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EDITORIAL

In focus in HCB

Douglas J. Taatjes[1] · Jürgen Roth[2]

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In this month's editorial we are pleased to highlight three Original Papers, describing a detailed morphometric analysis of the capillary network in lean and obese mouse gluteus maximus muscle, an analysis of the effect of commercially available culture media on some physiological and morphological characteristics of primary human endothelial cells, and a combined freeze-fracture immunoelectron microscopic investigation of the GPI-anchored SAG1 protein in the parasite *Toxoplasma gondii.* We hope you enjoy these brief synopses, and encourage you to read the full-length articles, as well as the additional interesting articles in this November issue.

Morphometric evaluation of muscle-associated capillaries in obese mice

The capillarity of skeletal muscle fibers has been shown to be altered in a variety of pathological conditions (see for instance Cebasek and Ribaric 2019; Taatjes and Roth 2019). In this regard, given the world-wide increase in obesity and concomitant type 2 diabetes, an investigation of the potential correlation between obesity and muscle capillarity is of great interest. Umek et al. (2019) have performed an extensive morphometric analysis of the capillary network of the mouse gluteus maximus muscle from lean healthy mice *versus* high-fat-diet (HFD)-induced insulin-resistant littermates. Thick (100 µm), transverse sections of muscle were stained with an anti-collagen IV antibody to visualize basement membranes, and with *Griffonia simplicifolia* lectin I to visualize capillary endothelial cells, followed by acquisition of 3D stacks of images by confocal microscopy. From these stacks of images, several morphometric parameters were determined, including muscle fiber diameter, surface area, and volume, capillary length related to muscle fiber length, surface area, and volume, the anisotropy index of the capillaries, and muscle fiber size (small or large). Myosin heavy chain expression in the myocytes was determined by immunohistochemical staining with isoform-specific antibodies. The results from these very detailed analyses demontrated that (1) capillarization of the gluteus maximus muscle was higher in obese mice compared to lean mice; (2) the capillary lengths related to muscle fiber length and volume were higher around small muscle fibers (< 40 µm) in obese mice compared to lean animals; and (3) increased capillarization around smaller muscle fibers, with a shift towards fast type myosin isoforms (type 2b fibers) in obese mice. Other morphometric parameters were not significantly different between obese and lean mice. The authors conclude that these results suggest that insulin-resistant obese mice display alterations in skeletal muscle capillarization and muscle fiber type, as a consequence of impaired glucose metabolism related to type 2 diabetes.

Is behavior related to culture?

Continuing on the theme of vascular-related research, we will now switch from the in vivo model just described, to the in vitro use of endothelial cell cultures. Endothelial cells lining the vasculature represent a heterogeneous family of cells, displaying a variety of phenotypes dependent upon their vascular and tissue anatomic origin (Minami et al. 2019; Trotman et al. 2011). They function in myriad physiological processes in both health and disease, including providing a barrier function, establishing vascular tone, assisting leukocyte transmigration, and angiogenesis. Thus, given their heterogeneity and importance in the above mentioned processes, they are often studied as primary cells in a culture model. Of crucial importance for the reproducibility of these

✉ Douglas J. Taatjes
douglas.taatjes@med.uvm.edu

[1] Department of Pathology and Laboratory Medicine, Larner College of Medicine, University of Vermont, Burlington, VT 05405, USA

[2] University of Zurich, 8091 Zurich, Switzerland

investigations is the composition of the culture medium, which is often purchased from commercial sources and contains a variety of growth-related components. Leopold et al. (2019) have now investigated the effects of the contents of culture media from seven different commercial sources on outgrowth, viability, angiogenesis, and phenotype on primary human endothelial cells derived from feto-placental chorionic arteries (fpEC). The purchased media could be divided into two specific catagories: (1) *undefined, complex media* which contained endothelial cell growth supplement, and a poorly defined supplement containing growth factors (for instance, extracts from bovine brain, pituitary, or hypothalamus); and (2) *defined medium* which contained specific concentrations of growth factors, such as epithelial growth factor (EGF), fibroblast growth factor (FGF2), vascular endothelial growth factor (VEGF), or insulin-like (IGF1) growth factor. Isolated fpEC cells were cultured in the seven different commercial media, then analyzed for the characteristics described above. FACS analysis demonstrated that surface markers for endothelial subtypes was not affected by the composition of the culture media. However, in contrast, the detailed results revealed that the optimum culture media for primary endothelial cells differed dependent upon the analysis performed; i.e., parameters such as colony outgrowth, proliferation, and angiogenesis were strongly affected by the media in which the cells were grown. This study clearly demonstrates the need to test a variety of commercially-available cell culture media, containing variable mixtures of supplements, to determine the optimum conditions for specific experimental requirements involving cultured primary endothelial cells.

Nanoscale analysis of GPI-anchored SAG1 in the plasma membrane of living *Toxoplasma gondii*

Freeze-fracture (FF) electron microscopy has revolutionized morphological membrane research (for a recent review see Meier and Beckmann 2018). In combination with immunogold labeling (Roth et al. 1978), the FF immunogold labeling (Fujimoto 1997) permits high resolution 2D analysis of membrane constituents in aldehyde-fixed samples. A major step forward for the supravital analysis at nanoscale resolution of membrane proteins, and in particular of membrane lipids, was the introduction of physical fixation by quick-freezing of samples prior to FF immunogold labeling (Fujita et al. 2007). By using their technique, Fujita and colleagues (Kurakawa et al. 2019) have analyzed the distribution pattern of GPI-anchored SAG1 in the plasma membrane of quick-frozen living *Toxoplasma gondii*. SAG1 is a major surface protein and may play a role in the invasion of host cells (Grimwood and Smith 1992). Immunogold labeling for SAG1 was observed in the E-face (exoplasmic leaflet) of the plasma membrane, but no significant labeling was detected in the P-face (cytoplasmic leaflet) of the plasma membrane, or in any leaflet of the inner membrane complex (see cover image). By using Ripley's K-function (Ripley 1977; Philimonenko et al. 2000) for point pattern analysis, the distribution of SAG1 labeling in the E-face of the plasma membrane was determined to be totally random. This result was confirmed by nearest neighbor distance analysis. In addition, the glycosphingolipids GM1 and GM3 were undetectable in the plasma membrane of *Toxoplasma gondii*, which was in contrast to their presence in the plasma membrane of mouse fibroblasts. Furthermore, when analyzed by quick-freezing-FF immunogold labeling, the major lipid raft components GM1 and GM3 occurred in clusters of < 100 nm in diameter in the exoplasmic leaflet of the plasma membrane of mouse fibroblasts (Fujita et al. 2007). The authors postulated that endogenous SAG1 does not associate with lipid rafts and that this results in a constitutively active state of SAG1 in the plasma membrane of *Toxoplasma gondii.*

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