increased Trx1 expression in heart and bone was aslo demonstrated by RT-PCR; and (4) elevation of Trx1 serum levels; and (5) treatment of animals with the glutathione precursor *N*-acetyl cysteine resulted in a reduction of Trx1 immunoreactivity in bone marrow precursor hematopoietic

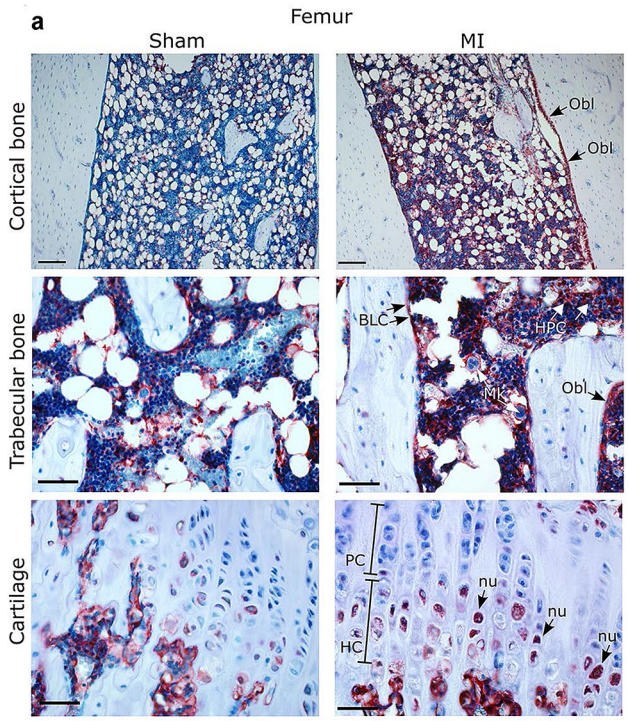

Fig. 3 **a** Immunohistochemical localization of Trx1 in rat femur in sham-operated and myocardial infarcted animals. Obl, osteoblasts; Mk, megakaryocytes; HPC, other hemaopoietic cells; BLC, bone lining cells; PC, zone of proliferating cartilage; HC, zone of hypertrophic cartilage; nu, nucleus. From Godoy et al. (2021)

cells; and (6) no significant alterations in the expression of the other Trx and Prx family proteins was observed. The authors propose a model illustrating the mechanism of Trx1 upregulation in the bone marrow following myocardial infarction, illustrating the remote organ effect of ischemia–reperfusion injury.

Identification of avian cell lineages sharing markers by double ISH and IHC

The chemokine receptor CXCR4 is involved in the control of cell migration during limb and cloacal muscle formation (Hunger et al. 2012) as well as of sympathetic ganglia progenitor cells, (Kasemeier-Kulesa et al. 2010). The migratory path of the mesodermally and neural crest-derived cells occurs in close spatial relationship and in addition the two cell lineages have markers in common. Thus, the in-situ identification of the two cell lineages poses some difficulties. In their present work, Yahya et al. (2021) report a protocol for double whole-mount ISH combined with immunoperoxidase staining for analyzing gene expression pattern in mesodermal and neural crest cells during forelimb development in avian embryos (Fig. 4).

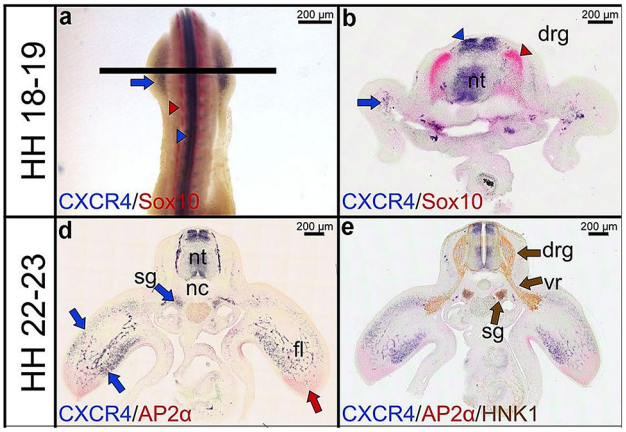

Fig. 4 Combined double whole-mount ISH and immunostaining for CXCR4, Sox10, Ap2α, and HNK1 in stage HH18-19 and HH 22–23 embryos. CXCR4 appears in blue, Sox10 and Ap2α in red, and HNK1 in brown. From Yahya et al. (2021)

Specifically, they analyzed the expression of CXCR4, Myf5, Pax3, Sox10, Ap2α and Slug as well as Nkx2.2, HNK1 and desmin. For ISH, digoxigenin- and FITC-conjugated riboprobes were used and together with immunoperoxidase IHC this permitted the simultaneous detection of specific mRNAs and the respective proteins. As an example of the application of the protocol, the expression pattern of mesodermal and neural crest cells during forelimb development in embryos ranging from HH18 to HH25 stage was analyzed.

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News

Springer Nature and ***Histochemistry and Cell Biology***

are proud to announce that the

Histochemistry and Cell Biology Lecture 2021

at the 16th International Congress of Histochemistry and Cytochemistry in Prague, Czech Republic will be delivered by

Professor Takehiko Koji
Nagasaki University Graduate School of Biomedical Sciences
Nagasaki, Japan

"Global changes in epigenomes and their significance in mouse spermatogenesis"

The Robert Feulgen Prize 2020 of the Society for Histochemistry

has been awarded ex aequo to

Dr. Christian Mühlfeld
Hannover Medical School, Hannover, Germany

for the development of new stereological and 3D image analysis techniques and their application to the quantitative analysis of the lung alveolar capillary network.

Dr. Hari Shroff
National Institute of Biomedical Imaging and Bioengineering, NIH, Bethesda, MD, USA

for significant improvement of spatial resolution and speed of image acqui- sition for super-resolution microscopes and their application in the generation of a 4D atlas of neurodevelopment in *C. elegans* embryos.

The Editors of *Histochemistry and Cell Biology* wish to convey their heartfelt congratulations to Drs. Mühlfeld and Shroff on the receipt of this honor.

https://www.microscopy-conference.de/

Invitation

Dear Colleagues,

It is a great pleasure to officially invite you to participate in the Microscopy Conference 2021 (MC2021), held in Vienna, Austria, from August 22 to 26, 2021. After the very successful preceding meetings MC2009 in Graz and MC2013 in Regensburg, MC2021 will be a combined conference of Dreiländertagung and Multinational Congress on Microscopy again. The conference will be jointly organised by ten microscopy societies from 11 countries (Austria, Croatia, Czech Republic, Germany, Hungary, Italy, Serbia, Slovakia, Slovenia, Switzerland and Turkey).

The MC2021 aims at bringing together leading experts and emerging young researchers, highlighting new developments in instrumentation and methods as well as providing a forum for new directions in the field of life or materials sciences. It will be an EMS extension, and it will focus especially on young scientists by providing very affordable fees. We are confident that at least 1,300 participants from all over Europe and overseas will attend the conference and guarantee a major scientific exchange.

The scientific programme will consist of plenary talks on important current topics. Latest developments in the fields of instrumentation and methods, materials science and life science will be highlighted by invited talks and also oral and poster presentations submitted. In addition to the scientific programme, workshops, an industrial exhibition, a conference dinner and award ceremonies in conjunction with award lectures will complement the programme.

The venue of MC2021 is the Congress Center Messe Wien, next to the Viennese Prater and within a 7-minute ride to the city center. The MC2021 will host a large exhibition, which aims to show the latest equipment from the manufacturers of all different kinds of microscopy and microscopy techniques, along with suppliers of consumables and accessories as well as publishers in the field. The exhibition will be embedded within the poster and catering area and will become an integral part of the conference.

Our goal is to organise a memorable microscopy event that gives you the opportunity of gathering information in science, networking and even perhaps enjoy some culture. We cordially welcome you to Vienna and to the MC2021!

Johannes Bernardi
Conference Chair

Michael Stöger-Pollach
Conference Co-Chair

Stefan Löffler
Conference Co-Chair

Dear Colleagues,

In the light of the COVID-19 pandemic, the ICHC 2020 organizers and IFSHC Executive Council decided to postpone the ICHC 2020 to **5 - 8 September 2021**. The ICHC 2021 will take place as originally planned in the Cubex Centre, Prague, Czech Republic. The safety of all participants is our top priority. We are sorry for any inconvenience the postponement might have caused you.

The ICHC is held every four years under the auspices of the International Federation of Societies for Histochemistry and Cytochemistry (IFSHC), which continually strives to provide grounds for communication and cooperation among scientists all over the world in the areas of cyto- and histochemistry, cell and tissue biology, microscopy, pathology and other relevant fields.

The city of Prague, also known as the heart of Europe, provides easy access for scientists from all over the world. The congress venue, Cubex Centre Prague which offers technologically and visually unique space, promises to leave everyone with an unforgettable experience. Of course, Prague prides itself with its beautiful historical architecture, technical monuments, celebrated cafés, great food, and beer. This will be underlined by the ICHC gala dinner in the famous Art Nouveau Municipal House, and a free beer party organized in the premises of the Staropramen brewery.

We hope that you will join us in Prague to discuss together your latest achievements and that the venue will provide great opportunities for specialists at all levels of their career, bringing lots of opportunities for strengthening international collaborations. Special attention will be therefore given to the presentations of students. We also expect a rich commercial exhibition where new and emerging technologies will be presented.

We are delighted to inform you that the following speakers will present a lecture at the congress:

Stefan Hell, a Nobel Prize laureate, Max Planck Institute for Biophysical Chemistry, Germany (keynote speaker)
Alev Erisir, Department of Psychology, University of Virginia, USA
Toyoshi Fujimoto, Juntendo University, Nagoya, Japan
Hans-Joachim Gabius, Institute of Physiological Chemistry, Ludwig Maximilians University of Munich, Germany
Bozena Kamińska, Nencki Institute of Experimental Biology PAS Warszawa, Poland
Takehiko Koji, Department of Histology and Cell Biology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
Ohad Medalia, Department of Biochemistry, University of Zurich, Switzerland

See you all in Prague, September 2021!

Hinke Multhaupt, President of the IFSHC
Klara Weipoltshammer, President of the Society for Histochemistry
Pavel Hozak, Chair of the Local Organizing Committee

Contacts
We will keep the current domain:
www.ichc2020.com

If you have any questions about registration, please contact: registration@ichc2020.com

If you have any questions about abstracts, please contact: abstracts@ichc2020.com

Other inquiries and comments about the conference, please contact: info@ichc2020.com

ANNOUNCEMENT

The Society for Histochemistry

Invites scientists to apply for the 2021 Robert Feulgen Prize. The prize is awarded for an outstanding achievement in the field of histochemistry.

The contributions may be either towards the development of new histochemical and cytochemical techniques or in the application of existing technology towards solving important problems in biology and/or medicine. Addressed are scientists working in microscopical sciences (in the widest sense) as well as in biochemistry, cell biology, endocrinology, in situ molecular techniques, and neurosciences. Scientists in their mid-career (assistant or associate professor, priv. doz.) are encouraged to apply. The prize is not intended for lifetime contributions.

The Prize consists of a monetary prize of €2,000

All applications should be submitted before January 31, 2021 via the electronic submission system at: https://www.greception.com/form-login-window/191a281d/

The application should contain a short curriculum vitae, a 1,000 word summary of the contributions of the applicant and PDF reprints of the pertinent publications. Full description of conditions is available on the Society website: http://histochemistry.eu/description_of_conditions_.html