Histochemistry and Cell Biology (2021) 156:79–82
https://doi.org/10.1007/s00418-021-02017-5

EDITORIAL

In focus in HCB

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

Accepted: 15 July 2021 / Published online: 20 August 2021

In this August issue of the journal, we will highlight two review articles on the topics of (1) stereological tools to investigate the pulmonary vasculature (Robert Feulgen Prize 2020 awardee) and (2) a proposed classification scheme for human enteric neurons, as well as two original articles describing (1) the immunoelectron microscopic localization of the cell signaling lipid molecule phosphatidylinositol 4,5-bisphophate in yeast cells using quick-freezing freeze-fracture replica labeling, and (2) modified immunohistochemical protocols to provide augmented staining in aged archived paraffin human tissue blocks.

In addition, we are delighted to inform our readership that the 2-year 2020 impact factor for *Histochemistry and Cell Biology* has risen to 4.304. We would like to thank all of the authors for contributing their work leading to the success of the journal.

Publishing Open Access in *Histochemistry and Cell Biology*

Histochemistry and Cell Biology published by SPRINGER NATURE has recently become a Transformative Journal, which is committed to transitioning to fully Open Access. For authors, this means that they may publish their manuscript as Open Access with the fees covered by their institution provided it has a Transformative Agreement. To find out if your institution has such a Transformative Agreement, please access:

https://www.springernature.com/gp/open-research/institutional-agreements

Additional benefits for authors publishing their work as Open Access in *Histochemistry and Cell Biology* are:

- The higher visibility and greater impact of your work, since Open Access articles are accessed four times as frequently on average, and cited 1.6 times more often on average in Springer's Transformative Journals.
- Easy compliance with mandates, since many funders require Open Access, and some take compliance into account when assessing future grant applications.
- Last but not least, the copyright remains with you as author, since most Open Choice articles are published under the liberal Creative Commons Attribution 4.0 International license.

3D visualization and quantification of the pulmonary vasculature: from initial disappointment to the Robert Feulgen Prize 2020

The Robert Feulgen Prize is an internationally announced prize of the Society for Histochemistry and is awarded for work of outstanding scientific merit in the field of microscopic histochemistry. The prize was presented for the first time half a century ago, in 1971, jointly to O. von Deimling and H. Madreiter of the Institute of Pathological Anatomy, Ludwig Aschoff-Haus, University of Freiburg i. Br., Germany, for their work on the histochemical demonstration of esterases.

The 2020 Robert Feulgen Prize has been awarded ex aequo to Ch. Mühlfeld, Hannover Medical School, Hannover, Germany, and to H. Shroff, National Institute of Biomedical Imaging and Bioengineering, NIH, Bethesda, MD, USA.

In this issue of the journal, Mühlfeld (2021) presents an overview of his prize-winning work and of the current state of the art of 3D visualization and quantification of the microarchitecture of the pulmonary vasculature, with emphasis on the alveolar capillary network. The review nicely illustrates

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

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

[2] University of Zurich, CH-8091 Zurich, Switzerland

the development and application of stereological and morphometric principles and the use of a variety of microscopic techniques including light microscopy, transmission electron microscopy, scanning electron microscopy, and micro-computed tomography to the study of normal and diseased lung (Fig. 1).

The prize-winning work of H. Shroff will be published in a forthcoming issue of *Histochemistry and Cell Biology*.

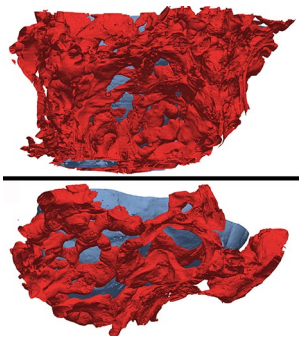

Fig. 1 3D reconstruction of the alveolar capillary network (red) from serial block-face scanning electron microscopic data sets of 14-day-old mice. Capillaries from normoxic (upper panel) and hyperoxic (lower panel) animals are presented. From Mühlfeld (2021)

The human aspect of the enteric neurons

The enteric neurons are well studied in terms of their morphology, function, and chemistry in different animal species, and both regional and species-specific differences are well known (Furness 2006). For obvious reason, studies on human enteric neurons are more limited (Brehmer 2006). In his inaugural review article, Brehmer (2021) begins by critically discussing various aspects and limitations of the classification of enteric neurons in only two morphologically distinct subpopulations: Dogiel type I and type II neurons. In the main part of the manuscript, he reviews in great detail recent morphological, immunohistochemical (Fig. 2), and

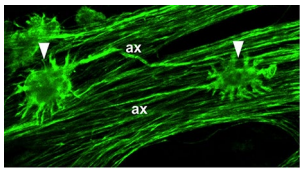

Fig. 2 Two "Dogiel type I" neurons (filled arrowheads) immunostained for neurofilaments. From Brehmer (2021)

functional analyses of human myenteric and submucosal neurons and their regional peculiarities.

Despite the abundance of new data and the emergence of some general principles, many blank spots will remain until a truly satisfactory classification is achieved. This means that, as for enteroendocrine cells (Fothergill and Furness 2018), establishing a satisfying classification for enteric neurons will remain a field of intense research.

The budding topic of yeast budding plasma membranes

Yeasts are often employed as model organisms in biomedical research due to their facile growth conditions, well-known genetics, and frequent biochemical similarities to mammalian cells (Mager and Winderickx 2005). Kurokawa et al. (2021) have used the budding yeast model to investigate the membrane distribution of the cell signaling lipid molecule phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2). Since conventional aldehyde fixation does not preserve lipid moieties well, the authors resorted to quick-freezing

freeze-fracture replica labeling (QF-FRL) combined with quantitative immunoelectron microscopy for precise nanometer-level resolution of membrane-associated PtdIns(4,5)P_2 in wild-type and mutant temperature-sensitive yeast strains. The authors provide a very detailed description of the QF-FRL protocol for budding yeast cells, including a most informative diagram. Their immunolabeling freeze-fracture results demonstrate that PtdIns(4,5)P_2 immunoreactivity was preferentially localized to the furrowed cytoplasmic surface of the plasma membrane (Fig. 3), and restricted from the hexagonal region (in yeast, three distinct areas of the cytoplasmic surface of the plasma membrane can be recognized: furrowed, hexagonal, and undifferentiated flat).

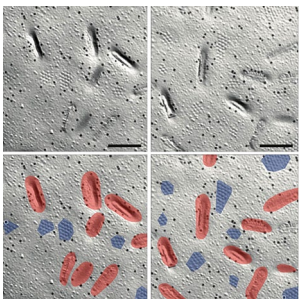

Fig. 3 Distribution of PtdIns(4,5)P_2 labeling in the cytoplasmic leaflet of the yeast plasma membrane. The immunogold labeling for PtdIns(4,5)P_2 is absent from the hexagonal areas (blue), whereas it is observed around the furrowed areas (orange) and the undifferentiated flat areas. From Kurokawa et al. (2021)

Previously this same group had used similar ultrastructural techniques to demonstrate that PtdIns(4)P [the precursor molecule which is phosphorylated by a kinase to yield PtdIns(4,5)P_2)] was localized to the undifferentiated flat area of the cytoplasmic side of the plasma membrane (Yoshida et al. 2016). In the present study, they also found that although the immunolabeling density for PtdIns(4)P was significantly diminished in the plasma membrane of temperature-sensitive mutant strains, that for PtdIns(4,5)P_2 was comparable to that seen in wild-type yeast. Taken together, their results indicate that in the yeast plasma membrane, PtdIns(4)P is likely immediately phosphorylated to yield PtdIns(4,5)P_2.

The waxing and waning of tissue antigens

Practitioners of immunohistochemistry are all too familiar with the loss of antigenicity which oftentimes occurs during the steps of tissue fixation and processing (Larsson 1988; Burry 2010). This is particularly true for tissues fixed in formalin followed by paraffin embedding (FFPE), the routine protocol for human biopsy samples. Such samples typically require antigen retrieval methods (heating, boiling, enzymatic treatments, etc.) to "restore" antigenicity of many proteins, notably those with a membrane or nuclear localization (Grillo et al. 2017a). Ki67, one such protein with a nuclear localization, is routinely employed in both research and clinical applications to assess cell proliferation. Grillo and colleagues (2021) have now combined their dual interests in preservation of antigenicity in archived FFPE samples (Grillo et al. 2015) with the use of Ki67 as an immunohistochemical marker to assess the grade of neuroendocrine tumors (Grillo et al. 2017b) by exploring methods to increase the immunoreactivity of Ki67 in old archival paraffin blocks (Fig. 4).

Paraffin blocks processed 40 years ago were either reprocessed with a modern automated tissue processor or simply re-embedded, since the old paraffin blocks mounted in plastic moulds could not be sectioned on newer microtomes. Some of the blocks were re-embedded by flipping the block upside down to allow sectioning from the opposite side. Sections were cut at depths of 500 µm down to 1000 µm, and subjected to various heat-based antigen retrieval protocols, followed by immunostaining for Ki-67, and finally assessment of the staining intensity. Their results showed that (1) the best method for increasing Ki-67 immunostaining

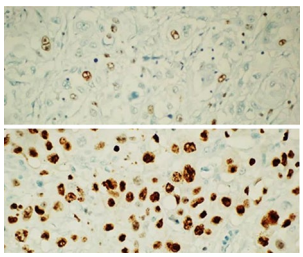

Fig. 4 Ki67 immunoperoxidase staining, 40-year-old archival paraffin tissue block. Standard 34-min heat-induced antigen retrieval results in faint and limited nuclear immunostaining in a section prepared from the periphery of the paraffin block (upper panel), whereas prolonged 64-min heat-induced antigen retrieval gives intense nuclear immunostaining in a section prepared from the deeper block region. From Grillo et al. (2021)

in aged archived FFPE tissue blocks is the combination of deeper sectioning (down to 1000 µm) and prolonged heat-based antigen retrieval; (2) re-embedding or reprocessing of the tissue blocks did not have a significant effect on subsequent Ki-67 immunostaining intensity; and (3) in sections from larger tissue samples, Ki-67 immunostaining was heterogeneously expressed in the central area compared to the periphery. While this study only focused on one antigen (Ki-67), it is hoped that the described method will be applicable to the recovery of other diagnostically related antigens in aged FFPE clinical archives.

References

Brehmer A (2006) Structure of enteric neurons. Adv Anat Embryol Cell Biol 186:1–94

Brehmer A (2021) Classification of human enteric neurons. Histochem Cell Biol. https://doi.org/10.1007/s00418-021-02002-y

Burry RW (2010) Immunocytochemistry—a practical guide for biomedical research. Springer Science+Business Media, New York

Fothergill LJ, Furness JB (2018) Diversity of enteroendocrine cells investigated at cellular and subcellular levels: the need for a new classification scheme. Histochem Cell Biol 150:693–702. https://doi.org/10.1007/s00418-018-1746-x

Furness JB (2006) The enteric nervous system. Blackwell, Oxford

Grillo F, Pigozzi S, Ceriolo P et al (2015) Factors affecting immunoreactivity in long-term storage of formalin-fixed paraffin-embedded tissue sections. Histochem Cell Biol 144:93–99. https://doi.org/10.1007/s00418-015-1316-4

Grillo F, Bruzzone M, Pigozzi S et al (2017a) Immunohistochemistry on old archival paraffin blocks: is there an expiry date? J Clin Pathol 70:988–993. https://doi.org/10.1136/jclinpath-2017-204387

Grillo F, Valle L, Ferone D et al (2017b) Ki-67 heterogeneity in well differentiated gastro-entero-pancreatic neuroendocrine tumors: when is biopsy reliable for grade assessment? Endocrine 57:494–502. https://doi.org/10.1007/s12020-017-1364-8

Grillo F, Campora M, Pigozzi S et al (2021) Methods for restoration of Ki67 antigenicity in aged paraffin tissue blocks. Histochem Cell Biol. https://doi.org/10.1007/s00418-021-01987-w

Kurokawa Y, Konishi R, Tomioku K, Tanabe K, Fujita A (2021) The distribution of phosphatidylinositol 4,5-bisphophate in the budding yeast plasma membrane. Histochem Cell Biol. https://doi.org/10.1007/s00418-021-01989-08

Larsson L-I (1988) Immunocytochemistry: theory and practice. CRC Press Inc, Boca Raton, FL

Mager WH, Winderickx J (2005) Yeast as a model for medical and medicinal research. Trends Pharmacol Sci 26(5):265–273. https://doi.org/10.1016/j.tips.2005.03.004

Mühlfeld C (2021) Stereology and three-dimensional reconstructions to analyze the pulmonary vasculature. Histochem Cell Biol. https://doi.org/10.1007/s00418-021-02013-9

Yoshida A, Shigekuni M, Tanabe K, Fujita A (2016) Nanoscale analysis reveals agonist-sensitive and heterogeneous pools of phosphatidylinositol 4-phosphate in the plasma membrane. Biochim Biophys Acta 1858:1298–1305