



## In focus in HCB

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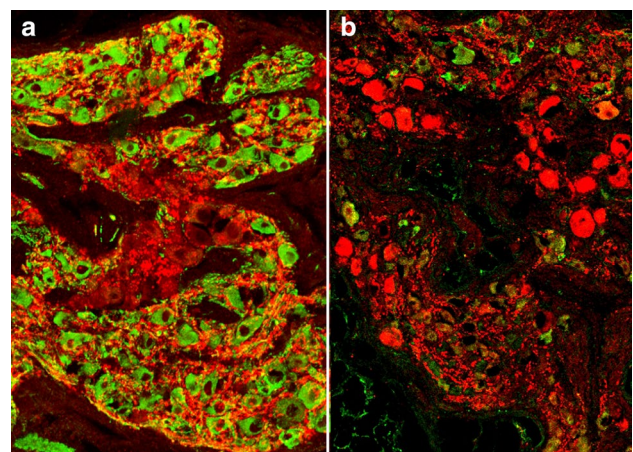
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In this third Editorial of 2020, we provide a brief synopsis of two Original Papers and one Short Communication illustrating multiple contemporary investigative techniques. We highlight (1) a detailed multi-fluor confocal microscopic and quantitative real-time PCR investigation of the effects of neonatal castration on anterior pelvic neurons in a pig model, followed by (2) a fluorescence microscopy and semi-quantitative image analysis approach to examine the result of dietary supplementation of polyunsaturated fatty acids on renal tubular epithelial cells in a diabetic rat model, and finally (3) presentation of detailed protocols for combined RNAscope ISH and IHC on sections from human diffuse large B-cell lymphomas. As always, we hope you enjoy the entire issue!

### Castration incites apoptosis of pelvic neurons

The effects of androgens on neuronal circuits in the central nervous system are well documented (Heberden 2017). In contrast, much less is known about the influence of gonadal steroids on peripheral neurons (Keast 2000). Kaleczyc and colleagues (2020) have analyzed the effect of androgen deprivation on neurons of the pelvic plexus following castration of 1-week-old male pigs. The anterior pelvic ganglion of the pelvic plexus of pig consists of androgen-dependent adrenergic and cholinergic neurons (Kaleczyc et al. 2019) and supplies the testis and vas deferens as well as the urinary bladder (Pidsudko 2014). The anterior pelvic ganglion plexus of male pigs was analyzed 3 and 6 months after castration by multiple labeling immunofluorescence

(Fig. 1) and quantitative real-time PCR. Specifically, the protein gene product 9.5 as a general neural marker was analyzed, dopamine- $\beta$ -hydroxylase as an adrenergic marker, vesicular acetylcholine transporter as a cholinergic marker, VIP, galanin and calcitonin gene-related peptide as sensory markers, and cleaved caspase-3 as an indicator of apoptosis. Three months after castration, 74% of adrenergic and 31% of cholinergic neurons of the anterior pelvic ganglion were immunopositive for caspase-3 and the number of perikarya that expressed calcitonin gene-related peptide, galanin and VIP was greatly increased. Six months after castration, about 90% of neurons and intraganglionic nerve fibers were lost, most probably due to apoptosis. The immunohistochemical data were corroborated by the results of quantitative real-time PCR. Collectively, these data show the profound effect of castration-induced androgen deprivation on pelvic neurons. The authors concluded that the excessive loss of pelvic neurons due to apoptosis may have consequences for the normal functioning of the urinary bladder and urethra. In more general terms, gonadectomy as well as reproductive



**Fig. 1** Double confocal immunofluorescence for vesicular acetylcholine transporter (green) and dopamine- $\beta$ -hydroxylase (red) in the anterior pelvic ganglion of a 1-week-old male pig (a) and 6 months after castration (b). From Kaleczyc et al. (2020)

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hormone disorders may result in dysfunction of the lower urinary tract.

## A “connexin” between diet and diabetic nephropathy

It is well known that dietary control is important in ameliorating the chronic effects of diabetes on multiple organs (Evert et al. 2014). The kidneys represent a main target for the complications of diabetes, with the possibility of the onset of diabetic nephropathy, with cell–cell communication proteins often the target of pathological changes. Earlier investigations have established the central role of dietary supplements such as polyunsaturated fatty acids (PUFAs) in regulating the expression of integral plasma membrane proteins such as connexins and pannexins (Puebla et al. 2017; Samuels et al. 2013). Recently, Vitlov Uljevic et al. (2019) demonstrated that dietary supplements with *n*-3 form of PUFAs resulted in an alleviation of renal distal tubular cell damage in a rat experimental model of diabetes mellitus. This same group has now extended this study by investigating a possible positive effect of dietary supplementation with differing *n*-6/*n*-3 PUFA ratios on diabetic nephropathy through an alteration in connexin and pannexin protein expression (Luetic et al. 2020). The study involved staining tissues from groups of diabetic rats fed a diet incorporating varying ratios of *n*-6/*n*-3 PUFA for immunofluorescence microscopy with several different anti-connexin and -pannexin antibodies, followed by semi-quantitative image analysis, focusing on the renal cortex. The results of their imaging and analyses showed that (1) in the renal cortex from diabetic rats versus control animals, immunostaining for connexin 40 (Cx40) and Cx43 was diminished, whereas that for pannexin 1 (Panx1) was increased; (2) dietary addition of a high *n*-6/*n*-3 PUFA ratio to diabetic animals resulted in decreased immunostaining for Cx45 and Panx1; and (3) feeding of a low *n*-6/*n*-3 PUFA ratio supplement to diabetic rats led to an increased expression of Cx43 immunoreactivity. Their results underscore the potential importance of dietary PUFAs supplementation in lessening the causes of diabetic nephropathy.

## Combined RNAscope ISH and immunohistochemistry to characterize tumors

RNAscope ISH technology has provided the technological means to perform in situ hybridization on paraffin-embedded tissues to localize even small amounts of RNA with diminished background signals (Wang et al. 2012). Recently, the combination of RNAscope ISH together with

immunohistochemical (IHC) antigen localization has provided even more detailed characterization of the expression of these molecules in various cell types (Grill et al. 2019). In this issue, Annese and colleagues (2020) have provided a detailed set of protocols for optimizing dual RNAscope ISH for STAT3 mRNA with IHC for protein FVIII in the characterization of angiogenesis in diffuse large B-cell lymphomas (DLBCLs). Additionally, they detail how the sections were scanned by whole slide imaging and automated image analysis using a Fiji plugin to count the number of probes per nucleus. Figure 1 included in their manuscript provides a very detailed workflow for a 2-day protocol for the combined RNAscope ISH–IHC techniques, which can be easily followed in other laboratories. Given the heterogeneous nature of tumors and the tumor microenvironment, these dual staining techniques should prove to be most useful for dissecting their detailed molecular analyses and possibly diagnostic features.

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## News from the Society for Histochemistry

# 16<sup>TH</sup> INTERNATIONAL CONGRESS OF HISTOCHEMISTRY AND CYTOCHEMISTRY

30 August - 2 September  
**PRAGUE 2020**

Dear Colleagues,

On behalf of the board of the Society for Histochemistry, it is our great pleasure to invite you to the world 16th International Congress of Histochemistry and Cytochemistry (ICHC), to be held on August 30 – September 2, 2020, in Prague, Czech Republic.

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.

Looking forward to meeting you in Prague,

Klara Weipoltshammer, President of the SfH  
Pavel Hozak, Chair of the Local Organizing Committee, Secretary of the SfH

### Contacts

Home: [www.ichc2020.com](http://www.ichc2020.com)

If you have any questions about registration, please contact: [registration@ichc2020.com](mailto:registration@ichc2020.com)

If you have any questions about abstracts, please contact: [abstracts@ichc2020.com](mailto:abstracts@ichc2020.com)

Other inquiries and comments about the conference, please contact: [info@ichc2020.com](mailto:info@ichc2020.com)

## ANNOUNCEMENT

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**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](http://histochemistry.eu/description_of_conditions_.html)