In focus in HCB

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In this issue, we highlight the results and significance of manuscripts investigating the cell biology of differentiation of murine keratinocytes in 3D culture and pituitary gland cells, an updated technique for improving the Golgi stain specificity for neurons and glial cells, and a characterization and comparison of traditional and newer fluorescence light sources for microscopy, including their environmental impact.

Developing a thick skin

One of the very intriguing and interesting differences between the human and murine digestive system involves the epithelial covering in the early hollow organs of the digestive tract: the stratified epithelium present in the oral cavity, esophagus, and forestomach are keratinized in the mouse, but not in the human. Ozaki et al. (2019) have now investigated this keratinization process in the mouse by employing a sophisticated 3D culture model of murine epidermal keratinocytes (COCA cells) together with conventional histology, multilabel confocal microscopy, immunoblot analysis, and transepithelial electrical resistance measurements. Since components in serum are known to effect the proliferation of keratinocytes in 2D culture models (Bertolero et al. 1986; Borowiec et al. 2013), their effects on tissue morphology, expression of tight junction proteins (occludin, ZO-1, E-cadherin, and claudins-1 and -4), keratinocyte differentiation markers (loricrin and keratin-4) and transepithelial resistance in the air–liquid 3D cultures were also examined. The authors found that employing calcium-depleted serum (in several different concentrations) to the 3D COCA cell

cultures resulted in: (1) alteration in differentiation marker expression from loricrin (marker for keratinization) to keratin-4 (a marker for non-keratinization); (2) reduced expression of claudin-1, while expression of claudin-4, occludin, ZO-1 and E-cadherin were all upregulated; and (3) reduction of transepithelial resistance (indicative of paracellular permeability) by approximately one-half. Incredibly, these effects emblematic of a loss of epithelial keratinization were induced by incubation in as little as 0.1% fetal bovine serum. Further results demonstrated that inhibition of retinoic acid (a metabolite of vitamin A) present in serum resulted in enhanced expression of loricrin and keratinization of the cell surface. In conclusion, the authors suggest that the keratinization of the epithelium of the murine oral cavity, esophagus, and forestomach may be the result of inhibition of retinoic acid receptor-mediated signaling involving vitamin A.

Histone H3K9 acetylation is implicated in pituitary gland cell dynamics

The synthetic nonsteroidal estrogen diethylstilbestrol (DES) is known to induce proliferation of prolactin (PRL) cells in the anterior pituitary gland of rodents which may result in the formation of prolactinomas (Cauwenberge et al. 2001; Matsubara et al. 2001). In addition, DES was found to increase the number of PRL cells via transdifferentiation of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) cells (Shukuwa et al. 2006). Here, Tun et al. (2019) set out to clarify the mechanism through which DES results in cellular proliferation and transdifferentiation in the anterior pituitary gland of male mouse. Since it is known that DES acts through binding to ER α and that this interaction changes the acetylation state of lysine residues in histones (Fiskus et al. 2007; Kuo and Allis 1998; Strasak et al. 2009), they investigated the degree of histone H3 acetylation in the pituitary gland of DES-treated mice as compared to control animals. In a first step, Tun et al. (2019) studied the acetylation level of histone H3 by single and double

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labeling immunohistochemistry. In control mice, strong nuclear staining for acetylated histone H3K9 was observed in PRL, LH and FSH cells. A quantitative analysis revealed that DES-treatment significantly reduced the signal intensity of acetylated H3K9 per each cell type. This finding was substantiated by the use of two histone deacetylase inhibitors (sodium phenylbutyrate and valproic acid) showing that they neutralized the effect of DES on H3K9 acetylation level and prevented the DES-induced increase in PRL cells. Furthermore, and unexpected, histone deacetylase inhibition by valproic acid significantly decreased H3K9 acetylation in all PRL, LH, and FSH cells, which was apparently related to the production of ROS, likely causing hypoacetylation.

Taken together, these findings indicate an essential role of epigenetic factors such as the acetylation status of histone H3K9 in preserving the differentiation state of various cell types in the anterior pituitary gland.

Improving specificity and reliability of the Golgi staining technique for neurons and glial cells

A milestone for the field of neurocytology was the development of the “reazione nera”, a silver-osmium-based technique by Camillo Golgi (1873), which also resulted in the discovery of the “apparato reticolare interno”, the Golgi apparatus (reviewed by Dröschner 1998). The Golgi staining technique and its various modifications provide an extraordinary contrast which permits the visualization of the complex structural organization of neurons and glial cells, as nicely illustrated by the cover picture of this issue depicting Purkinje cells. However, the Golgi staining techniques are also known for unpredictable and inconsistent results, and their reputation is furthermore hampered since they are time-consuming and costly. In a detailed methodical investigation, Czechowska et al. (2019) worked out conditions that improved both cell type-specificity and result reliability of various modifications of the classical Golgi staining technique using the cerebellum as a test tissue. Of importance, their updated protocols are also time saving. The authors compared Bubenaite's, Fox's as well as published variations of the Patro Golgi staining protocols and the commercially available FD Rapid GolgiStain™ kit. In all these protocols, aldehyde fixation was used. For the Patro protocol, the effect of pH, silver nitrate concentration and incubation time during impregnation on the staining quality was assessed. Standardized conditions for image acquisition and quantification were applied. Although the various staining protocols resulted in the preferential staining of distinct cell types, the authors recommend the use of the Golgi-Colonnier method in combination with a Quick Golgi fixative for a reasonable

specific staining of granule cells, Bergmann glia or Purkinje cells.

Fluorescence light sources for microscopy: characterization and environmental impact

Mubaid et al. (2019) provide an informative and timely analysis of the stability of a variety of fluorescence light sources for light microscopy. With the steady transition from the standard mercury and xenon arc lamps to solid-state light-emitting diodes (LED)-based technologies for fluorescence microscopy, an in-depth analysis of the properties of these newer light sources is warranted. In this manuscript, the authors compared both the power output of four commercially available LED light sources on multiple time scales, and their electrical power consumption with those of mercury arc lamps and metal halide bulbs. Their analyses demonstrated that when used at lower power outputs, the LEDs provided enhanced light stability with less power consumption than the traditional mercury arc lamps and metal halide bulbs. Their superior light stability also renders them the light source of choice for quantitative fluorescence microscopy. Moreover, other positive attributes of LED sources include highly stable power output, rapid warm-up periods, and can be rapidly switched on and off. The authors provide detailed protocols for all of their testing methods, so that we can all perform similar analyses in our own labs as we consider light sources for fluorescence microscopy. Be sure to also read their “Life Cycle Analysis” provided in the online supplemental file, comparing the overall environmental costs for LED-based and metal halide bulb light sources.

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