In focus in HCB

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Two original papers, one describing a fixation protocol to optimize the identification and labeling of tunneling nanotubes (TNT) in cells and tissues, and the other investigating the cystine–glutamate exchanger in rodent eye, as well as one Review Article describing insights into the cell biology and morphology of chymase-containing mast cells will be featured in our overview of the October issue of HCB. But first we will begin with a *mea culpa*...

Nobody is perfect—2019

The Editorial published in the July issue of *Histochemistry and Cell Biology* (Taates et al. 2019) not only covered aspects of the history of the journal and its fruitful affiliation with the Society of Histochemistry as its official journal, but also some journal statistics. For the latter, Table 5 enumerated articles that were published between 2008 and 2018 with an arbitrarily chosen threshold of ≥ 2500 PDF downloads. Since this table was man-made, and not created by an algorithm or another automated process, it was error-prone. In fact, the original paper entitled: “Dynamic imaging of cancer growth and invasion: a modified skin-fold chamber model” from Peter Friedl’s group (Alexander et al. 2008) with 3700 PDF downloads and 147 cites was omitted by mistake, for which the Editors of *Histochemistry and Cell Biology* offer their sincere apologies. As the statistics prove, the prediction of the authors that “This modified window model will be suited to address mechanisms of cancer invasion and metastasis, and related experimental therapy” was proven true, for which we offer our heartfelt congratulations!

Tunneling nanotubes (TNT) are dynamite and treble their cytoskeleton

Tunneling nanotubes (TNT) are cellular protrusions made of membranous tubes for contact-mediated intercellular communication through which signaling molecules, vesicles, viruses, protein aggregates and certain organelles are transferred (Gurke et al. 2008; Mattes and Scholpp 2018). TNT appear to exist in two types: short and thin containing an F-actin cytoskeleton, and long and thick containing microtubules in addition to F-actin bundles. Their morphological analysis has been challenging since TNT are not only transient structures, which in cell cultures hang free in the medium, but are sensitive to mechanical stress, chemical fixation and prolonged exposure to light (Gurke et al. 2008). Resnik et al. (2019) report a modification of the protocol published by Vielkind and Swierenga (1989) for the detection of various cytoskeletal elements by single and double immunofluorescence. In their investigation for triple immunofluorescence labeling of F-actin, microtubules and keratins, Resnik et al. (2019) used a simultaneous formaldehyde fixation/Triton X-100 permeabilization protocol comprising the microtubule-stabilizing PEM buffer (Soltys and Borisy 1985). When applied to whole-mount urinary bladder pieces, cryosections of urinary bladder, or cultured normal and cancer urothelial cells, both the cell and tissue structure and that of TNT, and the reactivity towards antibodies against α -tubulin and keratins 7 and 20, as well as binding of AlexaFluor 350 Phalloidin were optimal (see cover image). Hence, the protocol is proposed to be of general use for tissues and cell cultures. Of note, it could be shown that the TNT cytoskeleton may be composed of up to three cytoskeletal elements: F-actin, microtubules, and keratins.

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Lightening the cystine–glutamate exchanger in the mouse eye

The equimolar exchange of extracellular cystine for intracellular glutamate is mediated by the cystine–glutamate exchanger (system xc[−]) (Langford et al. 2010; Lewerenz et al. 2013). Here, Martis et al. (2019) have mapped, by confocal immunofluorescence, the expression of xCT, the light chain subunit of system xc[−] which confers transport specificity, in the different parts of C57BL/6J mouse eye and analyzed the role of xCT using knockout mice. They also report the distribution of the second subunit, the ubiquitous heavy chain 4F2hc anchoring the exchanger to the membrane. The use of the xCT knockout tissue was instrumental for validating the various commercial and custom-made xCT antibodies. Among the tested antibodies, the anti-N-terminus xCT antibody from TransGenic Inc. (Kobe, Japan) produced immunofluorescence in wild-type cornea and lens, which was completely absent in the respective tissues of knockout mice. However, similar to all other tested xCT antibodies, it labeled the retina and ciliary body from both wild-type and xCT knockout mice, indicating lack of specificity. By RT-PCR, xCT transcripts were detected in the cornea and lens. By immunofluorescence, the basal cells, wing cells and superficial cells of the corneal epithelium were positive for xCT. In the lens, xCT immunoreactivity was localized to the apical–apical interface of the epithelium/fiber cell border and the broad sides of the fiber cells of the outer cortex, a pattern more restricted than that observed in lens of embryonic (E16) and postnatal (P3 and P21) animals. The immunofluorescence was corroborated by Western blot analysis. A comparison of wild-type and knockout mice showed no significant differences in levels of cystine in the cornea, lens and retina. However, about fourfold higher levels of cystine were measured in the aqueous humor in the xCT knockout mice, which was interpreted to indicate that the anterior tissues of the eye were exposed to a heightened oxidative environment.

Chymase-containing mast cells: morphology and immunohistochemical properties

Mast cells are a type of white blood cell, often associated with immune and allergic conditions. They are typically identified at both the light and electron microscopic level by the morphological appearance of abundant cytoplasmic secretory granules containing several types of proteases. Indeed, mast cells can be characterized as a heterogeneous cell population, based upon the enzymatic contents of their

secretory granules. In an “In focus” editorial from 2017 (Taatzes and Roth 2017), we highlighted a manuscript by Atiakshin et al. (2017) in which they described a variety of histochemical and immunohistochemical staining methods to identify mast cells in multiple tissues of human and rodent origin. These same authors have now provided a timely review focusing on the identification and cell biology of mast cells containing the protease chymase within their secretory granules (Atiakshin et al. 2019). They review in detail multiple aspects of mast cell-associated chymase cell biology, including secretory granule and chymase biogenesis, potential models and mechanisms explaining granule cell release and secretion, and features and location of enzymatic activity. They also delve into other aspects of chymase function and activity in the tissue microenvironment, including extracellular chymase effects on other proteins, cells and tissues. They highlight the role which chymase-containing mast cells may play in both the normal physiology and pathology of organ systems such as cardiovascular, respiratory, gastrointestinal, musculoskeletal, and integumentary, including potential diagnostic implications. The review is beautifully illustrated with panels of images displaying mast cells immunohistochemically stained for chymase, as revealed by both chromogenic and fluorescence dyes.

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