TRIB3 DEFICIENCY INCREASES PLAQUE STABILITY BY ALTERING MACROPHAGE FUNCTION

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Background Metabolic dysfunction and chronic inflammation under obesity are key factors promoting atherosclerosis. Macrophages are the major immune cell type found in atherosclerotic lesions, where plaque macrophages engulf oxidised lipids forming foam cells and also regulate plaque stability. Tribbles (Trib) 3 is a pseudokinase known to be involved in the regulation of adipose homeostasis, lipid and glucose metabolism and macrophage lipid uptake. We propose that Trib3 has a role in regulating metabolic and immune responses to obesity ultimately affecting atherosclerosis development.

Methodology. Trib3KO and Trib3WT mice were injected with rAAV8/mPCSK9 and fed with western diet for 12 weeks to promote atherosclerosis development. Atherosclerotic plaques were quantified using Oil-red-O and aortic root lesions were characterised histologically. Primary macrophages from bone marrow (BMDMs) were isolated from these strains and human monocyte derived macrophages (MDMs) were used to further characterise the effects of Trib3 regulating macrophage function.

Results En face analysis of the aortae showed no statistical differences in atheroma burden, while histological examination of the plaques indicated an increase in plaque stability in the absence of Trib3. Trib3KO BMDMs showed differential expression of genes involved in macrophage metabolism and regulation of the cytoskeleton. The differential expression of these genes was assessed in human MDMs following control or siTRIB3 treatment.

Conclusion These results suggest a role for Trib3 in the regulation of macrophage function promoting plaque stability, and may also have an impact on atherosclerosis development.

CONFLICT OF INTEREST None

ASYMMENTRIC DIMETHYLARGININE INCREASES CALCIUM SENSING RECEPTOR SIGNALLING TO PROMOTE ENDOTHELIAL NO PRODUCTION

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Objective The post-translational methylation of arginine residues by PRMT enzymes can yield asymmetric dimethylarginine (ADMA); which, following proteolysis is released into the cytosol and enters the circulation. Elevated plasma ADMA concentrations are recognised as a risk factor for cardiovascular disease including hypertension, atherosclerosis and stroke. In part, this is due to ADMA acting as a competitive inhibitor of the NOS enzymes reducing NO production and contributing to endothelial dysfunction. However, recently we have identified the Calcium sensing receptor (CaSR) as a target for physiological concentrations of ADMA. CaSR is a GPCR which binds both Ca2+ and amino acids, and has been shown to positively modulate NO production in endothelial cells.
Our objective was to determine whether physiological concentrations of ADMA had a positive effect on NO production via CaSR stimulation.

Methods Inducible CaSR overexpressing HEK293 cells (Hek-CaSR) were generated using the FLP-IN T-REX transfection method. Intracellular Ca2+ mobilisation was assessed using the Ca2+ sensitive dye CAL520, while cAMP accumulation was measured using an ELISA kit. CaSR was stimulated by Gd3+ in the presence of 10µM ADMA. ADMA-CaSR signalling in ECs was investigated using human lung microvascular ECs (HuLMVECs). NO production was assessed using the fluorescent sensor DAF-AM. The CaSR antagonist NPS-2143 was used to show specificity to CaSR signalling.

Results Gd3+ releases stored intracellular Ca2+ and suppresses cAMP production in a dose dependent manner in HEK-CaSR cells. Incubation with 10µM ADMA for 1 hour prior to Gd3+ stimulation was found to sensitise CaSR and shift the dose-response curve for Ca2+ mobilisation leftward (CTRL EC50 0.16mM, ADMA EC50 0.05mM p=0.01). ADMA pre-treatment suppressed forskolin induced cAMP production to 70% of the maximal response. Ca2+ stimulation of HuLMVECs releases intracellular Ca2+ in a dose dependent manner, in the presence of 10µM ADMA maximal fluorescence is increased with no change to the EC50. Given cytosolic Ca2+ release increases NO activity we determined whether low concentrations of ADMA (10µM) promoted NO production. At maximal CaSR stimulation ADMA increased NO production by 40% (p=0.04). Consistent with previous reports higher concentrations of ADMA (100µM) blocked NO production through inhibition of NOS. The action of ADMA was blocked by the addition of the CaSR inhibitor NPS-2143.

Conclusion Physiological concentrations of ADMA increase CaSR-mediated intracellular Ca2+ release and increase CaSR-mediated suppression of cAMP synthesis. In ECs, physiological concentrations of ADMA increase Ca2+ stimulated NO synthesis. In contrast pathophysiological concentrations directly inhibit EC NO synthase. These data suggest that physiological concentrations of ADMA positively regulate vascular reactivity while high concentrations of ADMA seen in cardiovascular disease reduce vascular function through NO blockade.

Conflict of interest None