The function of the 130kDa MLCK in regulating in vivo vascular permeability and angiogenesis.

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Disruption of endothelial integrity is an essential component of vascular inflammation, angiogenesis, atherosclerosis, and tumor metastasis. Many studies have shown that activation of myosin light chain kinase (MLCK) in endothelial cell is correlated with increase in vascular permeability. Currently, most research in endothelial cells has focused on the 220kDa MLCK isoform which is the predominant isoform present in cultured endothelial cells. However, in freshly isolated uncultured endothelial cells, the 130kDa MLCK predominates. Yet nothing is known about the roles of the 130kDa MLCK isoform in endothelial cells. Therefore, our goal is to determine the role of the 130kDa MLCK in regulating vascular permeability and angiogenesis in vivo. To do this we will generate an endothelial cell-specific 130kDa MLCK knockout mice. As transcripts encoding the 130 and 220kDa MLCK isoforms are produced by independent promoters within the same *mylk1* gene, I will selectively knockout the 130kDa MLCK by deleting unique cis-acting gene regulatory elements required for the expression of this transcript. A key element identified within the intron following the first exon of the 130kDa MLCK transcript has been flanked by LoxP sites such that Cre recombinase (Cre) mediated recombination will delete the element and attenuate expression of the 130kDa MLCK. By crossing these floxed mice with Tie2-Cre mice which express Cre specifically in endothelial cells, I will obtain endothelial cell-specific 130kDa MLCK knockout mice. In vivo vascular permeability and angiogenesis assays on these mice will allow me to determine the role played by the 130kDa MLCK in these processes. This study will not only help to identify specific functions of the 130kDa MLCK isoform, but also determine if this is a drug target for developing novel treatments of vascular diseases and cancer.