Histochemistry and Cell Biology (2021) 156:405–408
https://doi.org/10.1007/s00418-021-02044-2

EDITORIAL

In focus in HCB

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

Published online: 3 November 2021
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

In this November issue of the journal, we will highlight three Original Articles describing (1) the effects of overexpression of the nuclear pore complex component nucleoporin Nup88 on the malignant phenotype, (2) the possible protective effect of ascorbic acid treatment on *Porphyromonas gingivalis*-induced periodontitis, and (3) an exhaustive validation analysis of five commercial antibodies raised against N-terminus or C-terminus regions of the cannabinoid receptor 1 protein and; one Short Communication describing a simple method for the cryopreservation of human granulosa cells for subsequent cell culture experimentation. We hope you enjoy these brief highlights along with the entire issue.

Nucleoporin Nup88: from nucleo-cytoplasmic transport to malignant phenotype

The nuclear pore complexes in the nuclear envelope are macromolecular assemblies composed of about 30 nucleoporins, which not only function in the bidirectional macromolecular exchange between the nucleoplasm and cytoplasm, but also are involved in various cellular signaling pathways as well as in cell migration and differentiation (Jühlen and Fahrenkrog 2018). Therefore, not surprisingly, mutations in nucleoporin genes have emerged as the cause of many human hereditary and developmental disorders (Jühlen and Fahrenkrog 2018). Moreover, since some nucleoporins, such as Nup88, have been found to be overexpressed in malignant tumors, they have been linked to tumorigenesis and malignant transformation (Simon and Rout 2014; Xu and Powers 2009). In this issue of the journal, Makise and colleagues (2021) report the consequences of the overexpression of Nup88 in two human cervical cancer cell lines, HeLa and CaSki cells. HeLa cells overexpressing either GFP-fused Nup88 or GFP alone as a control, as well as Nup88-knockdown HeLa cells, were analyzed by scratch wound healing assays and an invasion assay using BD Matrigel® Invasion chambers. The authors found that the overexpression of Nup88-GFP slightly but significantly promoted HeLa cell migration compared to control HeLa cells overexpressing GFP alone. This was substantiated by live-cell single-cell tracking showing enhanced migration of Nup88-overexpressing HeLa cells. In contrast, Nup88 knockdown suppressed migration of HeLa cells. Moreover, overexpression of Nup88 resulted in a 1.29-fold increased invasion of HeLa cells (Fig. 1), whereas Nup88 knockdown reduced the number of invasive cells by 50%.

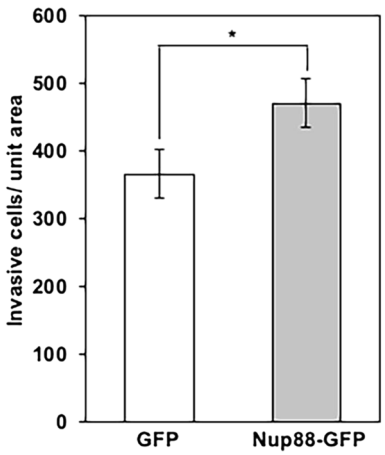

Fig. 1 Overexpression of Nup88 in HeLa cells affects cell invasion. On average, 471 Nup88-GFP overexpressing invasive cells were counted per unit area compared to 366 GFP alone overexpressing control cells (*$p < 0.05$). From Makise et al. (2021)

✉ 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, 8091 Zurich, Switzerland

Importantly, the authors found that neither nuclear NFkB translocation or enhanced expression nor stimulation of EMT (as analyzed by the expression of E-cadherin, vimentin, Snail1, or Twist1) were involved in these phenotypical changes. However, the expression level (mRNA and protein) of 15 out of 23 matrix metalloproteinases was increased in Nup88-overexpressing Hela and CaSki cells as well as the prostate cancer cell lines LNCap and PC-3. A more detailed analysis of matrix metalloproteinase-12, including the use of a specific enzyme inhibitor, indicated that it is involved in the promotion of the malignant phenotype resulting from Nup88 overexpression in human cervical cancer cell lines. The main conclusion of the authors is that overexpression of Nup88 stimulates migratory and invasive activity of cervical cancer cells with the matrix metalloproteinase-12 actively involved.

Ascorbic acid reverses *P. gingivalis*-induced epigenetic modifications in oral stem cells

Ascorbic acid as a reducing agent and antioxidant fulfills many biological functions and has been shown to exert an effect on cell proliferation and mesenchymal stem cell regeneration (Fujisawa et al. 2018; Wei et al. 2012). Furthermore, ascorbic acid has been shown to be an important epigenetic modulator (Lee Chong et al. 2019). In their present study, Pizzicannella and colleagues (2021) analyzed the effect of ascorbic acid on the inflammatory cascade caused by *P. gingivalis*, the major pathogen of periodontitis that affects the teeth-supporting tissue and causes bone and tooth loss. They treated cultures of human gingiva-derived mesenchymal stem cells (hGMSCs) and endothelial-differentiated hGMSCs (e-hGMSCs) established from tissue of healthy donors with ultrapure lipopolysaccharide prepared from *P. gingivalis* (LPS-G) and using immunofluorescence (Fig. 2) and Western blotting observed an upregulation of histone acetyltransferase p300 and downregulation of DNA methyltransferase 1, both representing epigenetic markers.

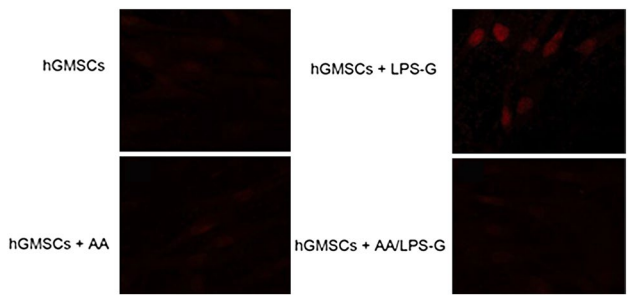

Fig. 2 Immunofluorescence for p300 in cultured hGMSCs is increased following LPS-G treatment and normalized following combined LPS-G—ascorbic acid treatment. AA: ascorbic acid; LPS-G: lipopolysaccharide from *Porphyromonas gingivalis*. From Pizzicannella et al. (2021)

However, no differences in the methylation patterns of *CCL5* and *ICAM1* gene promoters were detected in hGMSCs and e-hGMSCs treated with LPS-G compared to nontreated cell cultures. Furthermore, reactive oxygen species (ROS) production as measured using the cell-permeant ROS probe 2′,7′ dichlorodihydrofluorescein diacetate was found to be increased in cultures of hGMSCs and e-hGMSCs following LPS-G treatment. The effects of PLS-G on p300 and DNA methyltransferase 1 expression and ROS levels observed in hGMSCs and e-hGMSCs were reversed by combined PLS-G and ascorbic acid treatment. Thus, the authors concluded that ascorbic acid treatment nullified the inflammation-related epigenetic modifications caused by an oral pathogen. However, the underlying mechanism remains to be established.

Getting (anti)bodies "fit-for-the-purpose": An in-depth analysis of binding specificity for five commercial cannabinoid receptor 1 antibodies

The many issues arising from lack of antibody validation and specificity on the reliability of immunohistochemical experiments have been frequently highlighted in journal editorials and articles over the years (Couchman 2009; Kalyuzhny 2009; Saper 2009). This has led to many and frequent proposals calling for specific methods for the validation of antibodies for binding to their specified targets in a variety of labeling techniques (O'Hurley et al. 2014; Uhlen et al. 2016; Voskuil 2014; Weller 2018). In particular, the manuscript by Uhlen and colleagues (2016) represents the consensus opinion of an ad hoc International Working Group for Antibody Validation, recommending the use of five conceptual "pillars" for antibody validation in an application-dependent manner. Indeed, we recommend the specific use of these "pillars" to demonstrate the validation of antibodies used in immunolabeling experiments in manuscripts submitted to *Histochemistry and Cell Biology* (see "Instructions for Authors section on Policy on Antibody Validation": (https://www.springer.com/journal/418/submission-guidelines#Instructions for Authors). In this current issue of the journal, Echeazarra et al. (2021) have performed just such an extensive validation of five commercial antibodies raised against different amino acid sequences of the cannabinoid receptor 1 (CB1) and in different animal species; two were raised against N-terminal regions of the protein (N15 and H150) and three against the carboxy terminus (K15, Af380, and Af450). Validation assays for the antibodies included immunohistochemistry and immunofluorescence on sections from normal control rat cerebral cortex and control and transgenic CB1-knockout mouse cerebral cortex, immunofluorescence on human HEK-293 cells transfected with plasmids coding for the human CB1-receptor (Fig. 3), Western blotting, and radioligand binding assays.

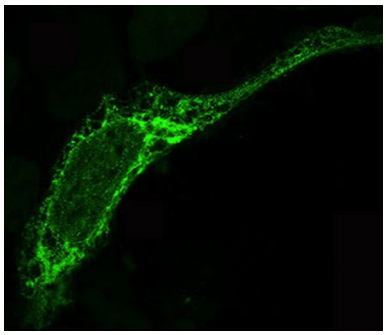

Fig. 3 Immunofluorescence for CB1 receptor in transfected HEK293 cells using the affinity-purified rabbit polyclonal anti-CB1 receptor antibody H150 raised against a peptide corresponding to amino acids 1–150 of the human receptor. From Echeazarra et al. (2021)

Their most voluminous and carefully controlled results showed that three of the five antibodies could be used reliably for very specific end-use applications. They provide a very detailed discussion seeking to explain discrepancies in antibody reactivity among the various assays performed, with well thought out points for consideration related to other publications as well. We highly encourage our readers who employ antibodies in their research to read the article in its entirety for its comprehensive treatment of possible antibody cross-reactivity with other proteins and information on how critical exhaustive controls are necessary to prove specificity of binding in various end-use assays. As responsible scientists, it is our duty to ensure that reagents, such as antibodies, used in our experiments are adequately validated and thus adhere to the underlying principles of rigor and reproducibility.

"Putting the freeze" on human follicular granulosa cells

The follicular aspiration performed during assisted reproductive technology yields granulosa cells as a by-product of the procedure. These granulosa cells are key components of the follicle and, due to their characteristics and function, can provide a unique in vitro model to study the ovary (Bagnjuk and Mayerhofer 2019). However, once in culture they undergo rapid changes, and their heterogeneity arising from their human source makes them a difficult model for matching with specific patient conditions. In this light, Beschta et al. (2021) used the routine method for freezing cells in their laboratory (employing DMSO and fetal calf serum), applied it to freshly isolated human granulosa cells, and then compared the characteristics of these cells when subsequently cultured with freshly derived cells. Granulosa cells isolated from patients were divided into two samples per patient: one sample was immediately seeded for cell culture, while the other was subjected to cryopreservation via cooling to − 80 °C at a fixed rate and then transferred to liquid nitrogen for storage. The freshly cultured cells and the frozen/thawed cells were then subjected to comparative analysis using RT-qPCR (focusing on gap junctional cell contact, mitochondrial and steroidogenic markers), proteomics (5962 proteins detected), and progesterone measurements. Their results revealed no significant differences in cell growth and survival, transcript levels of selected genes, protein abundance, and progesterone production when comparing freshly isolated and cultured human granulosa cells to their frozen/thawed counterparts (Fig. 4).

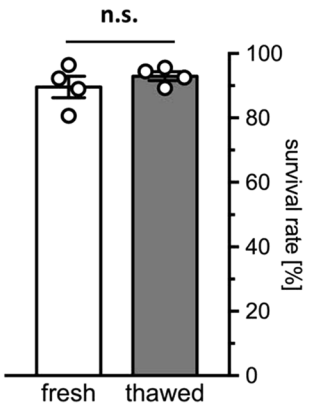

Fig. 4 Survival rate of fresh and frozen/thawed human granulosa cells after 3 days in culture is not significantly different. From Beschta et al. (2021)

The authors compare their cryopreservation method to others found in the literature and surmise that the method described in their manuscript is simpler and superior to other previously reported methods.

References

Bagnjuk K, Mayerhofer A (2019) Human luteinized granulosa cells—a cellular model for the human corpus luteum. Front Endocrinol (Lausanne) 10:452. https://doi.org/10.3389/fendo.2019.00452

Beschta S, Eubler K, Bohne N, Forner I, Berg D, Berg U, Mayerhofer A (2021) A rapid and robust method for the cryopreservation of human granulosa cells. Histochem Cell Biol. https://doi.org/10.1007/s00418-021-02019-3

Couchman JR (2009) Commercial antibodies: The good, bad, and really ugly. J Histochem Cytochem 57(1):7–8

Echeazarra L, Garcia del Cano G, Barrondo S et al (2021) Fit-for-purpose based testing and validation of antibodies to amino- and carboxy-terminal domains of cannabinoid receptor 1. Histochem Cell Biol. https://doi.org/10.1007/s00418-021-02025-5

Fujisawa K, Hara K, Takami T, Okada S, Matsumoto T, Yamamoto N, Sakaida I (2018) Evaluation of the effects of ascorbic acid on metabolism of human mesenchymal stem cells. Stem Cell Res Ther 9(1):93. https://doi.org/10.1186/s13287-018-0825-1

Jühlen R, Fahrenkrog B (2018) Moonlighting nuclear pore proteins: tissue-specific nucleoporin function in health and disease. Histochem Cell Biol 150:593–605. https://doi.org/10.1007/s00418-018-1748-8

Kalyuzhny AE (2009) The dark side of the immunohistochemical moon: Industry. J Histochem Cytochem 57(12):1099–1101

Lee Chong T, Ahearn EL, Cimmino L (2019) Reprogramming the epigenome with vitamin C. Front Cell Dev Biol 7:128. https://doi.org/https://doi.org/10.3389/fcell.2019.00128

Makise M, Uchimura R, Higashi K et al (2021) Overexpression of the nucleoporin Nup88 stimulates migration and invasion of HeLa cells. Histochem Cell Biol. https://doi.org/10.1007/s00418-021-02020-w

O'Hurley GO, Sjöstedt RA et al (2014) Garbage in, garbage out: A critical evaluation of strategies used for validation of immunohistochemical biomarkers. Mol Oncol 8:783–798. https://doi.org/10.1016/j.molonc.2014.03.008

Pizzicannella J, Marconi G, Guarnieri S et al (2021) Role of ascorbic acid in the regulation of epigenetic processes induced by *Porphyromonas gingivalis* in endothelial-committed oral stem cells. Histochem Cell Biol. https://doi.org/10.1007/s00418-021-02014-8

Saper CB (2009) A guide to the perplexed on the specificity of antibodies. J Histochem Cytochem 57(1):1–5

Simon DN, Rout MP (2014) Cancer and the nuclear pore complex. Adv Exp Med Biol 773:285–307. https://doi.org/10.1007/978-1-4899-8032-8_13

Uhlen M, Bandrowski A, Carr S et al (2016) A proposal for validation of antibodies. Nat Meth 13(10):823–827

Voskuil JLA (2014) Commercial antibodies and their validation [version 2; peer review: 3 approved]. F1000Research 3:232. https://doi.org/10.12688/f1000research.4966.1

Wei FL, Qu CY, Song TL, Ding G, Fan ZP, Liu DY, Liu Y, Zhang CM, Shi ST, Wang SL (2012) Vitamin C treatment promotes mesenchymal stem cell sheet formation and tissue regeneration by elevating telomerase activity. J Cell Physiol 227(9):3216–3224. https://doi.org/10.1002/jcp.24012

Weller MG (2018) Ten basic rules of antibody validation. Anal Chem Insights 13:1–5. https://doi:https://doi.org/10.1177/1177390118757462

Xu S, Powers MA (2009) Nuclear pore proteins and cancer. Semin Cell Dev Biol 20(5):620–630. https://doi.org/10.1016/j.semcdb.2009.03.003

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.