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RESEARCH HIGHLIGHT

Vascular Procr$^+$ stem cells: Finding new branches while looking for the roots

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Generation and growth of the blood vasculature network is a highly synchronized process, requiring coordinated efforts of endothelial cells and pericytes to maintain blood vessel integrity and regeneration. In a recent paper published in *Cell Research*, Yu *et al*. identified and characterized bipotent Procr-expressing vascular endothelial stem cells, which give rise to both endothelial cells and pericytes.

The vasculature is a complex transport system, composed of a remarkable network of arteries, veins and connecting capillaries of the blood system that supply every organ with oxygen and nutrients. Blood vessels also regulate organ development, regeneration and stem cell behavior. Endothelial stem cells are highly plastic and can rapidly switch from long-term quiescence to active angiogenic growth upon metabolic and stress stimulations. Vascular growth and remodeling are vital for successful organ growth and play a major role in tissue repair. However, this dynamic property also holds dark sides as vascular sprouting provides survival and growth cues to allow tumor progression and metastasis spreading. Identifying the root origin of the vascular segments could potentially make it possible to aid in the development of therapies to promote vascular repair or to prevent blood vessel growth within tumors. During the last decade, much effort has been devoted toward isolating and characterizing the adult stem cells that give rise to the vascular endothelium. A growing body of evidence indicates that tissue-resident endothelial stem and progenitor cells are the major source of mature endothelial cells for vascular renewal and repair, over bone marrow-derived endothelial precursors [1]. The report by Yu *et al*. [2] provides strong evidence to support this concept and identifies protein C receptor-expressing (Procr, also known as endothelial protein C receptor — EPCR) endothelial stem cells as the apex of the bipotent lineage tree for both endothelial cells and pericytes, providing new understanding on the root of adult neovascularization in homeostasis and injury repair.

To identify vascular endothelial stem cells (VESCs), Yu and colleagues took advantage of the mammary gland which develops mostly in the postnatal stage. The authors demonstrate that Procr-expressing endothelial stem cells exhibit robust *in vitro* clonogenic potential and that their stemness capacities can be retained in culture. Importantly, when transplanted into an empty fat pad of pubertal recipients, Procr-expressing endothelial stem cells could functionally incorporate into an existing vessel and form new vessels in the mammary fat pads, demonstrating robust regenerative capacity of Procr$^+$ VESCs. The authors extend their observations and show that Procr is a common marker for VESCs in the vasculature of other adult tissues, including the skin and retina. Targeted ablation of Procr-expressing endothelial cells revealed the functional importance of Procr$^+$ VESCs for blood vessel development and regeneration during normal retinal development and in vascular restoration upon hind limb ischemic injury. To further verify the clonogenicity of Procr$^+$ endothelial cells *in vivo*, Yu *et al*. elegantly carried out lineage tracing experiments, revealing that Procr$^+$ endothelial cells not only significantly contribute to adult neovascularization, but unexpectedly, are bipotent stem cells which can also give rise to pericytes in normal development and homeostasis. To the best of our knowledge and understanding, this is the first documentation of an adult bipotent common vascular progenitor that gives rise to both endothelial and periendothelial cells. These results are in line with previous observations revealing a common bipotent embryonic vascular progenitor cell that differentiates into both endothelial and smooth muscle cells [3].

Procr has been identified as a stem cell marker also in various other tissues including long-term repopulating hematopoietic stem cells (LT-HSCs) in the fetal liver during embryo development [4] and adult bone marrow [5, 6], and more recently in mammary stem cells [7]. Procr has clear endothelial characteristics, and its ligation with the serine protease, activated protein C (aPC), induces classic PAR1-mediated anticoagulation with anti-apoptotic and anti-inflammatory activities, as well as promotes endothelial barrier protection and stabilization [8]. Current evidence reveals that the aPC/EPCR pathway plays an important role in both fetal hematopoietic stem cell survival [4] and adult LT-HSC retention in the bone marrow and protection from myelotoxic insult [9]. Protection from DNA damaging agents via aPC/EPCR/PAR1 signaling limits nitric oxide generation

and Cdc42 activity, leading to enhanced VLA4-mediated adhesion and stem cell retention to ensure preservation of the bone marrow stem cell pool [9]. Preservation of Procr expression among different stem cell types strongly implies that Procr provides common stem cell signals, which may preserve the 'stemness' phenotype (Figure 1).

The cancer stem cell paradigm refers to the ability of cancer cell subpopulation to initiate tumorigenesis by undergoing extensive self-renewal and differentiation [10]. It becomes clear that the high frequency of relapse predicts that cancer stem cells are resistant to standard therapy [11], therefore making strong claim that targeting the stem cell population will likely lead to improved patient outcomes. Procr was identified as a marker not only for healthy mammary stem cell population [7], but is also highly expressed by a cancer stem cell-like populations in aggressive human breast cancer cells [12]. Since cancer-initiating cells are the lineage tree origin for chemotherapy resistance and disease relapse, a better understanding of how a single Procr$^+$ stem cell remains dormant within its niches and how it is protected will broaden our knowledge of how to eliminate the hiding cancer stem cell.

In light of these important observations, the study by Yu *et al*. [2] opens up new horizons, but raises questions at the same time. Based on the bipotent potential of Procr$^+$ endothelial cells, what is the mechanism that triggers the endothelial regeneration process of Procr-expressing stem cells? What are the signals that enable coordinate differentiation to both endothelial and pericyte cell population? Whether resident Procr$^+$ stem cell pool becomes exhausted in disease states, aging and upon vascular injury remains to be determined. Differences in the gene expression profile of Procr$^+$ VESCs versus hematopoietic and mammary stem cells may contribute to our global understanding on 'stemness' identity and to further determination of their phenotypic properties. The study by Yu *et al*. may also have therapeutic implications as neovascularization holds the key to wound healing, and angiogenesis is a target for antitumor treatments. For example, does induction of Procr by Wnt signaling [7] or activation by its ligand aPC initiate vascular growth? On the other hand, could targeting Procr by neutralizing antibodies be beneficial in fighting cancer? Finding an answer to those potentially clinically-relevant questions might yield an exciting finding and a rewarding journey.

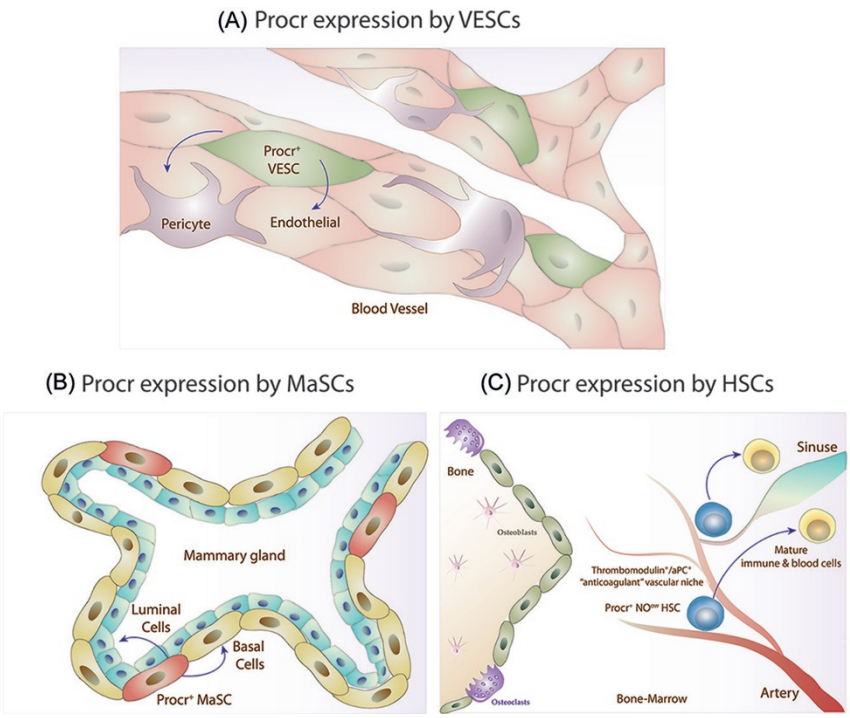

Figure 1 Procr is widely expressed by stem cells in various tissues. **(A)** Procr is expressed by vascular endothelial stem cells (VESCs). Procr$^+$ VESCs are bipotent, giving rise to endothelial cells and pericytes. **(B)** Procr is expressed by mammary stem cells (MaSCs), and can be differentiated into all lineages of the mammary epithelium including basal and luminal cells. **(C)** Procr (EPCR) is highly expressed by bone marrow hematopoietic stem cells (HSCs) located in a thrombomodulin- and aPC-enriched arterial microenvironment. EPCR signaling, manifested by aPC ligation, promotes stem cell retention in the bone marrow and protection from cytotoxic damage.

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References

1 Rinkevich Y, Lindau P, Ueno H, *et al*. *Nature* 2011; **476**:409-413.
2 Yu QC, Song W, Wang D, *et al*. *Cell Res* 2016; **26**:1079-1098.
3 Yamashita J, Itoh H, Hirashima M, *et al*. *Nature* 2000; **408**:92-96.
4 Iwasaki H, Arai F, Kubota Y, *et al*. *Blood* 2010; **116**:544-553.
5 Kent DG, Copley MR, Benz C, *et al*. *Blood* 2009; **113**:6342-6350.
6 Balazs AB, Fabian AJ, Esmon CT, *et al*. *Blood* 2006; **107**:2317-2321.
7 Wang D, Cai C, Dong X, *et al*. *Nature* 2015; **517**:81-84.
8 Esmon CT. *Semin Immunopathol* 2012; **34**:127-132.
9 Gur-Cohen S, Itkin T, Chakrabarty S, *et al*. *Nat Med* 2015; **21**:1307-1317.
10 Lapidot T, Sirard C, Vormoor J, *et al*. *Nature* 1994; **367**:645-648.
11 Ding L, Ley TJ, Larson DE, *et al*. *Nature* 2012; **481**:506-510.
12 Schaffner F, Yokota N, Carneiro-Lobo T, *et al*. *PLoS One* 2013; **8**:e61071.