In focus in HCB

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In this first Editorial of 2020 we are pleased to highlight three Original Papers, describing the role and regulation of the tight junction proteins lipolysis-stimulated lipoprotein receptor (LSR) and tricellulin (TRIC) in human pancreatic cancer cells, an investigation of the morphological appearance and cellular behavior of clonal human breast cancer cells overexpressing $\beta 4$ -*N*-acetyl-galactosaminyltransferase 4, and the cellular analysis of macrophage phenotypes in aged rats. As you peruse this Editorial and the other contents of this issue, we would like to take the opportunity to wish you all the best for the New Year.

The “long and short” of “Function at the Junction” (with apologies to R&B singer Shorty Long)

The alteration of epithelial cell membranes, especially at tight junctions, resulting in modified barrier function and migratory properties is a topic of great relevance in cancer biology (Martin 2014). When three epithelial cells interact in a polarized epithelium, tricellular tight junctions (tTJs) form (Furuse et al. 2014). These junctions are composed of multiple protein complexes, whose expression levels may influence cellular behavior. Since pancreatic cancer cells display aggressive invasive and metastatic properties, Kyuno et al. (2020) have focused on the role and regulation of two specific tTJ proteins in cultured pancreatic cells and tissue sections from human pancreatic cancers: the barrier function protein lipolysis-stimulated lipoprotein receptor (LSR) and tricellulin (TRIC), a molecular component of tTJs recruited by LSR. In their experiments, they employed multiple

assays, including RNA interference and cell transfection, immunohistochemistry on tissue sections, immunofluorescence on cultured cells, transmission electron microscopy, transepithelial electrical resistance measurement (TEER), western blotting, Matrigel invasion assay, and a wounded cell migration assay. Highlights of the results from these numerous assays demonstrated that (1) treatment of cells with EGF or TGF- β led to an increased expression of LSR, but had no effect on TRIC expression, and promoted the relocalization of both proteins to bicellular tight junctions from tTJs; (2) knockdown of LSR using siRNA techniques resulted in an increase in cell migratory, invasion, and proliferation properties, and a concomitant decrease in barrier properties; (3) cells treated with the LSR ligand peptide angubindin-1 displayed a decrease in LSR expression and barrier function, and an increase in invasiveness; (4) TRIC knockdown by siRNA techniques resulted in decreased cell migration and barrier function; and (5) by immunohistochemical analysis, both LSR and TRIC were detected at cell membranes of normal pancreatic ducts and well-differentiated pancreatic ductal adenocarcinomas (PDAC), whereas in poorly differentiated PDAC, LSR was found only weakly expressed in the cell cytoplasm. The authors conclude that in human pancreatic cancer cells LSR is regulated via the activity of multiple growth factors, and its loss of expression promotes cell invasion, migration, and proliferation. They posit that LSR may be a target candidate for pancreatic cancer therapy.

A sweet mesenchymal–epithelial transition

Glycosylation is a most frequent and structurally highly varied post-translational modification on proteins and lipids (Corfield 2017; Kopitz 2017). The high degree of structural variability of glycans on proteins is reflected by their well-established myriad biological functions (Gabius and Roth 2017) such as in the quality control of protein folding in the endoplasmic reticulum (Roth and Zuber 2017) and cell

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surface-mediated communication, signaling, adhesion and migration in health and disease (Bhide and Colley 2017). Following previous work showing that a correlation exists between the disaccharide GalNAc β 1 $\rightarrow$ 4GlcNAc (LacdiNAc) on *N*-glycans of cell surface proteins of human breast cancer and tumor progression (Kitamura et al. 2003), and the malignant properties of the human breast cancer cell line MDA-MB-231, Hirano et al. (2020) report now the effect overexpression of LacdiNAc has on the morphological appearance and adhesive properties of this breast cancer cell line. The two clonal cell lines overexpressing LacdiNAc, as verified by FITC-labeled *Wisteria floribunda* agglutinin, showed a morphological change typical of a mesenchymal–epithelial transition when compared to mock-transfected cells. The epithelial-like cell phenotype was associated with an increased expression of cell surface E-cadherin and a decreased expression of *N*-cadherin, vimentin, α -smooth muscle actin and ZEB1. Furthermore, the LacdiNAc-overexpressing cell clones showed a lower migratory activity compared to mock-transfected cells by wound-healing assay. The authors also observed increased adhesion to extracellular matrix proteins such as fibronectin, collagen type I, collagen type IV, and laminin, as well as highly efficient spreading on laminin. Thus, an enhanced expression of LacdiNAc on glycans of cell surface proteins in a human breast cancer cell line, and by inference in human breast cancer, may decrease their malignant potential.

M&Ms (1 and 2) in the liver of aging rats

Macrophages may be roughly classified as either of the M1 subtype (involved in pro-inflammatory functions), or M2 subtype (involved in anti-inflammatory functions) (Murray et al. 2014). Alterations in the tissue microenvironment in which macrophages are located can result in phenotypic changes (also known as polarization). Since aging is associated with an enhanced pro-inflammatory and pro-oxidative state environment, Bloomer et al. (2020) have now expanded upon earlier studies (Bloomer et al. 2009; Hilmer et al. 2007; Maseo-Diaz et al. 2018) to perform a detailed histochemical and morphometric characterization of macrophage phenotype in aged animals. Macrophage subtypes were determined by careful evaluation of immunostaining using well validated antibodies (see Materials and methods section of manuscript for description of detailed antibody validation). Additionally, they sought to determine if the increased numbers of hepatic Kupffer cells found in aging animals may be a source for the associated elevated oxidative stress. To perform this set of experiments, they treated animals with gadalonium chloride (GdCl₃), a compound known to affect the activity and viability of Kupffer cells (Hardonk et al.

1992). Their detailed multi-fluorophore immunostaining and semi-quantitative cell analysis revealed that (1) in the periportal hepatic region, and to a lesser extent in the perivenous hepatic region, aged rats had greater numbers of both M1 and M2 macrophages compared to young animals; and (2) in general, treatment of both young and aged animals with GdCl₃ resulted in decreases in both M1 and M2 subtype macrophage markers; however in the aged animals a marked shift towards M2 phenotype overall was observed. Interestingly, contrary to their hypothesis, no change in oxidative stress was observed following treatment with GdCl₃ even though a specific decrease in macrophage-specific markers was detected.

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