Delineating the transcriptional regulators of *KLF2* using enhancer analysis

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The development of a functional vascular system is critically dependent on shear stress, a hemodynamic force exerted onto the vascular endothelium by flowing blood. Although one of the key effects of shear stress-mediated signal transduction is modulation of gene expression, our understanding of transcriptional networks governing endothelial responses to shear stress remains incomplete.

Krüppel-like factor 2 (KLF2) is the central transcriptional regulator of the endothelial shear stress response. The expression of *KLF2* gene is robustly upregulated after the onset of shear stress in arterial endothelial cells and endocardial cushions, both areas of high shear stress. Both global and endothelial-specific *Klf2* deletion result in embryonic lethality between E12.5 and E14.5 due to vascular maturation defects leading to embryonic heart failure. Despite the crucial role in the development of a functional endothelium, the mechanisms by which shear stress signalling regulates *KLF2* expression remain controversial. Activation of *KLF2* by shear stress was previously hypothesised to occur through a MEK5-ERK5-MEF2 pathway thought to interact directly with the *KLF2* promoter, thereby inducing *KLF2* expression. However, KLF2 is expressed in multiple cell types and the promoter is common to all, suggesting cell type-specific *KLF2* expression might be regulated by yet uninvestigated distal enhancers. Further, MEF2 factors are not only widely expressed beyond the vasculature where they control differentiation of many cell types, but also regulate the expression of endothelial genes independent of shear stress in different settings, suggesting additional transcriptional regulators may interact with MEF2 to induce context-specific *KLF2* expression.

Here we show that endothelial *KLF2* is transcriptionally regulated by two distal enhancers independently of its core promoter during development. Each *KLF2* enhancer directs a distinct pattern of endothelial-specific reporter gene expression corresponding to expression of the endogenous *KLF2*. Manipulation of blood flow *in vivo* suggest that reporter gene expression driven by these enhancers changes in response to shear stress. Phylogenetic foot printing combined with mutational analysis of both *KLF2* enhancers has identified a functional MEF2 binding motif alongside a number of other conserved transcription factor binding motifs essential for enhancer-driven reporter expression. This work demonstrates previously unappreciated complexity in the transcriptional regulation of *KLF2*, and provides an opportunity to better understand transcriptional dynamics at the locus of this disease-relevant gene.