In focus in HCB

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In this month's editorial, we highlight one beautifully illustrated Review Article describing the many functions of the myosin motor protein MYO6 during spermiogenesis in flies, worms, and mammals, and three Original Articles describing (1) the arrangement of the splenic microvasculature from the 3D serial section reconstruction of an adult human spleen analyzed by virtual reality; (2) the possible role of syndecan proteins in the remodeling of the nodes of Ranvier following peripheral nerve injury; and (3) factors involved in osteoblast to osteocyte differentiation in sites of bone modeling and remodeling. We hope you find these highlights of interest, and motivate you to read the full articles.

Myosin VI in spermiogenesis: multiple functions of an unusual myosin

During spermiogenesis, unpolarized spermatids differentiate into sperms, and actin and associated actin-binding proteins are of critical importance during this process. Furthermore, myosin motor proteins moving along actin filaments and acting to mediate interactions between the actin network and organelles are making a major contribution by generating force and movement. Zakrzewski and colleagues (2021) review the functions of myosin VI during spermiogenesis in flies, worms and mammals (Fig. 1).

Myosin VI is noteworthy since unlike other myosins, it moves towards the minus end of actin filaments (Wells et al. 1999). Following a short section highlighting functions of the actin cytoskeleton and myosin Va as well as myosin VIIa, the authors summarize general aspects of the structure and function of myosin VI. They then present a concise

account of the diverse roles of myosin VI during *Drosophila* spermatid individualization and spermatid differentiation in *C. elegans*. The major part of the Review is devoted to the subcellular distribution and roles of myosin VI during the Golgi, cap, acrosome and maturation phase of mouse spermiogenesis. The Review also provides a highly informative account of the structural and functional consequences of the loss of myosin VI, which results in a reduced fertility in mouse, and infertility in *Drosophila* and *C. elegans*.

Splenic red pulp microvasculature meets virtual reality

The arterial side of the red pulp of the human spleen consists of an intricate system of terminal arterioles followed by sheathed capillaries that feed a capillary network with many open ends (Steiniger 2015). The sheathed capillaries and sinuses are spleen-specific structures. In previous investigations, Steiniger and colleagues (2007, 2014, 2018) have established the cellular phenotype as well as the location and form of capillary sheaths in 3D. In their present study (Steiniger et al. 2021), they used multiple labeling immunohistochemistry to detect smooth muscle α -actin, endothelial

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Fig. 1 Localization of myosin VI in mouse developing spermatids during the Golgi and cap phases (From Zakrzewski et al. 2021)

cells with anti-CD34, resident sheath cells with anti-CD271, and B lymphocytes with anti-CD20 (Fig. 2).

Immunohistochemistry was performed on serial sections (approximately 80 sections) for subsequent 3D reconstruction and evaluation of the 3D model in virtual reality based on surface mesh representations (Lobachev et al. 2017). The authors emphasize that their virtual reality visualization tool allows the inspection of the original sections blended into the mesh-based 3D reconstruction. Their main finding is “that sheathed capillaries are specialized vascular structures located distal to branching points of terminal arterioles in the human splenic red pulp. The sheaths consist of capillary endothelial cells and pericytes surrounded by special CD271⁺ stromal sheath cells mimicking a cuboidal epithelium, by B lymphocytes and by macrophages.” According to these observations, sheathed vessels should be referred to as sheathed capillaries. Only few connections were found between the red pulp capillary network and red pulp venules. Therefore, the authors concluded that the human splenic red pulp is mainly an open microcirculation.

Fig. 2 Immunohistochemical staining of adult human spleen for smooth muscle α -actin, CD 34, CD271 and CD20 (From Steiniger et al. 2021)

The nerve of it all

The repair of peripheral nerves following injury is a complicated and multifaceted process. Of the many components thought to be involved in this process, heparin sulfate proteoglycans (HSPGs) have received much attention due to their potential roles in tissue repair via binding to extracellular growth factors (Murakami et al. 2015). The syndecan family of HSPGs are present in abundance in the peripheral and central nervous systems, with their expression regulated by matrix metalloproteinase 9 (MMP-9). Moreover, additional factors potentially involved in peripheral nerve tissue repair are voltage-gated sodium channels (Henry et al. 2007). In view of these insights, Liu et al. (2021) have now used RNA-seq, Western blotting, proximity ligation assays and confocal microscopy to investigate the expression of the HSPGs Syndecan-3 (SDC3) and Syndecan-4 (SCD4), the sodium channel subtype Nav1.6, and MMP-9 at the node of Ranvier in an injured rat model. In addition, they employed time-of-flight secondary ion mass spectrometry (TOF-SIMS) to measure the distribution of unlabeled sodium ions in nodal regions, which could be attracted by the negatively charged proteoglycan chains on the syndecan proteins. Through the incorporation of all of these methods they found: (1) upregulation of SCD3, SCD4, Nav1.6, and MMP-9 1 month following injury (with a decline 3 months post-surgery); (2) colocalization of SCD3 with Nav1.6 in axonal nodes in control animals, which decreased 1 month after injury, but began to reappear after 3 months; and (3) decreased sodium ion concentration at the nodes 1 month post-injury, followed by increased levels at 3 months to sham-operation levels (Fig. 3).

Overall, these results suggest that it is mainly SDC3 and Nav1.6 expressed at the node of Ranvier which are critical components during peripheral nerve regeneration following injury. The authors speculate that SCD3 may serve to stabilize Nav1.6 and effect the localization of sodium ions at nodal regions, resulting in larger neuronal action potential triggering and enhanced functional recovery during the repair process.

A bone to pick...

Akin to the injury-driven neuronal regeneration process mentioned above, bone development and repair is likewise a complicated process, driven by the interactions of numerous cells and factors (Flores-Silva et al. 2015). Two of these cell types, bone-forming osteoblasts and their mature counterparts the osteocytes, can interact and communicate via the osteocytic lacunar–canalicular system (OLCS). This system is thought to be important in effecting osteocytic function,

Fig. 3 TOF-SIMS images of regenerating axons. Ionic images of sodium (left panel) and corresponding DIC bright field images (right panel); serial longitudinal sections of repaired nerve (From Liu et al. 2021)

including mechanotransduction and bone remodeling. Moreover, osteoblasts interacting through the OLCS can undergo further differentiation into osteocytes, comprising approximately 90–95% of all bone cells (Flores-Silva et al. 2015). This has prompted Nagai et al. (2021) to perform a detailed multi-immunohistochemical analysis, combined with pre-embedding peroxidase immunoelectron microscopy to investigate the process of late-stage osteoblast differentiation into osteocytes. They chose to combine immunostaining for podoplanin, a transmembrane glycoprotein expressed in late-stage osteoblasts and pre-osteocytes, together with some of its purported binding partners. In particular, they were interested in ascertaining if podoplanin interacted with the hyaluronan cell membrane receptor CD44 to potentially promote migration of cells to areas of bone modeling and remodeling in murine femora and tibiae. For immunostaining methods at the light microscopic level, they used both enzyme-based for transmitted light, and fluorescence-based for super-resolution STED imaging (Fig. 4).

Their results indicated that (1) podoplanin is a reliable marker for late osteoblasts/pre-osteocytes; (2) podoplanin-positive osteoblasts displayed an intracellular pattern of distribution of actin filaments similar to osteocytes; and

Fig. 4 The distribution of actin filaments and podoplanin in osteoblasts and osteocytes shown by STED (From Nagai et al. 2021)

(3) in regions of bone remodeling, osteoblasts differentiating into osteocytes are not associated with CD44, but did display colocalization of podoplanin with phosphorylated ezrin (mediates podoplanin-mediated reorganization of cellular actin filaments). Thus, their results suggest that the interaction of podoplanin with CD44 may be involved in the differentiation of osteoblasts into osteocytes in regions of bone remodeling, but not in sites of bone modeling.

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