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EDITORIAL

In focus in HCB

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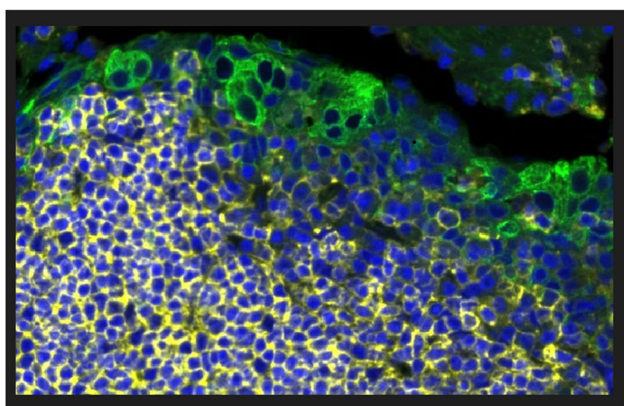

On behalf of the entire Editorial Board of *Histochemistry and Cell Biology*, we would like to wish our readers a healthy and prosperous 2024, and we look forward to receiving your manuscript contributions in the New Year. In this first editorial for 2024, we will highlight two original contributions describing studies with cultured cells illustrating (1) the effect of various culture media compositions on the response of a human renal proximal tubular epithelial cell line to treatment with TGF-β, and (2) a multiparameter comparison of biophysical and biological characteristics of U87 MG glioblastoma cells and astrocytes, as well as a review manuscript featuring updated information on the development of cerebellar inhibitory neurons since the advent of single-cell genetic analyses.

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Cells with culture…

In a recent "In focus in HCB" editorial (Taatjes and Roth 2023), we highlighted a manuscript by Stollmeier et al. (2023) extolling the virtues of using the air–liquid interface model for culturing epithelial cells versus conventional standard medium submersion. They described numerous metabolic and morphological differences in cells cultured using the two methods, once again illustrating how culture methods can influence cell physiology and morphology. Another factor to consider in this realm is the potential effect the composition of the culture medium may have on cellular characteristics and responses. In this light, Garmaa et al. (2024) have now reported studies illustrating the effects of various culture media on the response of HK-2 cells (a kidney proximal tubular epithelial cell line) to treatment with TGF-β. Since HK-2 cells are often employed as an in vitro model for kidney physiology and nephropathy, including fibrosis, they focused their attention on genes and proteins involved in the process of epithelial-to-mesenchymal transition (EMT), a pathological result of tubulointerstitial fibrosis. They tested six different culture media with composition as follows: (1) DMEM with added 2% fetal bovine serum (FBS), (2) DMEM with added 5% FBS, (3) DMEM with added 10% FBS, (4) DMEM/F12 with added 10% FBS (1:1 mixture of DMEM and Ham's F12 medium), (5) PTEC (hormonally defined; mixture of DMEM/F12 with added 10% FBS), and (6) keratinocyte serum-free growth medium (KSFM). We recommend that readers consult Table 1 in the manuscript by Garmaa et al. (2024) for a complete listing of all ingredients and components in each culture medium formulation. HK-2 cells were cultured in the six different culture mediums, TGF-β was introduced, and then cell morphology was assessed by phase contrast light microscopy, RNA was analyzed with qPCR, and protein by immunoblot and immunofluorescence. Although some very specific differences were noted in various assays dependent upon the culture medium employed, some of their results can be generalized as follows: (1) cells cultured without TGF-β

displayed a typical epithelial morphology, whereas those cultured in the presence of TGF-β were slightly elongated; (2) phenotypic changes toward EMT were attributable to treatment with TGF-β; (3) regardless of the medium formulation used, treatment with TGF-β led to increased expression of RNA for the EMT markers *ACTA2*, *TGFB*, *VIM*, and *CTGF*, as well as the inflammatory markers *COL4A1* and *EGR2*, whereas *PPARG* expression was reduced; (4) gene expression of *EGR1*, *FN*, *IL6*, and *C3* was either increased or decreased by TGF-β treatment depending upon the culture medium employed; and (5) by immunofluorescence, TGFB1 and vimentin protein were found to be overexpressed in response to TGF-β treatment in all culture media tested, whereas fibronectin expression and the nuclear translocation of EGR1 was dependent upon the medium. The authors conclude by emphasizing that the composition of cell culture media is critical for the evaluation of EMT for HK-2 cells exposed to TGF-β.

Biophysical analysis of cultured glioblastoma and astrocyte cells

Glioblastoma is a highly aggressive and devastating brain tumor. Over the years, many models have been proposed to study this cancer to seek therapeutic treatments. As recently summarized by Oraiopoulou et al. (2023), these models can be divided into (1) in vitro cell culturing of established cell lines or from patient-derived cells, either in conventional 2D monolayers, or from 3D or 4D spheroids (see, for instance, Khoshyomn et al. 1997; 1998 for information about spheroid models), (2) in vivo animal models, or (3) in silico designed predictive algorithms. Monolayer cell culture is still a very popular in vitro model to study glioblastoma treatment, with the patient-derived U87 MG established cell line being well known and characterized. To gain further information regarding the biophysical topology and chemistry of these aggressive glioblastoma cells, Ozdil et al. (2024) have now performed a comparison of U87 MG cells (of astrocytic origin) with a control astrocytic cell line (SVG p12) using phase contrast and fluorescence microscopy, atomic force microscopy (AFM), scanning electron microscopy (SEM) combined with energy dispersive X-ray spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), and RT-PCR. Among their results, they found (1) by fluorescence microscopy, the intensity of staining for the cytoskeletal protein F-actin was increased in U87 MG cells and its anisotropy (alignment of filaments within the cell) was different between the two cell types (higher in U87 MG cells); (2) no differences were found between the two cell types with respect to expression of a panel of genes related to the cytoskeleton; however, U87 MG cells had a higher expression of motility-related genes (as expected for a metastatic cell type); (3) AFM imaging revealed that U87 MG cells expressed a long and narrow phenotype, whereas the SVG p12 cells were shorter and had a greater measured surface roughness value; (4) by SEM analysis, U87 MG cells showed increased membrane filopodial protrusions, while EDS analysis did not reveal any significant elemental content differences between the two cell types; and (5) XPS (which probes the cell surface and analyzes elemental composition) revealed some differences between the cells with respect to the expression of the elements P, S, C, N, O, and Na. The authors conclude their interesting and detailed manuscript with the sentiment that a combination of spectroscopic and microscopic techniques may potentially serve as diagnostic tools for cancer cell characterization.

Reviewing the development of cerebellar inhibitory interneurons through single-cell genetic analyses

In contrast to Purkinje cells (Oberdick et al. 1990) and granule cells (Ben-Arie et al. 1997), the diverse group of inhibitory interneurons of the cerebellum is less well characterized in terms of their development, classification, and molecular characteristics as well as the importance of spatiotemporally patterned external signals for their diversification (e.g., Kano et al. 2018; Haldipur et al. 2022). The present review on the cerebellar inhibitory interneurons by Schilling (2024) builds on a previous review by Schilling et al. (2008) and represents the state of the art of this highly active field of research. The meticulously compiled review starts with a general introduction on developmental aspects of the cerebellar cortex. It is followed by 12 sections dealing with various aspects of the cerebellar inhibitory interneurons including (a) patterns in the cerebellar anlage, (b) differentiation and diversification of cerebellar inhibitory cells from Pax2 precursors as well as their migration and local integration, (c) key genes that pattern the cerebellar ventricular epithelium and may be used as markers of distinct inhibitory interneuron lineages, and (d) diversity of inhibitory interneurons residing in the Purkinje cell layer and Golgi cells, the molecular layer, and the cerebellar nuclei. The last section is a short and cautionary excursion in the evolving field of disease-related dysfunctions of cerebellar inhibitory interneurons. The accompanying figures show representative examples of the visualization of RNA markers by in situ hybridization and single-cell RNA expression analysis, and a revised version of the “instructive niche” model (Leto et al. 2010) as proposed by the current author. In the concluding perspectives, the author points to some conflicting results obtained by in situ hybridization and single-cell RNA expression studies and provides reasons for their possible (methodological) basis.

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