BMP-9 inhibits proliferation of lymphatic endothelial cells and reprograms them to blood vascular endothelial cells through downregulation of Prox1 expression

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Members of bone morphogenetic protein (BMP) family have been implicated in the formation of blood vessels. While members of BMP-2/4 and osteogenic protein-1 groups signal via activin receptor-like kinases (ALK)-2, -3, -6, BMP-9/10 signal via ALK-1. Of note, since circulating BMP-9 proteins in peripheral blood has been reported to be abundantly present and ALK-1 is one of the responsible genes for a human hereditary vascular disorder, hereditary hemorrhagic telangiectasia, the physiological roles of BMP-9/ALK-1 signals in the formation of vascular systems are intriguing. We have previously reported that BMP-9/ALK-1 signals enhance the proliferation of blood vascular endothelial cells (BECs). However, the roles of BMP-9/ALK-1 signals in lymphatic vessel formation remain largely unknown. Here we examined the effects of BMP-9/ALK-1 signals on lymphangiogenesis both in vitro and in vivo. BMP-9 significantly inhibited the proliferation of human dermal lymphatic endothelial cells (HDLECs) in vitro as well as mouse embryonic stem (ES) cell-derived lymphatic endothelial cells. BMP-9 increased the subpopulation of HDLECs which underwent apoptosis. Importantly, we found that BMP-9 decreased the expression of Prox1, a transcription factor critical for the differentiation and maintenance of LECs, concomitantly with decreased expression of lymphatic-specific genes such as VEGFR3 and with increased expression of blood vascular-specific genes such as VEGFR2. Microarray-based gene set enrichment analysis (GSEA) of differential gene expression between samples from HDLECs treated with BMP-9 and those treated with siRNA for Prox1 statistically demonstrated that LECs acquired blood vascular phenotype by BMP-9. In order to study the in vivo functions of BMP-9, we used multiple types of human cancer xenograft models. We observed the decreased formation of lymphatic vessels and the increased formation of blood vessels in tumors derived from tumor cells lentivirally transduced with BMP-9 as compared with those from control tumor cells. Furthermore, we observed that BMP-9 inhibited inflammation-induced lymphangiogegensis in the corneas and diaphragms of immunocompetent mice. Taken together, these results suggest that BMP-9 inhibits lymphangiogenesis both in vitro and in vivo through down-regulation of Prox1 expression, which leads to reprogramming of LECs to obtain BEC phenotypes.