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EDITORIAL

In focus in HCB

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We would like to begin this Editorial for the June issue by acknowledging the pioneering work by Dr. June Almeida and her colleagues who were the first to identify coronaviruses as a class using negative-staining transmission electron microscopy (Almeida et al. 1968). Now, on to this month's feature articles.

Chemokine signaling and anterior pituitary gland vascular remodeling

The anterior pituitary gland, like other endocrine glands, is rich in fenestrated capillaries (sinusoids) forming an intricate network into which various hormones are released. In addition to five endocrine cell types, the anterior lobe consists of S100β-positive cells as well as endothelial cells and pericytes forming the sinusoids. The majority of the S100β-positive cells are sex-determining region Y-box 2 (SOX2)-positive stem/progenitor cells. Previously, Horiguchi et al. (2012) showed that they may also express CD9 and the CXC chemokine ligand 12 (CXCL12) and its receptor CXCR4. Since chemokines are known to regulate angiogenesis (Strieter et al. 2006; Walenta et al. 2011; Zlotnik and Yoshie 2000), Horiguchi et al. (2020) now investigated by qPCR, ISH and IHC, siRNA knockdown, $CD9^+$ cell isolation, and electron microscopy whether C, CC and CX3C chemokine ligands and their receptors are expressed in the CD9/S100β/SOX2-positive stem/progenitor cells of the anterior lobe (Fig. 1).

They showed the presence of the CX3C chemokine ligand 1 (CXCL1) in 7.8% of the CD9-positive cells. By culturing CD9-positive cells on laminin-coated plates, they could demonstrate their differentiation into endothelial cells, which was accompanied by decreased CX3CL1 and increased endothelial cell progenitor marker Sox18, as well as the CX3CL1 receptor CX3CR1. Similar changes in the expression of these markers were found in vivo during the neovascularization in the prolactinoma rat model. The authors concluded that CX3CL1/CX3CR1 signaling in CD9/S100β/SOX2-positive cells of the anterior lobe plays an important role in resupplying endothelial cells for vascular remodeling.

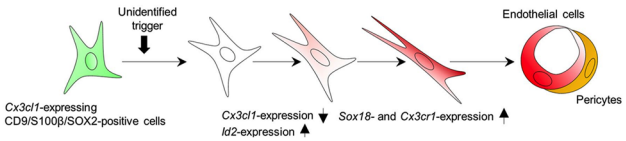

Fig. 1 Model of vascular remodeling of *Cx3cl1*-expressing CD9/S100β/SOX2-positive cells. From Horiguchi et al. (2020)

Cell cycle progression transcriptomic profile of ovarian granulosa cells

In a mature ovarian follicle, granulosa cells (GCs) form the follicle wall and surround oocytes. Besides their architectural tasks, they have important nutritional functions for oocytes during development and maturation (Jankowski et al. 2018). Furthermore, GCs have significant stem cell-like potential and express markers for mesenchymal stem cells and pluripotent stem cells (Kossowska-Tomaszczuk et al. 2009; Varras et al. 2012). Above all, isolated GCs can be grown as monolayer cultures, which can be subjected to molecular analyses. Kulus et al. (2020) report the results of a transcriptomic analysis of the expression of various genes regulating the cell cycle progression in porcine GCs with the use of Affymetrix microarrays (AffymetrixPorcine Gene 1.1 ST Array Strip), which was validated by RT-qPCR. This analysis was accompanied by detailed histological review of ovaries and separated follicles.

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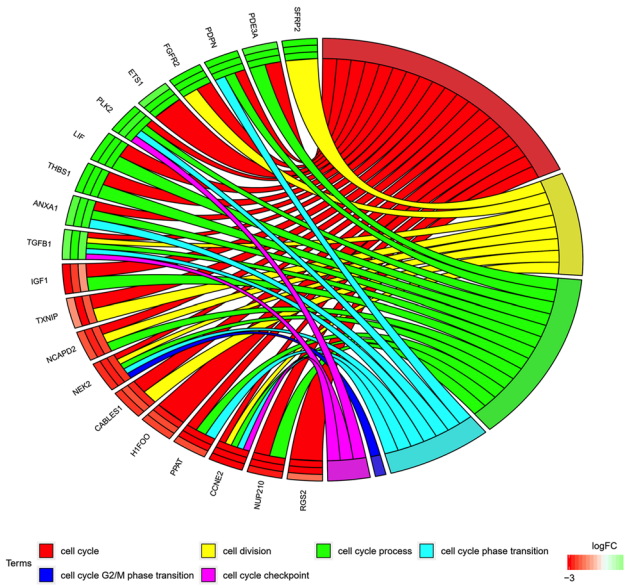

Fig. 2 Representation of the mutual relationship between 20 chosen genes that belong to various cell cycle progression GO BP terms. From Kulus et al. (2020)

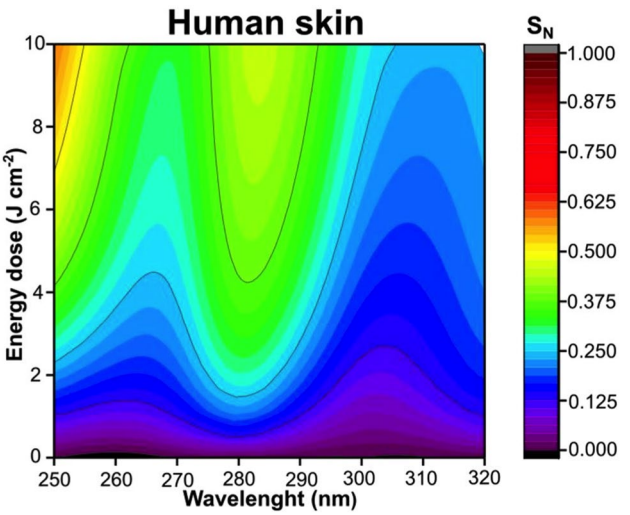

Fig. 3 Contour maps of free NO generated from human skin slices irradiated at 250–320 nm. S_N indicates normalized signal of free NO generated. Red, orange, yellow = high NO level; green = intermediate NO level; dark blue, light blue, purple = low NO levels. From Pelegrino et al. (2020)

Out of 27,558 transcripts analyzed at 48, 96 and 144 h of culture, 3380 transcripts showed a differential expression. These transcripts belonged to 344 Gene Ontology Biological Process (GO BP) terms. For a detailed analysis, the authors focused on "cell cycle checkpoint", "cell cycle G1/S phase transition", "cell cycle G2/M phase transition", "cell cycle phase transition", "cell cycle process", "cell cycle" and "cell division" GO BP terms (Fig. 2).

Furthermore, a STRING interaction network for the proteins encoded by the analyzed genes was generated and the functional interactions between selected genes were analyzed. As pointed out by the authors, the large amount of data obtained in this transcriptomic analysis will provide a reference for in vivo studies on the process of mammalian folliculogenesis and future clinical applications of GCs.

"NO skin off my back" to describe this work

Human skin contains a large amount of the small molecule nitric oxide (NO), stored in the more stable forms of nitrite (NO_2^-), nitrate (NO_3^-), and *S*-nitrosothiols (RSNOs), collectively referred to as NO_x. Upon stimulation by ultraviolet sunlight, the NO_x stores in the skin can be redistributed to the blood stream where they may exert a positive physiological response by lowering vascular blood pressure (Weller 2016). Weller and colleagues have been studying the aspects of this beneficial sunlight-induced process for a number of years (Liu et al. 2014; Mowbray et al. 2009), and have now investigated the most effective range of ultraviolet wavelengths for generating NO (Pelegrino et al. 2020). They used an NO meter with an electrochemical NO sensor to measure the release of free NO_x species following irradiation with a range of ultraviolet wavelengths from both a controlled chemical aqueous solution simulacrum and human skin biopsy samples (Fig. 3).

Their results showed that NO generation in human skin samples was most effectively achieved using ultraviolet light with a peak at 280–285 nm (in the UVB range). Moreover, they interestingly found RSNO formation in human skin slices using light at both 320 nm (in the UV region) and 700 nm (in the visible region). These results are of potential interest for clinical trials and photodynamic therapy, but must be measured against the known risk factor of increased exposure to ultraviolet light for developing skin cancer. Ultimately, achieving a balance between the opposing health effects exerted by ultraviolet light on human skin is certainly of great importance moving forward.

"Expansion" of the super-resolution microscopy toolbox

Super-resolution microscopy came to the forefront of imaging in the mid-to-late 2000s in a variety of implementations (Klein et al. 2014; also see the entire Special Issues of *Histochemistry and Cell Biology*: In Focus: Single-Molecule Super-Resolution Microscopy, June and July 2014; Volume 141, issues 5 and 6). Also referred to as single-molecule imaging, super-resolution microscopy techniques allow

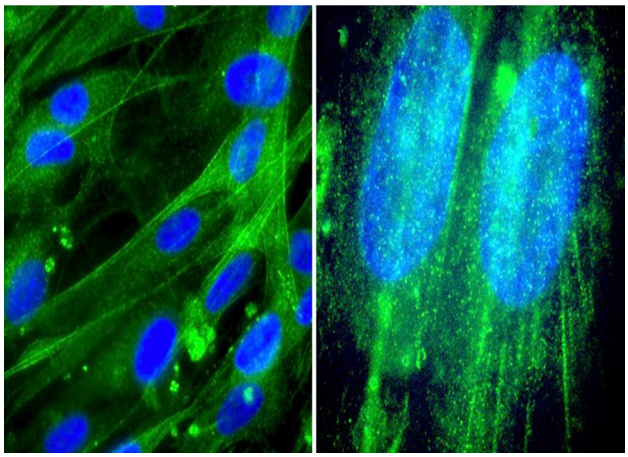

Fig. 4 Unexpanded (*left panel*) and expanded (*right panel*) cultured human skeletal muscle cells labeled with anti-myosin IIb antibody (*green*) and nuclear staining with DAPI (*blue*). Note the differential expansion of the cells obtained with the modified expansion protocol described in the manuscript. From Pernal et al. (2020)

sub-diffraction-limited imaging of fluorescently tagged molecules in cells and tissues. To achieve this enhanced resolution, instruments typically consisted of microscopes incorporating powerful lasers for the excitation of a specialized subset of fluorophores, together with a variety of software algorithms for constructing the final image (Klein et al. 2014). Recently, however, Boyden and colleagues have pioneered novel techniques, collectively referred to as "expansion microscopy" (ExM) for achieving super-resolution microscopy using both conventional diffraction-limited microscopy and fluorophores (Alon et al. 2019; Chang et al. 2017; Chen et al. 2015; Gambarotto et al. 2019; Tillberg et al. 2016; Wassie et al. 2019). Other groups have also contributed novel modifications to the expansion microscopy techniques (Li et al. 2018; Truckenbrodt et al. 2018). In essence, expansion microscopy involves introducing hydration-competent polymer gels to physically expand the sample (typically achieving fourfold lateral expansion), resulting in a physical magnification and enhanced super-resolution microscopy (lateral resolution ~ 70 nm) with a diffraction-limited microscope (Chen et al. 2015). Jena and colleagues (Pernal et al. 2020) have now provided additional modifications to the ExM protocol, referred to as "differential expansion microscopy" using rat liver tissue slices and cultured human skeletal muscle cells as samples. With their introduced modified protocol, they were able to achieve greater than 500-fold volumetric sample expansion, and demonstrate anisotropic expansion between tissues, between organelles, and also within organelles themselves (Fig. 4).

They also introduce machine learning approaches based on neural networks for automated processing and analysis of organellar morphometric differences attributable to ExM. Finally, they provide a detailed figure describing their modified expansion protocol, which should be of use for researchers wishing to implement it in their own labs. It is likely that further improvements in expansion and resolution of ExM will be forthcoming in the near future.

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News from the Society for Histochemistry

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
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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