Identification and Characterization of Endothelial Specific Genes

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ABSTRACT

Cardiovascular development and its proper function are essential for the development and survival of animals, while malformation of vasculature leads to a variety of diseases. The significance of vasculature during development and in adulthood has been delineated by investigating the functions of genes expressed in the vasculature. Endothelial cells lining the lumen of vessel tubes with a single layer, had long been considered inert, homogeneous cells. However, molecular and genetic studies have provided numerous pieces of evidence, which indicate that endothelial cells are active, dynamic, heterogeneous cells. Among these studies, molecular differences between arterial and venous endothelial cells were first revealed by the observation that *ephrin-B2* and its cognate receptor *EphB4* are restrictively expressed in arterial and venous endothelial cells, respectively. These genes are not only molecular markers of arteries and veins, but they also play essential roles in cardiovascular development.

To investigate whether the molecular difference between arteries and veins persists into adulthood, I analyzed *ephrin-B2* expression in adult tissues including pathological settings. These data indicate that the molecular distinction is maintained in adults, and *ephrin-B2* further distinguishes arterial smooth muscle cells from venous smooth muscle cells in adults.

Ephrin-B2 was serendipitously identified as an arterial marker; therefore, I performed a systematic screen to isolate novel arterial- and venous-specific genes, whose identification and characterization might improve current understanding of vascular biology. Through this screen, I isolated several novel arterial-restricted genes, and one of these genes, *Depp* (decidual protein induced by progesterone), was characterized in detail

by generating a knockout of the *Depp* locus. Although the homozygous mutant mice appear phenotypically normal, the detailed analysis of *Depp* expression reveals the heterogeneity of arterial endothelial cells from the early stage of vascular development.

I identified another novel gene, *D1.1*, through the screen; however, *D1.1* is expressed in both arterial and venous endothelial cells. The fact that *D1.1* is specifically expressed in endothelial cells and encodes a predicted transmembrane protein, prompted me to characterize *D1.1* in detail using a *tau-LacZ* knock-in to the *D1.1* locus. The data from the expression analysis suggest *D1.1* as a novel marker of adult neovasculature. In addition, the data using a soluble D1.1-Fc fusion protein in several different acute assays suggest that D1.1 may play a functional role in angiogenesis that is compensated in vivo by other, structurally distinct proteins.

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