In focus in HCB

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Published online: 16 September 2020
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Welcome to this month's Editorial in which we highlight three original articles describing (1) the tissue localization of the molecular chaperone Mdg1/ERdj4 during chick embryonic development, (2) developmental patterns for ABC transporter protein expression in the brain of fetuses, neonates, and young children when compared to adults, and (3) the role of the cyclic AMP response element-binding protein (CREB) in mouse primordial follicle activation. In addition, we highlight one short communication presenting a new immunohistochemical protocol to identify skeletal muscle fiber subtypes, the basal lamina, and capillaries in a single tissue section. As always, we hope you enjoy perusing all of the articles in this issue as the summer season winds to an end.

Molecular chaperone Mdg1/ERdj4 expression during chick development

Molecular chaperones, also called helper proteins, play a fundamental role in maintaining a functional proteome by assisting proteins to fold properly and thereby to prevent protein aggregation (Kim et al. 2013). One group of molecular chaperones is the Hsp70 system and its cycle is regulated by Hsp40 cochaperones. Besides their role in protein folding, molecular chaperones and cochaperones may have other functions as indicated by their presence in specific tissues and their regulated expression during development (Angelier et al. 1996; Vega-Nunez et al. 1999; Vos et al. 2008). Mdg1/ERdj4 is a vertebrate-specific cochaperone located in different cellular organelles (Berger et al. 2003; Dong et al. 2008; Pröls et al. 2001; Shen et al. 2002). Previously, Pröls et al.

(2001) have reported that this cochaperone is transiently upregulated in differentiating microvascular endothelial cells. In their present investigation (Daverkausen-Fischer et al. 2020), they have analyzed Mdg1/ERdj4 during chick embryonic development by immunohistochemistry. In the paraxial and lateral plate mesoderm of chick embryos at day 2 (HH stage 12 and 13), immunoreactivity for Mdg1/ERdj4 was evenly distributed in all mesenchymal cells (Fig. 1).

Fig. 1 Immunohistochemical Mdg1/ERdj4 protein pattern in the paraxial mesoderm. From Daverkausen-Fischer et al. (2020)

During mesenchymal-epithelial transition, immunostaining for Mdg1/ERdj4 became localized and concentrated in the apical and basal zones of the epithelial linings. In sclerotomal cells, after epithelial-mesenchymal transition, the apical-basal pattern of Mdg1/ERdj4 changed to the diffuse mesenchymal pattern. The detailed analysis of the developing central nervous system and the digestive tract revealed the epithelia-specific apical-basal and mesenchyme-specific diffuse Mdg1/ERdj4 distribution pattern. In the digestive tract, however, the polarized Mdg1/ERdj4 distribution seemed to be typical for the initial epithelialization process since it became diffuse with ensuing maturation. Moreover, the endothelia of the pharyngeal arch vessels and of the aorta, and the capillary endothelial cells, as well as serosa

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epithelium were positive and immunostaining for Mdg1/ERdj4 was also observed extracellularly in the mucus. From their observations, the authors drew two main conclusions: firstly, elevated levels of Mdg1/ERdj4 are essential for the induction of mesenchymal-epithelial transition and the maintenance of the epithelial organization during embryonic development, and secondly, constitutively elevated levels of the cochaperone are closely linked to cellular secretory activity.

Human blood–brain barrier and blood-cerebrospinal fluid barrier ABC membrane transporters exhibit specific developmental pattern

The human blood–brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB) display ATP-binding cassette (ABC) drug transporters which are of critical importance for the efflux of drugs (Morris et al. 2017). Although the localization of various ABC transporters in the adult human brain is well known (Nies et al. 2004; Morris et al. 2017), data for fetal, neonatal and young children's brains are sparse (Daaod et al. 2008; Lam et al. 2015; Strazielle and Ghersi-Egea 2015). Here, Verscheijden et al. (2020) report that BBB and BCSFB ABC transporter expression and location in maturing brains is different from adult brain. Using semi-quantitative immunohistochemistry, they analyzed the developmental patterns for the ABC transporters P-glycoprotein (Pgp), breast cancer resistance protein (BCRP), and multi-drug resistance-associated proteins (MRP) 1, -2, -4, and -5 in a unique cohort including fetuses, neonates and young children. They observed that in brain cortical BBB immunostaining increased with age for Pgp and BCRP, while in contrast, MRP1 and MRP2 staining intensity appeared higher in fetuses, neonates, and young children, as compared to adults (Fig. 2).

Transporter protein	PMA	GA	PNA
Pgp	$\rho = 0.5351, p < 0.0001$	$\rho = 0.4423, p = 0.0009$	$\rho = 0.2854, p = 0.0383$
BCRP	$\rho = 0.4303, p = 0.0013$	$\rho = 0.4350, p = 0.0011$	$\rho = 0.2604, p = 0.0597$
MRP1	$\rho = -0.1164, p = 0.4209$	$\rho = -0.1052, p = 0.4670$	$\rho = -0.1988, p = 0.1663$
MRP2	$\rho = -0.1556, p = 0.2708$	$\rho = -0.06476, p = 0.6450$	$\rho = -0.2340, p = 0.0917$

PMA postmenstrual age, GA gestational age, PNA postnatal age

ρ = Spearman's correlation coefficient. Bold = statistically significant ($p < 0.05$)

Fig. 2 Correlation of BBB staining intensity score with age. From Verscheijden et al. (2020)

The immunostaining for Pgp, MRP1, and MRP2 in the BCSFB appeared stable across age, while BCRP was not detected. MRP4 and MRP5 were not detected in BBB or BCSFB. Although the authors pointed out that their study has some technical limitations, they concluded that human BBB and BCSFB ABC membrane transporters show brain location and transporter-specific maturation. More specifically, Pgp and BCRP BBB expression is low and more variable around birth, potentially resulting in higher brain extracellular fluid concentrations of transporter substrates, whereas MRP1 and MRP2 expression appeared high around birth and diminishing towards adulthood.

A primer on primordial follicle activation

The development of the mammalian ovary is a highly regulated process occurring within a specific time frame. As very clearly described and illustrated by Wear et al. (2016), the process can be divided into five stages: (1) origin of germ cells and maintenance of pluripotency, (2) germ cell migration, (3) sex differentiation, (4) germ cell nest formation, and (5) germ cell nest breakdown and primordial follicle formation. The primordial follicles formed in stage 5 represent a pool of potential oocytes for fertilization following sexual maturity, maintaining a balance between follicle quiescence and activation. The process of primordial follicle activation is complex and regulated by a series of signaling events. Since previously Zhang and colleagues had discovered a role for the cyclic AMP response element-binding protein (CREB) in mouse oocyte maturation and cumulus expansion (Wang et al. 2016; Zhang et al. 2019), they have now expanded these studies to seek a possible role for CREB activity in primordial follicle activation (Li et al. 2020). In their detailed experiments, they employed multiple techniques, assays, and controls, including cultured ovaries, RNA interference, immunofluorescence and histological imaging and analysis, qRT-PCR, Western blotting, TUNEL assay for apoptosis, and chromatin immunoprecipitation. The results from all of these experiments and assays indicated that similar to mouse oocyte maturation and cumulus expansion, CREB is required for the activation of primordial follicles. Moreover, they determined that the mechanism of CREB activity was mediated through MAPK3/1-mTORC1 signaling-promoted KITL expression, and provide a model describing the signaling cascade involved in activation of primordial follicles (Fig. 3).

Fig. 3 Working model for the role of CREB in the activation of primordial follicles. From Li et al. (2020)

Finally, they provided a detailed Supplementary Table (1) providing specifications on all of the antibodies used in their kindly experiments, including validation procedures, serving as a valuable reference for future investigations.

New tissue section methods to identify muscle fiber subtypes and capillaries

The identification of muscle fiber subtypes, along with capillary density assessments is of critical importance in muscle training experiments, following nerve injuries (Cebasek and Ribaric 2019), or in relation to specific pathologies or disease entities (Umek et al. 2019). Techniques have been developed, and progressed over the years to perform these assays on muscle tissue sections through the use of histochemical, immunohistochemical, and lectin cytochemical means. Recently, the ability to clearly identify and distinguish type I fibers (slow oxidative), type IIA fibers (fast oxidative glycolytic), and type IIX fibers (fast glycolytic), together with capillary density measurements has become of interest in “response to training” studies. In a recent publication, Al-Shammari et al. (2019) presented a method to stain for types I, IIA, and IIX muscle fibers and the basal lamina using specific antibodies, while using the *Ulex europaeus* agglutinin (lectin; UEA I) to label capillary endothelial cells in the same tissue section. Bailly et al. (2020) have now presented a similar methodology, however replacing the UEA I lectin methodology with the endothelial cell-specific CD31 antibody. They tested five different protocols with various antibody cocktails and single- and two-step procedures to establish a time- and tissue-saving reliable immunohistochemical staining method for the three muscle fiber subtypes, the basal lamina (for muscle fiber morphometry measurements), and endothelial cells on a single tissue section (Fig. 4).

Fig. 4 Immunohistochemical staining of human skeletal muscle using method 1 (a) and method 3 (c) to reveal type I (blue), type IIA (green), type IIX (black) fibers, laminin (white), and endothelial cells (red). From Bailly et al. (2020)

Image analysis was performed to determine the number of capillary contacts (CC) per muscle fiber subtype (see Harris 2005 for review of the method). Based upon their extensive protocol evaluation, including aspects such as intra-experimenter reproducibility, they propose two methods, one a two-step procedure, and the other a one-step procedure. The two-step procedure has the advantage that the CD31 antibody staining (using a mouse monoclonal antibody) produces crisper images of the capillaries, making their identification simpler, whereas the one-step procedure is faster to perform. Detailed protocols for all of the methods are included as tables in the manuscript, providing a robust platform for investigators seeking to assess muscle fiber subtypes and associated capillary contacts in single muscle tissue sections.

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News from the Society for Histochemistry**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 acquisition 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.

16TH INTERNATIONAL CONGRESS OF HISTOCHEMISTRY AND CYTOCHEMISTRY

5 - 8 September
PRAGUE 2021

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

Bożena 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

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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

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