In focus in HCB

Douglas J. Taatjes¹ · Jürgen Roth²

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In this October issue of the journal, we will highlight three original articles, all featuring the use of stem cells and describing (1) the use of a chimeric rat pituitary tissue culture model to trace the potential migratory capacity of S100β-GFP-expressing cells; (2) the role of early B-cell factor-1 (EBF1) in the commitment of mesenchymal stem cells into vascular pericytes; and (3) investigation into whether porcine ovarian putative stem cells have the ability to differentiate into cells of an endothelial lineage. We wish you good reading!

Cell migration from the intermediate to the anterior lobe of the pituitary gland as a source of endocrine cells

The pituitary gland consists of the anterior lobe (AL), which together with the intermediate lobe (IL) forms the adenohypophysis, and the posterior lobe, the so-called neurohypophysis (Larkin and Ansorge 2017). The adenohypophysis synthesizes and secretes a variety of functionally important hormones. It is known that non-endocrine cells expressing sex-determining region Y-box 2 (SOX2) and S100β proteins constitute tissue-resident stem/progenitor cells that are important for the maintenance of the AL functions. SOX2/S100β-positive cells exist in two different stem/progenitor niches: (i) the marginal cell layer (MCL), facing Rathke's cleft between the IL and AL side, and (ii) throughout the AL (Vankelecom and Chen 2014). In previous work, Horiguchi et al. (2018; 2021a) established that the tetraspanin family proteins CD9 and CD81 are specific markers for SOX2/S100β-positive cells in both niches and that the cells in the IL-side MCL are more

immature than those in the AL-side MCL. To establish the migratory pathway of the CD9/CD81/SOX2/S100β-positive cells to the AL, Horiguchi et al. (2021b) have now applied chimeric pituitary tissue culture using S100β/GFP-transgenic rats and Wistar rats, and traced the S100β/GFP-expressing cells. They observed that the S100β/GFP-expressing cells migrated from the IL to the AL, but not in the opposite direction. Furthermore, the authors established tetraspanin 1 as a specific marker for IL side-derived CD9/CD81/SOX2/S100β-positive cells. Following siRNA-mediated knockdown of this tetraspanin, cell migration and the expression of the epithelial–mesenchymal transition marker *slug* were impaired. Hence, it was concluded that CD9/CD81/SOX2/S100β-positive cells in the IL-side MCL can be stem/progenitor cells that provide stem/progenitor cells to the AL-side MCL via SLUG-mediated EMT and cell migration (Fig. 1).

✉ 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

Fig. 1 Schematic of the pituitary stem/progenitor cell niches and the now established migratory process. From Horiguchi et al. (2021a, 2021b)

EBF1 contributes to pericyte cell commitment

Early B-cell factor-1 (EBF1) is a transcription factor important for cell lineage specification and commitment during early maturation to different cell types including B-cells, adipocytes, osteoblasts and neurons (Garel et al. 1999; Lukin et al. 2008; Jimenez et al. 2007). Based on previous findings that EBF3 is expressed in medulloblastoma and EBF, as detected by immunohistochemistry using a pan-EBF antibody in newly formed vessels of this highly malignant tumor (Corno et al. 2021), Pagani et al. (2021), in a highly detailed investigation, analyzed the expression EBF1-4 family members in blood vessels of a whole spectrum of malignant tumors and in various non-tumorous tissues. In all investigated tissues, the authors found immunostaining for EBF1 in blood vessels, and could identify the EBF1-positive peri-endothelial cells as pericytes by double immunohistochemistry with marker antibodies against SMA, PDGFR β , CD146, and NG2 (Fig. 2).

Fig. 2 Double immunostaining for EBF1 (brown, nuclear) and SMA or PDGFR β (blue, cytoplasmic) in sections from human placenta. From Pagani et al. (2021)

The immunohistochemical findings were verified by RT-qPCR and FACS analysis. Further experimental validation was obtained through EBF1-silencing of human brain vascular pericytes, which resulted in a significant reduction in the expression of the pericyte marker proteins PDGFR β and CD146, and in the reduced expression of various cytokines produced during angiogenesis and by differentiating or activated pericytes. However, no change for CD90, a protein mostly expressed by mesenchymal cells, was observed following EBF1 knockdown. In other experiments using cultures of human brain vascular pericytes and placenta-derived pericytes, the authors obtained further evidence for the involvement of EBF1 in the commitment toward pericytes. In addition to establishing its putative functional role for pericytes, this work also establishes EBF1 as a novel marker for pericytes that can be used to unequivocally distinguish this cell type from others, in particular of mesenchymal stem cells, of the vascular unit.

Differentiation of porcine ovarian mesenchymal stem cells into endothelial cells

The use of xenogeneic cells as therapeutic agents in treating numerous human diseases and pathologies has gained much interest of late (Huang et al. 2021). In this regard, cells derived from various porcine tissues have been viewed as particularly appropriate given the overall similarity in size and physiology of porcine organs compared to those from humans. Indeed, in recent publications, Wartalski and colleagues have shown that porcine ovarian putative stem cells (poPSCs) derived from the ovary cortex from sexually immature pigs display multipotency (Wartalski et al. 2016), with the ability under precisely controlled culture conditions to differentiate into neural-like cells (Wartalski et al. 2020). Given the need for a source of endothelial cells for tissue engineering and human regenerative medicine, the porcine model, which as mentioned above has been used previously for many xenogeneic procedures (see also for instance Gasek et al. 2021), appeared to represent a potential source for these cells. This led Wartalski et al. (2021) to extend their recent studies to investigate whether the multipotent high-plasticity poPSCs could also be directed to differentiate into endothelial-lineage cells. They isolated poPSCs from porcine ovarian cortex using an immunomagnetic approach with anti-SSEA-4 conjugated to the magnetic beads. The cells cultured initially were found by immunofluorescence and RT-qPCR to express several markers of mesenchymal stem cells (CD29, CD90, CD105, *ITGB1*, *THY*, and *ENG*). Following cell expansion in culture, the poPSCs cells were cultured in a modified medium designed to induce an endothelial cell lineage containing vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF), epidermal growth factor (EGF), ascorbic acid, and heparin. After 14 days in this culture medium, the cells were analyzed by immunofluorescence, Western blot, RT-qPCR, and migration and tube-formation potential (Fig. 3).

Fig. 3 The tube formation assay shows that SSEA-4⁺ poPSCs differentiate into endothelium under the influence of bFGF 1. The dextran microbeads are visible by transmitted light illumination. From Wartalski et al. (2021)

Using porcine-derived aortic tissue as a positive control, the authors found (1) positive immunofluorescence and Western blot detection of the endothelial cell markers vascular endothelial cadherin (VECad) and vascular endothelial growth factor receptor-2 (VEGFR-2); (2) RT-qPCR detection of the expression of VECad and VEGFR-2 mRNA (*CDH5* and *KDR*, respectively); (3) positive chemotaxis response indicating migratory ability; and finally (4) the ability to develop tube-like networks indicative of potential capillary formation. Overall, their results have demonstrated that poPSCs isolated from porcine ovarian cortex can be considered a heterogeneous population of MSCs, which can be driven to differentiate into cells from at least two germ layers represented by neural and endothelial cells. Thus, this model should serve as a useful donor source of specific cell types for xenogeneic transplantation into humans.

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