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EDITORIAL

In focus in HCB

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As we offer the first "In focus in HCB" Editorial for the new year, we would like to take this opportunity to wish our readership a peaceful and wholesome 2019. As *Histochemistry and Cell Biology* enters its 61st year, the four papers highlighted in the Editorial exemplify what the journal represents and will continue to stand for, namely the localization, identification and quantification of molecular components, supramolecular structures and organelles as well as metabolic activities of cells and tissues. Logically, this will encompass the improvement of existing techniques, and the development of novel methods to explore the complex relationships between gene expression, cell- and organelle-specific protein expression, enzymatic activities and metabolic reactions. For all these different aspects, *Histochemistry and Cell Biology* will continue to provide a scientific platform.

Gray's (Micro)-Anatomy

Liver tissue is composed of several cell types, with the hepatocyte representing the most abundant cell. However, across the liver lobules hepatocytes in different regions have been shown to perform specific cellular functions, and in fact to display significant differences in gene expression profiles. Interestingly, notwithstanding these observations, the nuclear chromatin pattern of these different hepatocytes appears similar by conventional light microscopic observation. Paunovic et al. (2019) have now investigated this issue by performing sophisticated image analysis on two distinct hepatocyte populations: those located perivenous and those located periportal. Sections from paraplast-embedded mouse liver were stained with a modified toluidine blue protocol, and images acquired with a scanner attached to a light microscope. Various textural features in the nuclear chromatin were then analyzed with the gray-level co-occurrence matrix (GLCM) module (Pantic et al. 2016) in CellProfiler software. They found significant differences in angular second moment, GLCM variance, and GLCM sum variance when comparing the chromatin structure of perivenous versus periportal hepatocytes. Additionally, some of the GLCM features determined in perivenous hepatocytes correlated with serum aminotransferase levels (indicative of hepatocyte damage), but not in periportal hepatocytes. Although some GLCM features were found to differ between the chromatin structure observed in perivenous versus periportal hepatocytes, the authors offered the following caveats to the results of this study: (1) image quality (such as focus) may affect the measurement of GLCM features; (2) small sample number (200 chromatin structures analyzed); and (3) the toluidine blue technique, which stains all nucleic acids, may not be as sensitive as the Feulgen stain which is specific for DNA. However, the authors do suggest that even with these limitations, the GLCM method may find utility in future studies of liver pathology and physiology.

Eph–ephrin gene expression during bone fracture repair

Eph receptors (Eph) represent a large family of receptor tyrosine kinases and exclusively bind membrane-tethered ligands termed ephrin proteins. They have important functions not only during development as regulators of morphogenetic processes but also for postnatal tissue homeostasis (Kania and Klein 2016). For instance, Eph–ephrin signaling has been shown to be involved in adult bone homeostasis by mediating the communication between bone-forming osteoblasts and bone-resorbing osteoclasts (Matsuo and Otaki 2012). Kaur et al. (2019) have now analyzed Eph–ephrin gene expression pattern and the location of the respective proteins during endochondral bone fracture repair in adult

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mice. Although they observed widespread gene expression in the fractured bone, only the ephrin A family members *Epha4, Epha5* and the ephrin B family member *Efnb1* showed significant changes in their expression: both *Epha4* and *Efnb1* were downregulated, whereas *Epha5* was upregulated. The respective ephrin proteins were detected in prehypertrophic chondrocytes and osteoblasts of the fracture callus, as well the periosteum and fibrous tissues. Furthermore, a positive correlation between the mRNA levels of *EfnB1* with *Col10* and *Epha5* with *Bglap* (osteocalcin) and the colocalization of the respective proteins was interpreted to indicate that *EfnB1* is a positive mediator of prehypertrophic chondrocyte development and that *Epha5* is involved in osteoblast-mediated mineralization of the fracture callus. It is concluded that EfnB1 and EphA5 comprise major mediators of fracture callus cartilage hypertrophy and ossification. However, the authors also emphasize that for a detailed characterization of ephrin functions in bone fracture repair further investigations including knockout studies with approaches that distinguish forward and reverse ephrin signaling are required.

Antibody specificity issues? Perhaps reach for RNAscope!

The readership of *Histochemistry and Cell Biology* should be well aware of the confounding issue of antibody specificity and the validation protocols required to ensure recognition of the appropriate molecular epitope (see for instance Griffiths and Lucocq 2014; Howat et al. 2014; Uhlen et al. 2016; Ma et al. 2017). Indeed, concerns surrounding antibody specificity resulted in the introduction of a new editorial policy at *Histochemistry and Cell Biology* whereby submitted manuscripts containing results from antibody-based protocols must provide details on the validation of the antibodies (see "Instructions for Authors" at https://www.springer.com/biomed/journal/418 for our policy).

In this issue of the journal, Grill et al. (2019) turned to in situ hybridization to investigate the localization of endocannabinoid receptors and enzymes during systemic and intestinal inflammation since several of the antibodies raised against these particular proteins display a lack of specificity. They utilized the RNAscope platform based on ZZ probes (Wang et al. 2012) for a sensitive and specific method for localizing low-copy RNA in their tissue samples. The RNAscope kit provides both positive and negative control probes, to ensure that the protocols are functioning appropriately and that cellular RNA has survived the tissue processing methods. The authors then performed immunohistochemical staining for cell-type specific antibodies together with the RNAscope localization of specific RNA probes identifying *cannabinoid receptors 1 and 2* (*$CB_1$and CB_2*), *G protein-coupled receptor 55* (*GPR55*), and *monoacylglycerol lipase (MGL)*. They found expression of these mRNAs in various cell types throughout the intestinal wall and immune system cells. Alterations in the cellular patterns of expression of these mRNAs, particularly for GPR55 and MGL were observed in the background of both intestinal and systemic inflammation. Their results provide compelling evidence that (i) RNAscope technology can be considered a viable option for assisting in protein localization studies where specific and validated antibodies may be unavailable and (ii) demonstrate a distinct cellular role in the regulation of the immune response to intestinal and systemic inflammation for GPR55 and MGL. The latter finding may be related to the observation that *Cannabis* appears to be of benefit for people with inflammatory bowel disease.

A traditional histochemical method permits quantification of mitochondrial coupling

Meijer and Vloedman (1980) published a simple histochemical method to determine the coupling state of mitochondria in skeletal muscle, which, however, was not amenable to quantification. Since due to technical reasons current methods to measure the mitochondrial coupling state are cumbersome, Peters et al. (2019) reconsidered whether Meijer and Vloedman's method conforms to the Lambert–Beer's law. When they performed the histochemical reaction for 10 min on 5-µm thick cryosections of rat myocardial tissue fixed in formalin-Macrodex, they observed by microdensitometry a linear correlation between the absorbance resulting from the histochemical reaction product on one side and the section thickness and incubation time on the other. Since the reaction product was not entirely due to mitochondrial F_1F_oATPase, ATPase background correction was introduced by subtracting the absorbance measured after section incubation with oligomycin. It is concluded that the histochemical method can be used to estimate ATP/O_2 ratios and this was shown for healthy rat myocardium and overloaded right-side myocardium of pulmonary hypertensive rats. Of note, the histochemical method can be performed with samples smaller than a milligram, and therefore, can be used for diagnostic tests of mitochondrial function in myocardial biopsies.

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