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Commentary

Emerging Role of VEGFC in Pathological Angiogenesis

Nao Nagai, Takashi Minami

Div. phenotype diseases analysis, IRDA, Kumamoto University, Kumamoto, Japan

Retinal neovascularization is accompanied by eye diseases such as macular degeneration and diabetic retinopathy. Over-sustained or inappropriate endothelial cells (ECs) activation, and dysregulation of the vascular network may cause severe retinal detachment and glaucoma, finally leading to blindness. Blindness caused by such age-related diseases is on the rise (Bourne et al., 2013), and should be an obstacle to overcome in the days ahead for eye health. In spite of the well-established role of VEGFA in pathological neovascularization, in this issue of *EBioMedicine*, Singh et al. revealed that another isoform of VEGF, VEGFC, is associated with retinal neovascularization (Singh et al., 2015).

Angiogenesis is a vascular formation process in which ECs sprout from the pre-existing blood vessels observed during development and wound healing, and conceptually separated from de novo vessel formation followed by EC differentiation from their precursors (vasculogenesis). However, angiogenesis may occur in pathological states (such as cancer), and promotes diseases—of which the best-known stimulatory axis is VEGFA and its receptor VEGFR2. In contrast, VEGFC-VEGFR3 axis is widely recognized as a promoter of lymph-angiogenesis in adult in spite of its angiogenic role in early development (Adams and Alitalo, 2007; Dumont et al., 1998).

With a more detailed cellular mechanism of angiogenesis, the "Tip-stalk" hypothesis has been proposed. In this concept, some ECs are selected as tip cells, characterized by high motility and low proliferation, to sprout in response to the chemo-attractant such as VEGFA. In contrast, stalk cells characterized by high proliferation and low motility, proliferate and elongate to form new blood vessels (Siekmann et al., 2013). Tip and stalk cells signal via the DLL4-NOTCH axis: DLL4 expression is up-regulated in the tip cells in response to VEGF and subsequently DLL4 activates NOTCH signaling in the surrounding stalk ECs to suppress the tip phenotype (Blanco and Gerhardt, 2013).

In this issue of *EBioMedicine*, Singh and colleagues show that VEGFC, popular as a regulator of lymphangiogenesis, promoted proliferation, migration and angiogenesis in hypoxic retinal ECs. Further, the authors illustrated the intra-cellular signaling pathway regulating the VEGFC-induced angiogenesis: 1) VEGFC stimulation causes phosphorylation of p38MAPK, 2) phosphorylated p38MAPK subsequently phosphorylates transcription factor CREB, and 3) CREB upregulates DLL4 and NOTCH1, which activates tip cells formation and consequently angiogenesis.

The authors firstly found that hypoxia, a major inducer of retinal neovascularization, induced the expression of VEGFC as well as VEGFA and VEGFB in cultured human retinal micro vascular endothelial cells. VEGFC indeed stimulated proliferation, migration and angiogenesis in vitro. They also found that VEGFC stimulation caused phosphorylation of CREB, previously reported to be associated with intra-tumor angiogenesis following the EGR3 expression (Suehiro et al., 2010), and demonstrated the necessity of CREB downstream of VEGFC using a dominant-negative form of CREB. The importance of VEGFC and CREB was further observed in oxygen-induced ischemic retinopathy (OIR) mouse model by using gene targeting technique with siRNAs.

Given that VEGFC was reported to activate NOTCH signaling via VEGFR3 during angiogenesis, Singh et al. next investigated whether CREB activation upregulated the NOTCH signaling pathway. They observed VEGFC-induced upregulation of DLL4, a NOTCH ligand, and activated form of NOTCH1 without upregulation of the other NOTCH-associated molecules. The activation of DLL4-NOTCH1 was again CREB-dependent; they identified a CREB-binding site at −193 bp upstream of the DLL4 promoter. In addition to the OIR mouse model, they also used drug-inducible EC-specific CREB targeted mouse to show the requirement of NOTCH signaling.

They lastly explored the kinase responsible for phosphorylating CREB. Between two kinases phosphorylated by VEGFC stimulation, p38MAPK was elegantly demonstrated to be the responsible kinase for VEGFC-induced CREB phosphorylation, since introduction of dominant-negative form of p38MAPK, not that of JNK1, canceled CREB phosphorylation and the subsequent upregulation of DLL4/NOTCH1. Phosphorylation of p38MAPK was indeed detected in hypoxic retina in OIC model. Taken together, this in vitro and in vivo evidence points to a model that VEGFC-induced p38MAPK-CREB-DLL4/NOTCH1 axis is strongly associated with retinal neovascularization.

The authors' work promotes some further questions. Firstly, how much VEGFC contributes to retinal neovascularization compared to conventionally well-reported VEGFA? While many papers have mentioned the impact of DLL4-NOTCH signaling as being downstream of VEGFA, interestingly, the authors found that VEGFA cannot induce expression of DLL4 and NOTCH1, despite the fact that VEGFA caused phosphorylation of CREB. Related to this question, it is unclear which receptor is the partner of VEGFC in the system described in this paper, given that VEGFC induces dimerization of VEGFR2 and VEGFR3 as well as the canonical VEGFR3 homodimer in the context of angiogenesis (Nilsson et al., 2010). In addition to the intercellular signaling via VEGFC, it will be of great interest to analyze whether VEGFR2- or VEGFR3-mediated phosphorylation cascades are dominant in retinal endothelial cells, for the future progression of this work.

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VEGFC is now increasingly implied as an angiogenic factor not only in developmental, but in pathological angiogenesis (Tammela et al., 2008). Now, Singh and colleagues contribute new evidence of VEGFC-induced pathological angiogenesis and have further clarified a signaling pathway critical for VEGFC-induced retinal angiogenesis, i.e. the p38MAPK-CREB-DLL4/NOTCH1 axis. This may greatly contribute to the development of novel strategies against retinal angiogenesis.

Conflict of Interest

The authors declare no conflicts of interest.

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