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EDITORIAL

In focus in HCB

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Stem cells figure prominently in two articles featured in the current issue. Andl et al. (2016) investigated the unique cell proliferation situation presented by human oral mucosa. Unlike epidermal stratified squamous epithelia where a proliferative basal cell layer is overlain by successively differentiated cell layers, the human oral mucosa contains a quiescent basal cell layer, with proliferation mostly restricted to parabasal and suprabasal cell layers. Andl et al. (2016) have now addressed the important and intriguing question relating to the function of such a quiescent basal cell layer. They performed an immunohistochemical and in situ hybridization characterization of the basal cell layer on sections from tissues embedded in paraffin, as well as in a human organotypic cell culture model of oral mucosal tissue. They found that cells located in the parabasal and suprabasal layers expressed markers related to cell cycle dynamics, whereas those cells found in the basal layer stained for molecular markers typically identified with stem cells. In particular, these basal layer cells expressed multiple proteins related to the TGFβ signaling pathway; indeed, 50 % of the overall markers displayed by cells in the basal layer were associated with SMADs. These findings are of particular interest since TGFβ is known to be involved in regulating quiescence in a variety of stem cell types. Their results also showed that basal layer cells expressed multiple molecules endowing the following protective effects on the cell: anti-apoptotic, regulating the response to oxidative stress and heat shock proteins, and the longevity regulating pathway. Further studies revealed that activation of the TGFβ signaling pathway in human keratinocytes in an organotypic culture model resulted in expression of markers characteristic of quiescent stem cells. Moreover, immunostaining of sections from the epidermal inflammatory hyperproliferative disease psoriasis demonstrated features of a quiescent basal stem cell layer akin to the normal human oral mucosa. The authors also made good use of data from the Human Protein Atlas (http://www.proteinatlas.org) for antibody screening and comparing some of their images with those published in the atlas. Finally, the authors propose a model in which the quiescent stem cell basal layer located in human oral mucosa may be equivalent to the quiescent reserve stem cells present in intestinal epithelial tissues, acting as a protective mechanism guarding the stem cell pool from high proliferation rates.

The connexins are a family of proteins highly expressed in all gap junctions. Since gap junctions appear to be highly mobile cellular structures, responding to changes in cell physiology and pathology, there is much interest in studying how they form and structurally respond to cellular perturbations or to differentiation and proliferation events. Beckmann et al. (2016) chose to study gap junction formation by employing a stem model consisting of clone number two human cord-blood-derived induced pluripotent stem cells (HCBiPS2). These stem cells are known to express connexin 43 (Cx43) following their differentiation into cardiomyocytes; however, whether or not the stem cells themselves form gap junctions or express Cx43 is unknown. Thus, to address these questions, Beckmann et al. (2016) used quantitative RT-PCR and immunofluorescence to assess the presence of Cx43 in undifferentiated HCBiPS2 cells, and freeze-fracture replica immunogold labeling (FRIL) to ultrastructurally investigate gap junction

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formation. The results of this study demonstrated that (1) gap junctions are already present in cultures of near confluent induced pluripotent stem cells derived from human cord blood, and (2) these gap junctions indeed express Cx43. Moreover, and quite fittingly, this study also elegantly demonstrates the modern use of the freeze-fracture technology, first described 55 years ago by Moor et al. (1961) combined with immunogold labeling to resolve a contemporary question in cell biology (refer to the cover image of this issue). This should serve as a reminder to us all that techniques from the twentieth century may still be quite relevant in the twenty-first.

The Golgi apparatus (GA) of eukaryotes typically is made of a stack composed of a varying number of smooth cisternae (or disks), which are interconnected laterally by tubules to form a large ribbon. In addition, tubular connections linking GA cisternae vertically and dynamic tubules emanating from the trans-Golgi network exist. In contrast, the GA of the yeast *Saccharomyces cerevisiae* is structurally simple and small-sized, and its compartments are dispersed. The details of cargo transport from ER-to-GA and between the different GA compartments in *S. cerevisiae* are intensely studied. Beznoussenko et al. (2016) report now the results of their 3D and immunoelectron microscopic analysis and discuss implications for ER-to-GA and intra-GA transport in *S. cerevisiae*. To better visualize thin membrane tubules and to achieve high-resolution 3D reconstruction, they applied improved protocols of high-pressure freezing and freeze-substitution including high OsO_4 concentration, and of image acquisition. In addition, correlative light and electron microscopy (CLEM) and quantitative immunolabeling was performed. For the cytoplasmic ER, tubular networks connecting several ER cisternae could be demonstrated and narrow pores as well as aggregates of them near the rims. In contrast, all GA structures appeared isolated. Only rarely, connections with the ER were found, and between GA compartments, but only when they were close to each other. GA cisternae with wide pores were classified as cis compartment and those with narrow pores as being the medial compartment. Furthermore, two types of vesicles could be identified: 45–50 nm vesicles enriched for Gosp1and assumed to be COPI-dependent and 35–40 nm vesicles derived from trans-GA clathrin-coated buds. It is proposed that the observed ER-to-GA and intra-GA connections are short-lived and that this would support the kiss-and-run model of ER-to-GA and intra-GA vesicle transport.

Typically, intercellular communication is mediated by gap junctions and chemical junctions as well as by tunneling nanotubes. More recently, different types of extracellular vesicles, the exosomes and the microvesicles, were shown to function in unconventional secretion and in intercellular communication. Exosomes have been shown to contain different types of bioactive molecules and are formed as a result of the fusion of classic multivesicular bodies with the plasma membrane. By fusing with other cells, exosomes can deliver their content and subsequently induce a specific response of the target cell. In contrast to exosomes, microvesicles bud and shed directly from the plasma membrane. Now, Junquera et al. (2016) report the existence of another type of extracellular vesicles, they named spheresomes, formed by gastrointestinal stromal tumors. The origin of spheresomes as shown by transmission electron microscopy is a structure very much resembling multivesicular bodies, which they termed multivesicular spheres. The extracellular spheresomes, in contrast to exosomes, are clusters of extracellular vesicles surrounded by a limiting membrane. It will be important to further analyze the protein composition of the multivesicular spheres as compared to endocytic multivesicular bodies and to determine the content of the spheresomes in order to establish their function.

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