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EDITORIAL

In focus in HCB

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Localization of ADP-ribosylation factor 6 in peripheral mouse tissues

ADP-ribosylation factors are members of the Ras small GTPase superfamily. This subfamily consists of six members (Arfs1–6), functioning as regulators of vesicular trafficking and actin remodeling. Arf6 is of particular interest, since it is believed to play a regulatory role in several cellular physiological functions, including cell–cell interactions (adhesiveness), motility, shape maintenance, and responses to environmental stimuli via cytoskeletal adaptations and vesicular trafficking. Previous investigations seeking to elucidate the intracellular and animal tissue distribution of Arf6 have been hampered by technical issues, prompting Katsumata et al. (2017) to optimize immunohistochemical techniques with heat-induced antigen retrieval (HIER) methods to re-evaluate the localization of Arf6 in a variety of mouse peripheral tissues. Importantly, the authors reaffirmed their previous characterization of the specificity of the anti-Arf6 antibody through transfection of HeLa cells with a FLAG-tagged Arf6 complex (Hara et al. 2016), as well as immunoblot analyses of multiple mouse tissues and organs. Using both immunofluorescence microscopy (confocal) and pre-embedding immunoelectron microscopy they performed an extensive evaluation of Arf6 localization in adult mouse peripheral tissues. Arf 6 was found to be distributed in epithelial cells from various tissues and organs, including esophagus, stomach, small and large intestine, trachea, kidney, epididymis, oviduct, and uterus. The polarized expression of Arf6 was readily apparent in simple and pseudostratified epithelia, where it was mostly restricted to the basolateral plasma membrane. Intracellularly, Arf6 was immunolocalized to a subset of cytoplasmic endosomes. The immunoelectron microscopic data provided new insights into the ultrastructural localization of Arf6 in specific cell types from various tissues. This very comprehensive study of the localization of Arf6 in multiple cell types from various mouse tissues, using optimized immunostaining protocols and antibody validation controls, should help elucidate the role of this small GTPase in multiple physiological functions.

Restricted distribution of histidine-rich glycoprotein in human skeletal muscle

Histidine-rich glycoprotein (HRG), a circulating serum protein, is synthesized in liver parenchymal cells (Hulett and Parish 2000). Previously, Sabbatini and colleagues demonstrated an interaction between HRG and the rabbit skeletal muscle protein AMP deaminase (AMPD1) (Ranieri-Raggi et al. 1997, 2003). Moreover, this same group also provided the first evidence that HRG is present in human skeletal muscle using an antibody raised against human plasma HRG (Sabbatini et al. 1999). This same group has now expanded upon these earlier investigations by performing a confocal immunofluorescence evaluation of the subcellular

The first two papers described in this installment of "In focus in HCB" illustrate once more the importance of performing adequate controls for immunostaining, and using multiple antibodies and optimized immunostaining protocols to ensure the veracity of the staining results.

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distribution of HRG in human skeletal muscle, and comparing its distribution to that of AMPD1 (Mattii et al. 2017). Importantly, the immunostaining patterns observed were consistent using both self-made and commercially purchased anti-HRG antibodies. By performing double immunostaining with antibodies recognizing distinct skeletal muscle molecules, they determined that anti-HRG immunoreactivity specifically localized to the I-band region of the sarcomeres. This area was also immunostained with antibodies against actin, although the two proteins did not show evidence for colocalization. The staining pattern for HMG similarly localized with that for AMPD1 over the I-bands, though AMPD1 was also observed to be localized to the A-band region of the sarcomeres as well. Moreover, they also detected immunoreactivity for HMG over cell nuclei, as demonstrated by co-distribution with To-Pro in confocal z-stack projections. Further experiments will be necessary to clarify the function of nuclear HMG.

Deficiency of the membrane skeletal protein 4.1G is associated with hypermyelination

The membrane skeletal protein family member 4.1G is located in the Schmidt–Lanterman incisures (SLIs) and paranodes of the myelin-forming Schwann cells of the peripheral nervous system (PNS) (Ohno et al. 2006). Protein 4.1G is essential for the molecular targeting of membrane palmitoylated protein 6 (MPP6) (Terada et al. 2012) and cell adhesion molecule 4 (CADM4) (Ivanovic et al. 2012) in SLIs. Saitoh et al. (2017) report now that in 10-month-old $4.1G^{-/-}$ mice, the sciatic nerve internodes exhibited hypermyelination as shown by both transmission electron microscopy and a significantly different g-ratio. Aberrant paranode structures were also observed. These structural abnormalities were accompanied by spastic leg extension in the tail-suspension test (10 month old mice) and a slower motor conduction velocity (4 month old mice). In addition, evidence was presented that in $4.1G^{+/+}$ mice, the 4.1G protein transports the scaffold Lin7c protein to SLIs and paranodes. It is proposed that the 4.1G protein functions as a signal for the proper formation of myelin in the PNS.

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