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EDITORIAL

# In focus in HCB

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In this issue, we highlight the results and significance of two manuscripts involving mitogen-activated protein kinases (MAPKs): the first investigates damage of the keratinocyte barrier by activation of MAPKs, and the second provides new methodology for preserving the phosphorylation state of MAPKs prior to tissue fixation for subsequent immunohistochemical analysis. The third manuscript highlighted describes the role of the components of a specific membrane protein complex in the formation of myelin in the sciatic nerve of the mouse peripheral nervous system.

## Chronic stress influences keratinocyte barrier function

In a previous investigation, Ozaki et al. (2019) have studied the influence of cell culture serum components on the keratinization process of 3D cultures of murine epidermal keratinocytes. This elaborate cell culture system served as a model for the keratinization of the epithelium of the murine oral cavity, esophagus, and forestomach. They demonstrated that lowering the calcium concentration in the culture medium inhibited the keratinization in the 3D cultures, while the inhibition of retinoic acid receptor signaling reversed this effect. In the current work from this group of investigators (Nikaido et al. 2019), the effect of anisomycin treatment on calcium-induced tight junction formation and composition as well as paracellular permeability was studied in 2D and 3D mouse keratinocyte cultures. Anisomycin is an activator of the stress-activated mitogen-activated protein kinases JNK and p38. Both enzymes are known to be involved in the assembly and disassembly of tight junctions in keratinocytes (Siljamaki et al. 2014). In 2D cultures, calcium-induced tight junction formation and maturation was observed in a time-dependent manner. Short-term (12 h) anisomycin treatment inhibited the maturation from zipper-like to string-like zonula occludens-1 tight junctions. This effect was also observed under anisomycin treatment followed by sole p38 inhibition. However, under such a condition, sole JNK inhibition did not interfere with tight junction maturation. Long-term (2 weeks) anisomycin treatment of 3D cultures, which activated only p38, inhibited the flattening of the superficial cells and reduced the transepithelial electrical resistance by about 30%. In addition, the distribution and amount of claudin 4, 6, and 7 was differentially affected as shown in the cover image. It is suggested that activation of stress-activated MAPKs by various chronic stimuli may damage the structure and function of the keratinocyte barrier.

## Method development for improved phosphorylated MAPKs immunolocalization

Mitogen-activated protein kinases (MAPKs) are a diverse enzyme family involved in a myriad of cellular physiological events (Cargnello and Roux 2011). During activation/deactivation cycles, MAPKs are both phosphorylated by MAPK-kinases, as well as dephosphorylated by a variety of cellular phosphatases. This has led to much interest in assessing the phosphorylation state of MAPKs, and to the generation of numerous anti-MAPK antibodies to recognize phosphorylation-specific sites on these enzymes. Unfortunately, the phosphorylation status of the MAPKs may be compromised by a multitude of cellular physiological events occurring during tissue procurement, fixation, and processing. Employing a rat model of ocular hypertension resulting in MAPKs' phosphorylation in the optic nerve head, Mammone et al. (2019) have investigated tissue processing methods to improve upon the retention of phosphorylated MAPKs for subsequent immunohistochemical detection in various levels of disease pathology. They perfused the

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tissue samples with a cocktail of tissue-permeable protein phosphatase inhibitors (PIs; sodium orthovanadate, sodium molybdate, sodium tartrate, imidazole, and sodium fluoride) prior to chemical fixation in an attempt to attenuate loss of the phosphoproteins in the interval between termination of the experiment and fixation with Davidson's fixative (formaldehyde, ethanol, and glacial acetic acid), followed by paraffin embedding. Paraffin sections were then stained with a battery of antibodies, including multiple phospho-specific anti-MAPKs representing the family subgroups p42/44 MAPK, SAPK/JNK, and p38 MAPK. While PIs in the perfusate resulted in no apparent effect on immunostaining for phosphorylated MAPKs in sections from untreated optic nerve head, they reduced the variability of staining observed on sections from treated animals, particularly those with less extensive pathological damage. They also stained for phosphoproteins unrelated to MAPKs, and likewise found an improvement in the consistency of immunostaining results when PIs had been administered prior to fixation. Interestingly, the addition of PIs to the perfusate prior to fixation did not have an effect on phosphoprotein immunolabeling in the retina from these animals; the authors provide several explanations for these results. However, for those wishing to immunolabel phosphoproteins on tissue sections, it would be prudent to consider treating the tissue with a perfusate containing PIs prior to fixation, in an attempt to maximally stabilize the phosphorylation state of these proteins.

## Membrane protein factors regulating myelin formation

Terada's group has been studying the role of membrane protein complexes in the formation of myelin sheaths in mouse peripheral nervous system (Terada et al. 2016; Saitoh et al. 2017). In a recent publication (Saitoh et al. 2017) using 4.1G-deficient mice, these authors demonstrated that the 4.1G protein component of the skeletal molecular complex 4.1G-membrane palmitoylated protein 6 (MMP6)-Lin7-cell adhesion molecule 4 (CADM4) functions as a signaling molecule for the proper formation of Schwann cell-associated myelin. This group has now extended these results by examining the role of the MMP6-protein component of the molecular complex in myelin formation by creating MMP6-deficient mouse strains (Saitoh et al. 2019). They used CRISPR-Cas9 genome editing to create MMP6 mutant mouse strains with either a deletion near the N-terminus start codon (MMP6-N) or near the PDZ domain (MMP6-P). The mice were characterized by genotyping analysis, and the protein expression was determined by western blotting and immunohistochemistry using a multitude of commercially available antibodies. Furthermore, they used transmission electron microscopy combined with morphometric analysis to investigate the myelin structure in sciatic nerve tissue. Their results showed that (1) the absence of MMP6 protein in the MMP6-P mutant animals resulted in a decreased representation of Lin7 protein by both western blot and immunohistochemistry, suggesting that MMP6 is involved in its production and localization; (2) the Schmidt–Lanterman incisure regions of Schwann cells were increased in number, but lower in height in the MMP6-P animals compared to controls; and (3) nerve fiber diameter was wider in MMP6-P animals compared to controls. Taken together, these results suggest that the MMP6-Lin 7 membrane complex is involved in the regulation of myelin formation in mouse sciatic nerve.

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