In focus in HCB

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In this issue, we offer brief synopses of four manuscripts highlighting machine-learning algorithms for transmission electron microscopy images of virus particles in cells, multi-label confocal microscopy of baroreceptors in carotid sinus, quantitative mass spectrometry of signaling components during ovary development, and transcriptomic analysis of genes regulating proliferation in cultured granulosa cells. These manuscripts nicely illustrate some of the wide-ranging and diverse technologies currently applied in the area of cell biology, and we hope that you enjoy learning more about them.

CNN update goes viral...

Machine-learning and neural network analyses are changing the way which images are quantitatively and visually analyzed (see for instance, Brent and Boucheron 2018; Brasko et al. 2018; Christiansen et al. 2018; Ounkomoi et al. 2018). As microscopists, these new techniques will most likely become incorporated into our normal research workflow. In this issue, Devan and colleagues (2019) provide a detailed account of their development of a machine-learning convoluted neural network (CNN) algorithm to assist in the recognition of secondary envelopment of human cytomegalovirus in images acquired by transmission electron microscopy. If you have ever attempted to differentiate virus particles from the other electron-dense particulate material abundant in cells visualized at the electron microscopic level, you are well aware of the challenges posed. Standard image analysis techniques exist to aid in virus identification based upon ultrastructural features, but they are quite time-consuming,

labor-intensive, and fraught with potential investigator subjective errors. Devan and colleagues therefore turned to the concept of deep learning, a subfield of machine learning and artificial intelligence to develop protocols for automated identification of viral capsids in their images. Since CNNs typically require large amounts of training data to be effective, and given the amount of effort required to prepare and image samples by transmission electron microscopy, they tested several “transfer learning” technique strategies to create image classification systems using a small training data set. In a series of complicated test trials, they validated their developed algorithms and compared them with each other and with the traditional image analysis techniques for their ability to correctly identify cytomegalovirus nucleocapsids in images from infected cells. Their results showed that transfer learning techniques applied in the generation of CNNs can indeed provide automated detection of viral capsids in electron microscopy images. Moreover, their proof-of-principle investigation of viral capsid identification indicates that, by altering the training data sets and fine tuning the algorithms, these CNN techniques should be useful for the automated detection of the other morphologically identifiable cellular structures in high-resolution images.

Multi-label confocal microscopy as a (baro) meter for pressure receptors

Arterial receptors for blood pressure (baroreceptors) are located in the carotid sinus of the carotid artery. Mapping of sensory nerve endings in the rat carotid sinus has previously been accomplished using silver impregnation (Yates and Chen 1980) and methylene blue staining (McDonald 1983). Yokoyama and colleagues (2019) have now re-investigated the distribution and morphology of sensory nerve endings in the rat carotid sinus using a multi-label confocal microscopy approach on both whole-mount preparations and thick cryosections. They used antibodies raised against the ionotropic ATP receptors P2X2 and P2X3, together with antibodies

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recognizing myelin basic protein (MBP) to identify myelin sheaths, as well as S100 and S100B to identify Schwann cells to establish the spatial relationships between the receptors of axon terminals and terminal Schwann cells. Importantly, to substantiate the results of the immunostainings, a very detailed description and validation of the antibodies was provided. The results of their immunostaining experiments demonstrating the localization of P2X3-positive nerve endings corroborated the earlier silver impregnation and methylene blue stainings with respect to the morphological distribution of the nerve endings. Dual P2X2- and P2X3-positive axon terminals were shown to be surrounded by Schwann cells in the carotid sinus, displayed complex leaf-like axon terminals, and originated from both myelinated and unmyelinated nerve fibers. New morphological results demonstrated that P2X3-immunostained axon terminals are composed of a variety of protrusion shapes projecting in three dimensions. Moreover, a brief functional characterization of the nerve endings was performed by measuring blood pressure changes following electrical stimulation of select morphological regions of the carotid sinus. Further studies are required to provide more information on the functioning of ATP in these P2X2/P2X3 presenting nerve fibers in the rat carotid sinus.

Quantitative mass spectrometry of WNT signaling in the human ovary

Wingless-type mouse mammary tumor virus integration site (WNT) signaling network is important during mammalian gonad development and sex differentiation (Maatouk et al. 2008; Ohinata et al. 2009). The canonical WNT signaling includes binding of an excreted WNT ligand to a frizzled receptor triggering the accumulation of non-phosphorylated β -catenin which, upon translocation to the nucleus, binds with lymphoid enhancer factor/T-cell factor (LEF/TCF) transcription factors. Its downstream targets promote proliferation or cell fate determination and differentiation in stem cells (Reya and Clevers 2005). Bothun and Woods (2019) have investigated the dynamics of WNT signaling, and identified various WNT signaling components in developing human ovaries by analyzing a comprehensive quantitative mass spectrometry data set of tissues at 47, 108, 122, and 137 days of development as well as in adult ovarian cortex. A total of 24 WNT signaling-related proteins were identified throughout ovarian development. When individually screened in ovarian tissues from specific stages, Frizzled 1 (FZD1), Frizzled 2 (FZD2), and Frizzled 7 (FZD7) had varying degrees of expression at each stage of development or adult tissue, whereas glycogen synthase kinase-3 beta (GSK3B), a protein kinase and negative regulator of WNT signaling, was detected at each analyzed developmental day,

but not in adult tissue. Finally, β -catenin (catenin beta-1, CTNNB1) was detected in all the tissues tested. By immunohistochemistry, GSK3B was nearly ubiquitously expressed during fetal development, FZD2 was specific to germ cell nests during early development, and β -catenin exhibited a change from primarily membrane bound during the early ovarian development to cytoplasmic and nuclear location in the early primordial follicles in fetal ovary. The latter β -catenin distribution pattern persisted in primordial follicles in adult ovarian tissue changing to membrane bound in secondary follicles. It is concluded that canonical WNT signaling may exist only in oocytes of primordial follicles and that it may have a role during human follicle formation and maintenance.

Transcriptome changes of human ovarian granulosa cells in long-term culture

The ovarian follicle consists of the oocyte and the surrounding granulosa cells. Although granulosa cells are often considered as waste material of in vitro fertilization, they exhibit stem cell-like potential when kept in long-term cell culture, and can be regarded as candidates for use in regenerative and reconstructive medicine (Kossowska-Tomaszczuk et al. 2009). Kranc and colleagues (2019) performed a transcriptome analysis of human granulosa cells obtained from human ovarian follicles hyperstimulated for in vitro fertilization purposes after 30 days of culture. Specifically, three differentially expressed gene ontology groups “cell differentiation” (GO:0030154), “cell proliferation” (GO:0008283), and “cell–cell junction organization” (GO:0045216) were investigated. During culture, the granulosa cells changed from epithelial-like to fibroblast-like morphology and lost LHR and FSHR as indicated by negative immunofluorescence at day 30 of culture. This kind of cellular dedifferentiation was accompanied by a significant up-regulation of various genes involved in proliferation, differentiation, and cell–cell junction maintenance, such as *FZD2* (*FRIZZLED CLASS RECEPTOR 2*), *VCL* (*VINCULIN*), *CDH2* (*CADHERIN 2*), *TGFB* and *TGFBR1* (*TYPE I TGF- β RECEPTOR*), or *SKI* (*ONCOGENE SK*). Altogether, this comprehensive analysis points to the possibility that granulosa cells during cell culture may acquire properties that permit them to differentiate into different cell types such as chondrocytes and osteoblasts.

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