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Grant Title: Functional Analysis Of Endothelial Heparan Sulfate In Angiogenesis In Vivo

Angiogenesis is a process of forming new blood vessels and is essential for development, tissue repair and pathological processes such as tumorigenesis. Understanding the mechanisms that control angiogenesis is essential for development of new therapeutics to treat angiogenesis-related human diseases. Heparan sulfate (HS) is abundantly expressed in developing and mature vasculature. Biochemical and *in vitro* cell-based studies have shown that HS interacts with both proangiogenic and anti-angiogenic factors, raising the question - what is the function of HS in angiogenesis *in vivo*? Examination of HS mutant mice has demonstrated that HS is cell-autonomously required for mural cell (MC) recruitment in angiogenesis and the enhancement of MC recruitment function correlates with the facilitation of both platelet-derived growth factor-B (PDGF-B) and transforming growth factor- β (TGF- β) signaling. However, the *in vivo* role of HS expressed by endothelial cells (EC), the leading and central cell type in angiogenesis in development, remains largely unknown (1, 2). In very recent studies, we examined EC-specific *N-deacetylase/N-sulfatase-1* knockout (*Ndst1*^{ECKO}) mice in which the N-, 2- and 6-O-sulfation of EC-HS is reduced by 20-60%, and observed that the mutant mice developed a vascular branch defect uniquely localized in diaphragm, thus documenting the first *in vivo* evidence showing that EC-HS functions to facilitate developmental angiogenesis (3). Our molecular mechanism studies uncovered that the EC-*Ndst1* ablation uniquely disrupts the Slit3-Robo4 signaling, a novel angiogenic pathway which we recently elucidated, that leads to the localized diaphragm vascular development defect in the *Ndst1*^{ECKO} mice (3, 4). Taken together, these studies established that EC-HS essentially facilitates Slit3-Robo4 signaling in developmental angiogenesis in diaphragm. However, the role of EC-HS in angiogenesis in other organs remains unknown. Since the *Ndst1* ablation only partially reduces EC-HS sulfation modification, we postulate that the residual EC-HS structure is functionally sufficient to sustain the angiogenic function of EC-HS in non-diaphragm tissue/organs and this was tested in current study. We generated EC-specific *Ext1* knockout (*Ext1*^{ECKO}) mice in which the expression of EC-HS is completely abolished. The *Ext1*^{ECKO} mice exhibit profound and severe developmental angiogenesis defects, including disruptions of EC migration and MC attachment in vasculature, and are embryonic lethal, showing that EC-HS is essentially required for developmental angiogenesis in various organs. Meanwhile, the much severe developmental angiogenesis defects, compared to the *Slit3* knockout (*Slit3*^{-/-}) and *Robo4* knockout (*Robo4*^{-/-}) mice, also suggest that EC-HS modulates other angiogenic signaling to essentially promote developmental angiogenesis. Mouse phenotype further observed that EC-HS deficiency leads to excessive vessel regression while lymph angiogenesis appears normal. Now we are further testing if EC-HS interacts with key angiogenic signaling, such as VEGF and PDGF signaling, to facilitate vascular development. We anticipate that our further studies will yield significant new insights into mechanisms that regulate angiogenesis and may also open a new direction to develop the urgently needed therapeutics that block multiple-angiogenic signaling by targeting only HS to effectively treat angiogenesis-related human diseases.

Reference:

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