TRISTETRAPROLIN POST-TRANSCRIPTIONALLY REGULATES TISSUE FACTOR EXPRESSION IN PRIMARY HUMAN AND MURINE MACROPHAGES

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Background Although monocyte-derived tissue factor (TF) plays critical roles in atherothrombosis, little is known about its post-transcriptional regulation. Tristetraprolin (TTP) binds the 3′UTR of target mRNAs and promotes their degradation, with its function being negatively regulated by p38 MAPK. Whether TTP post-transcriptionally regulates TF is unknown.

Methods We used human monocytes and bone marrow derived macrophages from TTP+/+ and TTP−/− mice. Procoagulant activity was determined using a clot turbimetric assay. mRNA decay was determined following transcriptional arrest using actinomycin D. TTP knockdown was achieved using siRNA transfection. RNA and protein interaction was determined using ribonucleoprotein (RNP) immunoprecipitation and RNA biotin pulldown assays.

Results p38 inhibition with SB203580 and SB202190 (1μM) reduced procoagulant activity, TF mRNA and protein expression in human macrophages (p<0.05). p38 inhibition reduced TF mRNA stability in both human and murine macrophages (p<0.05). TTP knockdown increased TF expression in human macrophages (p<0.05). Both TF mRNA and protein expression were significantly increased in TTP−/− versus TTP+/+ macrophages (p<0.05). Moreover TF mRNA decay was reduced in TTP−/− macrophages and p38 inhibition had no effect on this (p<0.01). RNP immunoprecipitation demonstrated TTP and TF mRNA interaction. Furthermore, a more specific interaction with TTP and TF 3′UTR was confirmed using RNA biotin pulldown techniques.

Conclusions These data provide evidence for the first time, that p38 and TTP post-transcriptionally regulate TF expression in macrophages. A better understanding of the post-transcriptional regulation of TF expression will provide novel insights into the interface between inflammation and thrombosis in vascular biology, and holds therapeutic potential.

TISSUE TRAIL DRIVES PULMONARY VASCULAR REMODELING AND ITS INHIBITION REVERSES EXPERIMENTAL PULMONARY ARTERIAL HYPERTENSION

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Background and aims Pulmonary arterial hypertension (PAH) is a fatal disease characterised by progressive narrowing of pulmonary arterioles, driven by aberrant cellular proliferation. Identifying key pathways in disease pathogenesis is required for the development of new-targeted therapies.

We have previously reported Tumour Necrosis Factor (TNF)-Related Apoptosis-Inducing Ligand (TRAIL) immunoreactivity within pulmonary vascular lesions from patients with idiopathic PAH and animal models. Since TRAIL induces endothelial cell apoptosis and smooth muscle cell proliferation, we hypothesised that TRAIL is an important driver of disease in PAH.

Methods Using the Paigen diet-fed ApoE−/− murine model, we first tested whether genetic deletion (ApoE−/−/TRAIRL−/−) and/or anti-TRAIL antibody treatment could modulate disease progression. Bone marrow transplantation (BMT) from ApoE−/− into sub lethally irradiated ApoE−/−/TRAIRL−/− mice, and vice versa was performed. Phenotyping included cardiac catheterisation (Right Ventricular Systolic Pressure) and immunohistological analyses of excised lung tissue.

Results ApoE−/−/TRAIRL−/− mice were protected from developing PAH (RVSP 23 mmHg v. 50 mmHg, P<0.001, n=6). Anti-TRAIL antibody treatment of ApoE−/− mice with established disease reversed PAH (RVSP 27 mmHg v. 83 mmHg, P<0.05, n=4). Blocking TRAIL significantly decreased cellular proliferation and increased apoptosis within pulmonary arterioles.

In chimaeras, only mice with expression of TRAIL restricted to tissue developed significant PAH (Mean RVSP 47 mmHg v. 26 mmHg, P<0.01, n=4–6). Mice with TRAIL only expressed by bone marrow derived cells showed no significant signs of PAH.

Conclusions Our studies are the first to determine the importance of TRAIL in the pathogenesis of PAH and demonstrate its potential for translation into a novel therapeutic target.