

THYMOSIN β 4 – A NOVEL REGULATOR OF LOW DENSITY LIPOPROTEIN RECEPTOR RELATED PROTEIN 1 (LRP1) IN VASCULAR DISEASE

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Introduction

Vascular diseases, like atherosclerosis and aneurysm, are a leading cause of morbidity and mortality. GWAS associated genetic variants of Low density lipoprotein receptor related protein 1 (LRP1), an endocytic regulator of PDGFR β signalling in vascular smooth muscle cells (VSMCs), with risk of abdominal aortic aneurysm (AAA), carotid and coronary artery disease. Endothelial damage triggers the release of growth factors, such as PDGF-BB, that induce VSMC contractile-synthetic phenotypic switching, to facilitate proliferation and migration. Thymosin β 4 (T β 4) is an actin monomer binding peptide and plays an important role in smooth muscle differentiation in the coronary, yolk sac and systemic vasculature.

Rationale

As a regulator of embryonic VSMC differentiation, we hypothesised that T β 4 may additionally function to maintain healthy vasculature postnatally and protect against disease.

Methodology and Results

Despite severe embryonic defects, most T β 4KO mice compensate and survive to adulthood; however, they display aortic VSMC phenotypic switching, elastin degradation and dilatation. We identified a putative interaction between T β 4 and LRP1 and validated close association by proximity ligation assay within medial VSMCs. We demonstrated predisposition to vascular disease in global and VSMC-specific T β 4KO mouse models of AAA (1mg/kg/day Angiotensin II) and atherosclerosis (ApoE^{-/-} hypercholesterolaemia), confirming that they closely phenocopy LRP1 mutants. Inflammation, ECM composition and elastin were analysed, alongside VSMC phenotype and signalling. T β 4KO displayed increased susceptibility to AAA, ranging from aortic dilatation to medial dissection and rupture in severe cases in <5 days. Similarly, in the aortic root and descending aorta, T β 4KO; ApoE^{-/-} developed more unstable atherosclerotic plaques, characterised by enhanced contractile-synthetic VSMC switching and dysregulated LRP1/PDGFR β signalling. *In vitro*, VSMCs lacking T β 4 were more sensitive to PDGF-BB, with downstream signalling enhanced in magnitude and more sustained in duration, consistent with augmented proliferation. Surface biotinylation assays in T β 4KO VSMCs and tracking the receptor complex through successive endocytic compartments confirmed altered trafficking, with increased recycling of LRP1-PDGFR β and reduced lysosomal targeting in the absence of T β 4. Finally, we evaluated the potential of exogenous T β 4 to protect against aortic disease. In the AngII AAA model, T β 4 treatment significantly reduced aortic dilatation and rupture and preserved VSMC phenotype and elastin integrity, associated with normalisation of PDGFR β signalling. While the initial inflammatory response to AngII was unaltered by T β 4, adventitial macrophage content post-VSMC dedifferentiation and apoptosis was diminished.

Conclusions

We identify T β 4 as a key regulator of LRP1-PDGFR β signalling, for maintaining vascular health. T β 4 may emerge as a promising candidate for treatment of vascular disease.