#### **EDITORIAL**



### In focus in HCB

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## Aquaporin 5 expression in cadmium injured alveolar epithelial cells

Cadmium is an environmental toxin which damages lung epithelial cells through activation of reactive oxygen species. Manifestations of cadmium-induced lung epithelial cell alterations include loss of cellular proteins such as aguaporin 5 (AOP5) (Kasper et al. 2004). Previously, Barth and colleagues (Ebeling et al. 2014) showed that activation of the ATP-gated cation channel purinergic receptor P2X7R resulted in an increase in alveolar type I cell AQP5 levels, while inhibition of the receptor resulted in a decreased amount of this receptor. This same group has now extended these findings to investigate the effects of cadmium treatment on the expression of P2X7R and AQP5 in cultured mouse lung epithelial cells and a precision-cut lung slice model (Heupel et al. 2018). Treatment of both cultured lung cells and precision-cut lung slices with cadmium chloride (CdCl<sub>2</sub>) resulted in an increase in both P2X7R and AQP5 levels; however levels of these two proteins decreased in the lung slice model after 72 h. Moreover, the increased levels of AQP5 following cadmium treatment were found to be P2X7R-independent. They also found that activation of P2X7R with an agonist resulted in a decrease in AQP5 protein content, as well as an increase in the number of apoptotic alveolar type II cells. They propose that the combination of cultured cells and lung slice cultures are useful models to study mechanisms of pulmonary toxicology outside of an animal model.

## A new probe for correlated light and electron microscopy

Correlative light and electron microscopy (CLEM) has become a powerful technique for investigating the localization of cellular constituents by diffraction-limited fluorescence microscopy, followed by precise identification at the ultrastructural level by transmission electron microscopy (Su et al. 2010; Rizzo et al. 2014). A variety of probes exhibiting optical recognition together with electron density have been utilized over the years to accomplish this dual microscopy imaging protocol. de Beer et al. (2018) have now introduced "FLIPPER-bodies" (FLuorescent Indicator and Peroxidase for Precipitation with EM Resolution) to serve as small protein probes to localize endogenous cellular proteins in a CLEM technique. These probes were developed with the aim of overcoming many of the limiting issues presented by the currently available CLEM reagents, including size-related diminished target efficiency. Cleverly, these multidomain probes are based on nanobodies (small antigen-binding proteins derived from the heavy chain of antibodies produced by camels and llamas) targeted to specific cellular proteins combined with a fluorescent tag and horseradish peroxidase. The FLIPPER-bodies are transfected into a stable cell line, and the secreted product is then used for staining of other cells as either a culture supernatant or in purified form, much like monoclonal antibodies. Following visualization of the fluorescent tag by light microscopy, the horseradish peroxidase component is rendered electron dense via reactivity with DAB for visualization by electron microscopy. The authors used various FLIPPER-bodies with different fluorescent tags to target and label nuclear and plasma membrane proteins, demonstrating their sensitivity and specificity. Given the reduced size of these probes compared to conventional antibodies used in immunocytochemical protocols allowing for enhanced penetration into cellular regions, as well as the genetic ability to target almost any protein, they will likely find widespread use in CLEM investigations.

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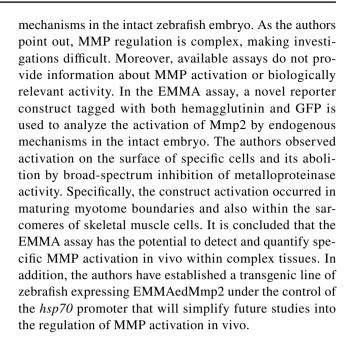
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## Simultaneous immunohistochemistry and enzyme histochemistry for ecto-nucleotidases

Proteins such as enzymes can be conveniently detected in cells and tissues by light and electron microscopic immunolabeling. Although silently assumed, immunolabeling inherently provides no information about the catalytic activity of the respective enzyme. In the case of glycosyltransferases, the combination of immunogold labeling and lectin-gold labeling permitted the study of the Golgi apparatus distribution of both the enzyme protein and its product of action (Roth et al. 1985). This combination was of critical importance in the case of unexpected, cell type-related Golgi apparatus distribution of terminal glycosyltransferases (Roth et al. 1986). Here, Villamonte et al. (2018) report an improved method for the simultaneous localization of ecto-nucleotidases by immunofluorescence and associated enzyme activity by classical substrate histochemistry in frozen sections of formaldehyde-fixed human oviduct. The concerted action of different ectonucleoside triphosphate diphosphohydrolases (NTPDases) and of ecto-5'nucleotidase/CD73 together with alkaline phosphatase results in the stepwise hydrolysis of ATP to adenosine. Hence, ecto-nucleotidases play a role in reproduction since a locally increased adenosine concentration is important for sperm capacitation in the oviduct (Burnstock 2014; Coy et al. 2012). NTPDases as well as ecto-5'nucleotidase were detected by immunofluorescence in ciliated epithelial cells, endothelial cells and smooth muscle cells. Reaction product for ATPase, ADPase and AMPase activity was confined to the same cell types. Of note, only the ciliated epithelial cells housed the complete set of enzymes required for the hydrolysis of ATP to adenosine and assigns an additional function in sperm capacitation to them. The authors also emphasize that the method should be useful for analyzing the effect of specific inhibitors developed for therapeutic use.

# An epitope-mediated matrix metalloproteinase activation assay for zebrafish embryo

Matrix metalloproteinases (MMP), a family of zincdependent endopeptidases, are important for extracellular matrix remodeling during embryonic morphogenesis (Apte and Parks 2015; Bonnans et al. 2014). Jeffrey and Crawford (2018) report now the epitope-mediated MMP activation (EMMA) assay and use it to investigate the activation of Mmp2 (gelatinase A) by endogenous



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