In focus in HCB

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Morphology-oriented epigenetic research

Kitazawa et al. (2018) present a most interesting and informative review on the current state-of-the-art in combining morphological and cellular observation together with epigenetic analysis. The authors define epigenetics as “heritable changes in phenotype (appearance) or gene expression caused by mechanisms other than changes in the underlying DNA sequence”. The review then focuses on gene regulation resulting from DNA methylation. In vertebrate animal species the majority of DNA methylation reactions are restricted to cytosine nucleotides; in particular those forming 5'-cytosine-phosphate-guanine-3' dinucleotides (CpG). These CpG sites are often located in clusters at the 5'-end of genes, and referred to as CpG islands. The methylation status of these CpG islands has been implicated in important cellular proliferation activities including development and tumorigenesis. However, since individual cells may be epigenetically heterogeneous, methods are required conjoining morphology with epigenetic analyses on a minimum of cells. The authors describe two of these methods in detail: (1) histoendonuclease-linked detection of methylated sites of DNA (HELMET), and (2) padlock probe and rolling circle amplification (RCA) and how they were used to detect methylated sites in different samples. They then finished with the description of a novel MeCP2-mediated gene-silencing mechanism through the addition of methylation to a single CpG locus upstream of a TATA-box illustrated via two examples.

The following two review articles appear in this issue as part of the 60th anniversary of *Histochemistry and Cell Biology*.

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Cell migration during cerebellum development

Schilling (2018) provides a comprehensive review detailing the processes of cell migration occurring during the development and histogenesis of the mouse cerebellum. The review begins with a general description of imaging methods and analysis protocols to monitor cell migration during development. Issues including the effects that image resolution may have on the accuracy of migration tracing analysis are explained in clear detail. Following this general introduction, the review focuses on specific aspects pertaining to cell migration in the embryo leading to development of the multiple layers and cell types composing the mature cerebellum. Results from experiments performed on fixed and processed tissue sections, as well as live cell models are detailed. The review is extensively supported by the literature citations detailing the experiments and tracing methods employed, including the phenotypic markers required to identify the individual cell types during their migration. The review concludes with the notion that although much information has been obtained detailing the development of the cerebellum, key aspects of the process, including the molecular mechanisms involved with cell migration still remain to be elucidated.

The endolysosomal system of human chordoma cells

Chordomas are malignant bone tumors derived from notochord remnants, which are highly resistant against conventional chemo- and radiotherapy (Salisbury 1993). Cell lines established from chordoma maintain characteristics of the primary tumors, such as a distinct cytoplasmic vacuolization (Erlandson et al. 1968; Gellner et al. 2016). Notochord cells typically contain fluid-filled organelles, the notochord vacuoles, which represent unique lysosome-related structures (Ellis et al. 2013). Since it has been demonstrated

that lysosomal sequestration of chemotherapeutic agents is a drug resistance mechanism for different kinds of tumors (Colombo et al. 2014), Kolb-Lenz et al. (2018) have performed a detailed analysis of the vacuolar system of three chordoma cell lines and have tested whether chloroquine alone or in combination with doxorubicin would prove cytotoxic. Using the LysoTracker® Red DND-99 and antibodies against the lysosomal membrane protein LAMP1, an enormous number of perinuclear acidic organelles including lysosomes, autophagosomes, endosomes and phagosomes was identified. However, the large vacuoles, although being LAMP1-positive, did not accumulate LysoTracker® Red DND-99, and were interpreted to represent remnants of notochord vacuoles. 3D reconstruction from high-pressure frozen and freeze-substituted cells revealed close association between lysosomes and mitochondria. Another feature of chordoma cell lines was the presence of large amounts of glycogen, some of which was removed by glycophagy. Finally, a possible synergistic effect of chloroquine and doxorubicin on chordoma cell growth was investigated using the highest single agent model. However, no clear effect of chloroquine could be observed.

Automated segmentation and quantitative cell-shape analysis by confocal microscopy

The shape of cells and their functional state are related, and thus accurate detection of shape changes is of crucial importance in many fields of experimental and clinical research. Kopanja et al. (2018) report the development of a new approach for accurate morphological analysis of cell shape and its use to estimate the effect of two toxins on the morphology of murine neuronal NG108-15 cells. As the authors have shown earlier (Vrecl et al. 2015), the toxins ostreolysin A and pleurotolysin B pore-forming complex cause extensive morphological changes in murine neuronal NG108-15 cells, including cell swelling and plasma membrane blebbing. The approach presented in this manuscript consists of a novel algorithm to extract cell contours, implemented in the software package MIPAR™ (Sosa et al. 2014), and the use of the modified circularity measure $C_2^n(S)$ for the cell shape from confocal images. The modified circularity measure $C_2^n(S)$, which defines the degree to which the shape of the cells differs from a perfect circle, enabled better detection of small changes in cell shape, allowing an

easily detectable numerical outcome. In addition, the authors showed that applying the described method of segmentation and using the measure $C_2^n(S)$ helps to detect cells with blebs, an important plasma membrane injury. Although the image analysis was applied to analyze the toxin effect on cultured neuronal cells, it is proposed that the approach should be more generally applicable for cell-shape studies.

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