In focus in HCB

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The Editorial of the first issue of *Histochemistry and Cell Biology* in 2022 starts with “Most popular articles published in HCB in 2019” and continues to highlight one Review about dynamic changes of histone methylation in mammalian oocytes and early embryos and three Original Articles reporting (1) that fat causes necrosis and inflammation in human steatotic liver, (2) the effect of a P2X7 receptor antagonist in a rat model of ulcerative colitis, and (3) *NANOS3* downregulation in Down syndrome hiPSCs. We wish you good reading!

Most popular articles published in HCB in 2019

As we began with the Editorial in December 2019 for the publications in *Histochemistry and Cell Biology* in 2017 (Taates and Roth 2019), here we look at the download number of manuscripts published in 2019 in the journal to ascertain their appreciation by our readership during the following 2 years. Noteworthy, 21% of the published papers fell into the category ≥ 1000 times downloaded (Table 1), and 34% were downloaded between 500 and 999 times.

Interestingly, the three topmost papers are methodological studies: the first emphasizing the influence of tissue section thickness for the outcome of immunoperoxidase staining, the second reporting an easy-to-use spectrophotometric trypan-blue-based assay, and the third analyzing the impact of different commercially available endothelial culture media on the growth behavior of primary human endothelial cells. As

can be deduced from Table 1, by applying histochemical, immunohistochemical, and in situ molecular techniques, the published papers cover a wide spectrum of topics, ranging for instance from the characterization of human ridged and non-ridged skin over the cellular localization and regulation of receptors and enzymes of the endocannabinoid system to the characterization of the blood–brain barrier integrity in a B-cell-dependent mouse model of multiple sclerosis.

The overall impact of the papers published in *Histochemistry and Cell Biology* is also reflected in the 2-year 2020 journal Impact Factor of 4.304. We would like to thank all of the authors for contributing their work leading to the success of *Histochemistry and Cell Biology*.

Reviewing histone methylation in mammalian oocytes and early embryos

Bilmez et al. (2021) present a comprehensive review on the spatiotemporal modulation and function of histone methylation by specific histone methyltransferases during mammalian oocyte maturation and early embryonic development (Fig. 1). The first part of their review provides a general insight into the chemistry and functional importance of histone lysine and arginine methylation and demethylation. This is followed by an in-depth presentation of the dynamics of lysine and arginine methylation in oocytes. The dynamics of H3K4, H3K9, H3K27, and H3K79 methylation in early embryos are then reviewed. Both sections are supplemented by highly informative tables.

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Table 1 Most popular articles published in *Histochemistry and Cell Biology* in 2019

<i>n</i> *	Article
4084	Libard et al.: Characteristics of the tissue section that influence the staining outcome in immunohistochemistry. <i>Histochem Cell Biol</i> 151, 91–96 (2019). https://doi.org/10.1007/s00418-018-1742-1
3921	Hammoudeh et al.: High-throughput quantification of the effect of DMSO on the viability of lung and breast cancer cells using an easy-to-use spectrophotometric trypan blue-based assay. <i>Histochem Cell Biol</i> 152, 75–84 (2019). https://doi.org/10.1007/s00418-019-01775-7
3770	Leopold et al.: Outgrowth, proliferation, viability, angiogenesis and phenotype of primary human endothelial cells in different purchasable endothelial culture media: feed wisely. <i>Histochem Cell Biol</i> 152, 377–390 (2019). https://doi.org/10.1007/s00418-019-01815-2
3265	Vela-Romera et al.: Characterization of the human ridged and non-ridged skin: a comprehensive histological, histochemical and immunohistochemical analysis. <i>Histochem Cell Biol</i> 151, 57–73 (2019). https://doi.org/10.1007/s00418-018-1701-x
2846	Grill et al.: Cellular localization and regulation of receptors and enzymes of the endocannabinoid system in intestinal and systemic inflammation. <i>Histochem Cell Biol</i> 151, 5–20 (2019). https://doi.org/10.1007/s00418-018-1719-0
2655	Luukkonen et al.: Osteoclasts secrete osteopontin into resorption lacunae during bone resorption. <i>Histochem Cell Biol</i> 151, 475–487 (2019). https://doi.org/10.1007/s00418-019-01770-y
2500	Abdellatif et al.: Indirect visualization of endogenous nuclear actin by correlative light and electron microscopy (CLEM) using an actin-directed chromobody. <i>Histochem Cell Biol</i> 152, 133–143 (2019). https://doi.org/10.1007/s00418-019-01795-3
2374	Fuchs et al.: Histological processing of un-/cellularized thermosensitive electrospun scaffolds. <i>Histochem Cell Biol</i> 151, 343–356 (2019). https://doi.org/10.1007/s00418-018-1757-7
2095	Mold et al.: Unequivocal imaging of aluminium in human cells and tissues by an improved method using morin. <i>Histochem Cell Biol</i> 152, 453–463 (2019). https://doi.org/10.1007/s00418-019-01809-0
1964	Kranc et al.: Genes responsible for proliferation, differentiation, and junction adhesion are significantly up-regulated in human ovarian granulosa cells during a long-term primary in vitro culture. <i>Histochem Cell Biol</i> 151, 125–143 (2019). https://doi.org/10.1007/s00418-018-1750-1
1535	Peters et al.: Meijer and Vloedman's histochemical demonstration of mitochondrial coupling obeys Lambert–Beer's law in the myocardium. <i>Histochem Cell Biol</i> 151, 85–90 (2019). https://doi.org/10.1007/s00418-018-1716-3
1231	Zalecki et al.: Gastric ulcer induced changes in substance P and Nk1, Nk2, Nk3 receptors expression in different stomach localizations with regard to intrinsic neuronal system. <i>Histochem Cell Biol</i> 151, 29–42 (2019). https://doi.org/10.1007/s00418-018-1715-4
1228	Czechowska et al.: An update on the Golgi staining technique improving cerebellar cell type specificity. <i>Histochem Cell Biol</i> 151, 327–341 (2019). https://doi.org/10.1007/s00418-018-01766-0
1205	Bell et al.: Characterization of blood–brain barrier integrity in a B-cell-dependent mouse model of multiple sclerosis. <i>Histochem Cell Biol</i> 151, 489–499 (2019). https://doi.org/10.1007/s00418-019-01768-6
1201	Taatjes et al.: <i>Histochemistry and Cell Biology</i> : 61 years and not tired at all. <i>Histochem Cell Biol</i> 152, 1–11 (2019). https://doi.org/10.1007/s00418-019-01796-2
1079	Papathomas et al.: Novel methods in adrenal research: a metabolomics approach. <i>Histochem Cell Biol</i> 151, 201–216 (2019). https://doi.org/10.1007/s00418-019-01772-w

*Threshold number of pdf downloads ≥ 1000 as per 30 November 2021

Fig. 1 Schematic depiction of the main functions of methylation on histone H3 lysine (K) residues in different developmental and intra-cellular events. From Bilmez et al. (2021)

The concluding paragraphs deal with aspects of the depletion or lack of histone methyltransferases and histone demethylases in oocytes and early embryos. The phenotypes resulting from the loss or knockdown of histone methyltransferases in mice and of histone lysine demethylase in different mammalian species are nicely summarized in additional tables.

A “phat” ultrastructural investigation of hepatic fat

The invention of the transmission electron microscope (TEM) by Ernst Ruska in 1933 (Ruska 1980) as part of his doctoral thesis research initiated the investigation of cell ultrastructure beyond the resolution diffraction limit

imposed by light microscopy. Early biomedical uses for the TEM prior to the introduction of sample plastic embedding and ultrathin sectioning were to visualize potential disease-causing agents such as viruses and bacteria (see, for instance, Lembke and Ruska 1940). The development of novel fixation methods, epoxy embedding resins, together with glass and diamond knives for ultrathin sectioning in the 1940s and 1950s (admirably recounted in the book by Saul Wischnitzer 1981), led to the widespread use of transmission electron microscopy for the observation of human tissues in health and disease (Pavelka and Roth 2015). In this issue, Wisse et al. (2022) continue this tradition with a beautifully illustrated TEM investigation of human nonalcoholic fatty liver disease specimens. Their study includes 217 liver needle biopsies collected over the course of 10 years employing several different primary tissue fixation protocols. The focus of the manuscript is on the ultrastructural investigation of fat droplets accumulated in the steatotic or early phase of the fatty liver samples. Since conventional immersion fixation results in collapse of some hepatic structures, rendering fine structural observation and analyses problematic, the authors sought alternative perfusion-type fixation protocols. They therefore compared samples fixed via immersion, “jet fixation,” and “injection fixation,” the latter two methods representing perfusion-type variants. Concerning these different fixation approaches, it was observed that both “perfusion-type” fixatives outperformed immersion fixation with regard to ultrastructural hepatic tissue preservation (Fig. 2).

Fig. 2 Transmission electron microscopy image of a human liver needle biopsy fixed with jet fixation exhibiting perfusion-fixation quality of fine structural preservation. From Wisse et al. (2022)

With these well-preserved samples, the authors made the following numerous and novel ultrastructural observations by carefully scrutinizing over 20,500 images (!): (1) necrosis of a single parenchymal cell containing one large fat droplet (“single-cell steatonecrosis”); (2) neutrophils and erythrocytes surrounding single fat follicles (“inflammatory fat follicle”); (3) fat droplets filling the sinusoid (“sinusoidal lipid emboli”); and (4) fat droplets incorporating filaments and other unknown materials, most likely representing the TEM equivalent of light microscopic Mallory bodies (“fatty Mallory bodies”). The authors also provide detailed quantitative evaluations of many of these observations, as well as a “Supplemental” 3D array tomography not to be missed. This manuscript reminds us once again that there is still much to be learned and appreciated from ultrastructural observations performed in a careful and thorough fashion with appropriately preserved and processed samples.

The brilliant effects of Brilliant Blue G on neurons in ulcerative colitis

The enteric nervous system (ENS), consisting of groups of neurons, axons, and support cells, is responsible for multiple intestinal physiologic functions, which can be disrupted by pathological processes occurring in inflammatory bowel diseases (Furness 2012). The ENS can be subdivided into two anatomically located ganglionic plexuses: Auerbach’s plexus (also called the myenteric plexus) and Meissner’s plexus (also called the submucosal plexus) (Furness 2012). The neurons comprising the ENS have been shown in prior investigations to express P2 (ATP-activated) purinergic receptors of the P2X family, particularly P2X7 (see, for example, Da Silva et al. 2015). Continuing their interests in the effects of ulcerative colitis on the ENS in a rat model (Da Silva et al. 2015), Castelluci and coworkers have now investigated the potential salubrious effects of treatment with Brilliant Blue G (BBG; an antagonist of the P2X7 receptor) on ENS neurons in experimental ulcerative colitis of the large intestine (Evangelinellis et al. 2022); previously, they have shown this compound to protect against neuronal cell death in the ileum of a rat model of inflammatory bowel disease (Souza et al. 2020). Animals were injected with 2,4,6-trinitrobenzene sulfonic acid (TNBS) into the colon to induce colitis, with BBG administered 1 h following TNBS injection, and for the subsequent 5 days (Fig. 3).

Fig. 3 Disease activity index scores of rats in the control and sham groups and in the ulcerative colitis group treated with Brilliant Blue G. From Evangelinellis et al. (2022)

Using conventional hematoxylin and eosin (H&E) staining, together with dual-stained immunofluorescence microscopy and semi-quantitative image analysis of the neurons in the myenteric plexus, they describe the following observations on colitis effects resulting from BGG treatment compared with nontreated animals: (1) the disease activity index (stool consistence and occult bleeding) decreased; (2) edema and inflammatory cell infiltration were diminished; (3) the numbers of neurons staining for neuronal nitric oxide synthase (nNOS), choline acetyltransferase (ChAT), and P2X7 receptor were restored to levels of control animals; and (4) profile areas of nNOS and ChAT-positive enteric neurons increased during colitis, but were restored to normal control sizes. The authors conclude that BGG may serve as an effective treatment to restore myenteric neurons in ulcerative colitis, and that the P2X7 receptor could possibly be a therapeutic target in this disease.

NANOS3 downregulation in Down syndrome primordial germ cell-like cells

NANOS3 encodes a NANOS-family zinc finger protein that plays a role in maintaining the undifferentiated state of germ cells and is essential in early embryos to protect migrating primordial germ cells (PGC) from apoptosis. Hence, it was reported that downregulation of *NANOS3* resulted in reduced human germ cell number (Angeles Julaton and Reijo Pera 2011) and its overexpression prolonged pluripotency and delayed differentiation of human embryonic stem cells (Panula et al. 2016). Human pluripotent stem cells have been used to recapitulate the human germ cell formation in vitro. In their present work, Abdyev et al. (2022) applied a male human pluripotent stem cell line with trisomy 21, the hallmark of Down syndrome, to induce primordial germ cell-like cells (hPGCLC) and compared the results with pluripotent stem cells having a normal karyotype (Fig. 4). The authors observed that the in vitro-derived germ cells expressed low levels of *EOME*, *SOX17*, and *PRMD14*, and continuously expressed elevated levels of *TFAP2C* and

Fig. 4 Immunofluorescence detection of various pluripotency markers in human induced pluripotent stem cells with trisomy 21. From Abdyev et al. (2022)

PRDM1. In contrast, *NANOG* (required for the acquisition of pluripotency; Silva et al. 2009) and *NANOS3* were downregulated. Since *NANOS3* might have a role during mitosis/meiosis (Angeles Julaton and Reijo Pera 2011), the current authors speculated that this might lead to mitotic and gametogenesis arrest that impedes hPGCLC formation and specification. Since the mechanisms underlying human germ cell development are largely unknown, it is proposed that the Down syndrome primordial germ cell-like cells could be used as a model to study human early germ cell development. Altogether, this study provides in vitro information on early germ cell development with trisomy 21 and the effect of gene dosage imbalance on differentiation of induced pluripotent stem cells with trisomy 21.

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