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Perspective

From nerve to blood vessel: a new role of Olfm2 in smooth muscle differentiation from human embryonic stem cell-derived mesenchymal cells

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Vascular smooth muscle cell (SMC) differentiation is an important process in vasculogenesis and angiogenesis during embryonic development. The alterations in the differentiated state in SMCs contribute to a variety of major cardiovascular diseases such as atherosclerosis, hypertension, restenosis and vascular aneurysm[1-2]. A better understanding of the cellular and molecular mechanisms that control SMC differentiation is essential to help develop new approaches to both prevent and treat these diseases. Therefore, development of reliable and reproducible *in vitro* cellular models in order to study the differentiation mechanisms is important although it has been challenging because of intrinsic peculiarities of SMC.

SMCs originate from at least eight different progenitors during embryonic development including neural crest, proepicardium, mesothelium, splanchnic mesoderm, secondary heart field, mesoangioblasts, somites and various stem/progenitor cells[1]. SMC populations from different embryological origins are observed in different vessels as well as within the same vessel segments although showing sharp boundaries with no intermixing of cells from different lineages[3]. Importantly, SMCs from different origins are regulated differentially and can exhibit a wide range of different phenotypes. Even in adult organs, SMCs are not terminally differentiated because the cells may undergo phenotypic modulation in response to alterations in local environmental cues including growth factors/inhibitors, mechanical influences, inflammatory mediators, cell-cell and cell-matrix interactions[2].

SMC differentiation is a complex but poorly defined process although much progress has been made in identifying molecular mechanisms controlling the expression of SMC specific genes. Accumulating evidence has shown that a precisely coordinated molecular network orchestrates the SMC differentiation program involved in a range of signaling pathways including TGF-β, retinoid, extracellular matrix, Notch, reactive oxygen species, histone deacetylase and microRNA signaling[4].

Several *in vitro* model systems have been developed to mimic the SMC differentiation *in vivo* including using C3H10T1/2 cells, neural crest cells, A404, embryoid body and embryonic stem cells. Although these models have significantly contributed to our understanding of SMC differentiation, each of these models has its limitations. In addition, human embryonic stem cell can differentiate to both endothelial cell (EC) and SMC populations in the same differentiation conditions. Though the cells are excellent for *in vivo* neoangiogenesis and regeneration of blood vessels, they may not be ideal for precisely dissecting the molecular mechanism governing SMC differentiation because SMCs differentiated from embryonic stems cells are heterogenic and thus contain a mixed population.

We recently developed a novel *in vitro* model for TGF-β-induced SMC differentiation from human embryonic stem cell-derived mesenchymal cells (hES-MCs). hES-MCs, derived from H9 human embryonic stem cells, are natural SMC progenitors for mesoderm-derived SMCs that account for most of

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the vascular SMCs[1]. hES-MCs have the capacity to produce the three lineages associated with mesenchymal stem cells including osteogenic, chondrogenic and SMC lineages. We found that hES-MCs can be robustly differentiated to SMC phenotype upon TGF-β stimulation and exhibit a morphology resembling functional SMCs. hES-MCs have the potential to be used for tissue engineering for regeneration of human SMCs due to their mesodermal origin.

Interestingly, the nervous and the vascular systems share many common features including a similar and often overlapping anatomy characterized by highly branched and ramified layouts, and common signaling pathways. Many similarities can also be found at the cellular and even extend to the molecular levels. There is strong evidence for coordination between the two systems[5]. In some cases this coordination may be achieved by utilizing the same cues or signals, suggesting that common molecules may regulate the development of both nervous and vascular systems.

Olfactomedin 2 (Olfm2), first found in the frog olfactory neuroepithelium, belongs to the family of Olfactomedin domain-containing proteins consisting of at least 13 members in mammals. Olfm2 expression is developmentally regulated. Blockade of Olfm2 reduces eye size, hinders optic nerve extension, and disrupts anterior central nerve system and head development including neural crest cell-derived cartilaginous structures of the pharyngeal arches in zebrafish[6]. In humans, a R144Q substitution in Olfm2 protein is thought to be the disease-causing mutation in Japanese patients with open-angle glaucoma. Effects of Olfm2 on eye development in developing zebrafish appear to be related to Pax6 signaling[6]. Pax6 is a master transcriptional factor for eye development and functions. Importantly, Pax6 has been shown to physically interact with TGF-β, which contributes to maintaining functional status of eyes. These results suggest a possible role of Olfm2 in the TGF-β signaling cascade during early eye development. Our recent study has shown that Olfm2 plays a role in vascular development, especially in TGF-β-induced SMC differentiation[7].

Olfm2 is dramatically upregulated during TGF-β-induced SMC differentiation of hES-MCs. *Olfm2* knockdown suppresses TGF-β-induced expression of SMC markers while Olfm2 overexpression promotes the marker gene expression. Interestingly, TGF-β induces Olfm2 nuclear accumulation, consistent with our finding that Olfm2 is abundantly expressed in nuclei of SMC in normal human aorta. Olfm2 expression is Smad2/3-dependent. In addition, Olfm2 acts as a nuclear cofactor binding to serum response factor (SRF) to promote SRF/CArG box interaction, leading to an enhanced transcription and expression of SMC marker genes. Olfm2 promotes SRF binding to SMC marker promoters through inhibiting the expression of HERP1 (Hrt2, Hey2, Hesr2, and CHF1) and thus attenuating the SRF association with HERP1, which is a downstream target of Notch signaling and a transcriptional repressor involved in SMC differentiation[8-9]. Our study indicates that the homeostatic balance between Olfm2 and HERP1 expression may be one of the factors that determine whether or not SMC marker genes can be effectively induced by TGF-β after the initial phase of SMC differentiation.

Interestingly, in addition to SMC differentiation, both SRF and HERP1 are also essential for nerve cell differentiation[10]. Our study identifies Olfm2 as a novel contributor that can regulate both processes. Further in-depth analysis of the Olfm2-SRF-HERP1 axis may provide new insights into the molecular networks coordinately regulating the neural and vascular development during embryogenesis. Moreover, identification of additional new factors that regulate both nervous and vascular systems is likely to unravel additional common mechanisms underlying the unique interaction between nerve and blood vessel.

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