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EDITORIAL

In focus in HCB

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In this issue, we highlight the results and significance of manuscripts involving the development of a novel correlative light and electron microscopy (CLEM) protocol for the high-resolution detection and localization of nuclear actin, the immunohistochemical analysis of skeletal muscle fiber subtypes and capillary density following nerve injury, the immunohistochemical localization and terminal carbohydrate characterization of the olfactory mucins Muc5ac and Muc5b, and finally the lack of correlation between the trace amine-associated receptor 1 (TAAR1) and breast cancer subtype.

Novel CLEM protocol for high-resolution localization of nuclear actin

Correlative light and electron microscopy (CLEM) is a powerful method for translating diffraction-limited fluorescence microscopy localization to the superior resolution of electron microscopy in the same biological sample (Giepmans 2008; Rizzo et al. 2014; de Boer et al. 2015). Abdellatif et al. (2019) developed a novel detailed and sophisticated CLEM protocol to investigate nuclear actin (Bajusz et al. 2018). To specifically label actin molecules, they transfected mouse fibroblasts grown on carbon-coated sapphire discs with a nuclear Actin-chromobody–GFP conjugate (nAC–GFP) which specifically identifies both monomeric G-actin and polymerized F-actin in the nucleus at levels far below the overexpression models normally utilized (Melak et al. 2017). Cells were physically fixed via high-pressure freezing, cryosubstituted and embedded in LR-gold low-temperature resin, and ultrathin sections prepared. CLEM images were acquired with a fluorescence microscope followed by ROI analysis by a dark-field detector to identify the 6-nm colloidal gold particles, and were then subjected to registration and quantitative image analysis. The results demonstrated: (1) precise high-resolution localization of the actin-chromobody in the cell nucleus, with only sparse labeling seen in the cytoplasm; (2) although gold particles were detected throughout the nucleoplasm, they were largely absent from the heterochromatin-rich regions stained with DAPI, suggesting a preferential distribution in the transcriptionally active euchromatin; (3) linear arrays of gold particles indicative of F-actin filaments were not observed, which may be the result of technical issues inherent in this experimental system; and (4) cells subjected to stress (DMSO treatment or uv irradiation) displayed an altered ratio of nuclear-to-cytoplasmic actin, whereby more signal was detected in the cytoplasm than in control non-stressed cells. The authors propose future studies to modify the multi-step labeling protocol by fusing the chromobody with an enzymatic tag, resulting in a much simplified single-step reaction procedure.

Muscle fiber subtypes and capillary density after nerve injury in rat

Skeletal muscle fibers are known to respond to injury in multiple ways, highlighting their inherent plasticity (Wu et al. 2014). Morphologically, these muscle fibers can be classified according to the type of myosin heavy chain (MyHC) protein they express. In rat skeletal muscles, four different MyHC isoforms can be differentiated: MyHC beta/slow (MyHC type-1), MyHC type-2a, MyHC type-2x, and MyHC type-2b (Schiaffino and Reggiani 1994). Altered physiological demands or insults can result in phenotypic alteration of the individual muscle fibers, as well as changes in capillary density surrounding the fibers. In a previous publication, Cebasek and Ribaric (2016) investigated the response of rat extensor digitorum longus (EDL) muscle fiber-associated

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capillaries to two different sciatic nerve injuries. However, since these capillary-associated changes were not analyzed with respect to individual MyHC muscle fiber subtypes, these same authors have now performed an extensive immunohistochemical/histochemical investigation of muscle fiber type and capillary response to nerve injury in rat muscle (Cebasek and Ribaric 2019). Two different types of injury were created: (1) a permanent injury by severing the sciatic nerve (NC), and (2) a transient injury by a double crush procedure (NCR). Four weeks after injury, the EDL muscles were processed for multi-label immunohistochemistry to identify individual muscle fiber MyHC subtypes (see cover image), and relate them to capillary response by quantitative image analysis. Briefly, amongst the large amount of quantitative data produced, the authors found (1) the NC procedure resulted in capillary rarefaction, whereas the NCR led to capillary proliferation; (2) in the NC group, the subtype MyHC-2x fibers were most sensitive to capillary rarefaction; and (3) in the NCR group, the greatest degree of capillary proliferation involved the type-2a fibers (oxidative fiber type), while the highest improvement in capillary supply occurred around the type-2b fibers (glycolytic type fibers). The authors speculate that a relationship exists between fiber size and capillary density following nerve injury, allowing the muscle cells to alter their metabolic response between oxidative and glycolytic states.

Differential terminal glycosylation and expression of Muc5 in nasal epithelium

The epithelial lining of hollow internal organs such as the respiratory tract is covered by a mucus layer containing highly glycosylated mucins. It not only functions as a protective barrier and for the mechanical removal of inhaled particles by its viscoelastic and gel-like properties, but also fulfills a broad range of biological functions achieved by the oligosaccharide side chains (glycans) of the mucins (Bhide and Colley 2017; Corfield 2017; Gabius and Roth 2017). Goblet cells and submucosal glands synthesize and secrete mucins in the airways (Andrianifahanana et al. 2006), whereby Muc5ac originates mainly from the former and Muc5b from the latter (Buisine et al. 1999). Here, Amini et al. (2019) have investigated the distribution of these two major airway mucins in the nasal epithelium of wild type, floxed Muc5b–GFP reporter mice, and Muc5b-deficient mice. Of note, the nasal epithelium consists of a respiratory and an olfactory epithelium and as the authors state the specific localization of Muc5ac and Muc5b is not clear. By analyzing transversal sections from the proximal to the distal region by immunohistochemistry, they observed that goblet cells in the respiratory epithelium were reactive for Muc5ac. In contrast, in the olfactory epithelium, the submucosal Bowman's gland produced Muc5b, but not Muc5ac. In the Muc5b-deficient mice, Bowman's gland was missing. Using lectin histochemistry (Manning et al. 2017) in combination with immunohistochemistry, in situ evidence was obtained that Muc5ac of the respiratory epithelium goblet cells is mainly fucosylated, whereas Muc5b of the olfactory region Bowman's glands is mainly sialylated. It is concluded that the region-specific distribution and differential terminal glycosylation of the two mucins may indicate different functions.

TAAR1 is related to breast carcinoma cell lines but not breast cancer subtypes

Trace amine-associated receptors (TAARs) belong to the family of G protein-coupled receptors (GPCRs) and are mainly expressed in the central nervous system, but also detectable in various other tissues and leukocytes (Berry et al. 2017; Regard et al. 2008). Recent data indicated a positive correlation between TAAR1 expression in human breast carcinoma, and both patient survival and HER2 overexpression (Vattai et al. 2017), which indicated that TAAR1 might be an independent survival predictor. Extending upon this finding, Pitts et al. (2019) have now performed an in-depth bioinformatics analysis of TAAR1 mRNA expression profiles to provide an independent verification of these findings. They report that mRNA expression levels in 20 breast carcinoma cell lines, 6 samples of normal breast tissue, and 31 breast carcinoma samples representing different carcinoma subtypes available within the Gene Expression Omnibus database did not show a correlation with breast carcinoma cell subtype, including HER2 positivity. Using a validated, highly specific monoclonal antibody against TAAR1 (Raab et al. 2016) for immunofluorescence and semi-quantitative flow cytometry analysis of various breast carcinoma cell lines (MCF-7,T47D, MDA-MB-231, SKBR3, MDA-MB-468, BT-474), the lack of correlation could be confirmed by the relative intensity of the immunofluorescence signal for TAAR1 protein expression which paralleled the mRNA levels. Although TAAR1 was detected intracellularly by immunofluorescence in all investigated breast carcinoma cell lines, in some (MCF-7, MDA-MB-468, and SKBR3), it was found to be located in the nucleus. The apparent difference between the results obtained by Pitts et al. (2019) and those previously reported by Vattai et al. (2017) seems to be due to lack of specificity of the commercially available rabbit anti-TAAR1 antibody, as shown in controls using a rabbit isotype control IgG and by shRNA knockdown.

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