Histochemistry and Cell Biology (2018) 149:193–195
https://doi.org/10.1007/s00418-018-1647-z

EDITORIAL

In focus in HCB

Douglas J. Taatjes[1] · **Jürgen Roth**[2]

Accepted: 2 February 2018 / Published online: 6 February 2018

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 aquaporin 5 (AQP5) (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 ($CdCl_2$) 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.

✉ Douglas J. Taatjes
douglas.taatjes@uvm.edu

[1] Department of Pathology and Laboratory Medicine, Larner College of Medicine, University of Vermont, Burlington, VT 05405, USA

[2] University of Zurich, 8091 Zurich, Switzerland

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 zinc-dependent 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 mechanisms in the intact zebrafish embryo. As the authors point out, MMP regulation is complex, making investigations difficult. Moreover, available assays do not provide information about MMP activation or biologically relevant activity. In the EMMA assay, a novel reporter construct tagged with both hemagglutinin and GFP is used to analyze the activation of Mmp2 by endogenous mechanisms in the intact embryo. The authors observed activation on the surface of specific cells and its abolition by broad-spectrum inhibition of metalloproteinase activity. Specifically, the construct activation occurred in maturing myotome boundaries and also within the sarcomeres of skeletal muscle cells. It is concluded that the EMMA assay has the potential to detect and quantify specific MMP activation in vivo within complex tissues. In addition, the authors have established a transgenic line of zebrafish expressing EMMAedMmp2 under the control of the *hsp70* promoter that will simplify future studies into the regulation of MMP activation in vivo.

References

Apte SS, Parks WC (2015) Metalloproteinases: a parade of functions in matrix biology and an outlook for the future. Matrix Biol 44–46:1–6. https://doi.org/10.1016/j.matbio.2015.04.005

Bonnans C, Chou J, Werb Z (2014) Remodelling the extracellular matrix in development and disease. Nat Rev Mol Cell Biol 15(12):786–801. https://doi.org/10.1038/nrm3904

Burnstock G (2014) Purinergic signalling in the reproductive system in health and disease. Purinergic Signal 10:157–187. https://doi.org/10.1007/s11302-013-9399-7

Coy P, García-Vázquez FA, Visconti PE, Avilés M (2012) Roles of the oviduct in mammalian fertilization. Reproduction 144:649–660. https://doi.org/10.1530/REP-12-0279

de Beer MA, Kuipers J, van Bergen en Henegouwen PMP, Giepmans BNG (2018) A small protein probe for correlated microscopy of endogenous proteins. Histochem Cell Biol. https://doi.org/10.1007/s00418-018-1632-6

Ebeling G, Blasche R, Hofmann F, Augstein A, Kasper M, Barth K (2014) Effects of P2X7 receptor knockout on AQP-5 expression in type I alveolar epithelial cells. PLoS One 9:e100282

Heupel J, Blasche R, Wesslau K-P, Kasper M, Barth K (2018) P2X7R-independent modulation of aquaporin 5 expression in $CdCl_2$-injured alveolar epithelial cells. Histochem Cell Biol. https://doi.org/10.1007/s00418-018-1637-1

Jeffrey EJ, Crawford BD (2018) The epitope mediated MMP activation assay: detection and quantification of the activation of Mmp2 in vivo in the zebrafish embryo. Histochem Cell Biol. https://doi.org/10.1007/s00418-018-1634-4

Kasper M, Seidel D, Knels L, Morishima N, Neisser A, Bramke S, Koslowski R (2004) Early signs of lung fibrosis after in vitro treatment of rat lung slices with $CdCl_2$and TGF-beta1. Histochem Cell Biol 121:131–140

Rizzo R, Parashuraman S, Luini A (2014) Correlative video-light-electron microscopy: development, impact and perspectives. Histochem Cell Biol 142:133–138. https://doi.org/10.1007/s00418-014-1249-3

Roth J, Taatjes DJ, Lucocq JM, Weinstein J, Paulson JC (1985) Demonstration of an extensive trans-tubular network continuous with the Golgi apparatus stack that may function in glycosylation. Cell 43:287–295

Roth J, Taatjes DJ, Weinstein J, Paulson JC, Greenwell P, Watkins WM (1986) Differential subcompartmentation of terminal glycosylation in the Golgi apparatus of intestinal absorptive and goblet cells. J Biol Chem 261:14307–14312

Su Y, Nykanen M, Jahn KA, Whan R, Cantrill L, Soon LL, Ratinac KR, Braet F (2010) Multi-dimensional correlative imaging of subcellular events: combining the strengths of light and electron microscopy. Biophys Rev 2:121–135

Villamonte ML, Torrejón-Escribano B, Rodríguez-Martínez A, Trapero C, Vidal A, Gómez de Aranda I, Sévigny J, Matías-Guiu X, Martín-Satué M (2018) Characterization of ecto-nucleotidases in human oviducts with an improved approach simultaneously identifying protein expression and in situ enzyme activity. Histochem Cell Biol. https://doi.org/10.1007/s00418-017-1627-8