presumably through its involvement in Hedgehog signalling. Ongoing analysis will further define the mechanisms through which HHIPL1 contributes to atherosclerosis.

B DKK3 STABILISES ATHEROSCLEROTIC PLAQUE VIA PROMOTING VASCULAR PROGENITOR AND FIBROBLAST DIFFERENTIATION TO SMOOTH MUSCLE CELLS

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Atherosclerosis, a chronic condition that can be converted into an acute clinical event caused by plaque rupture and thrombosis, has been the primary cause of mortality and morbidity worldwide. Dickkopf 3 (DKK3), a 36-kD secreted glycoprotein, has a role in cell differentiation, but little is known about its involvement in vascular disease. In the present study, we utilised a murine model of atherosclerosis ($ApoE^{-/-}$) in conjunction with $DKK3^{-/-}$ to assess the effects of DKK3 on plaque stability.

We found that the absence of DKK3 leads to vulnerable unstable atherosclerotic plaques, due to a reduced number of smooth muscle cells (SMCs) and reduced matrix protein deposition, as well as increased haemorrhage and macrophage infiltration. Using a cell linear tracing method, vascular progenitors and fibroblasts from SM22-LacZ transgenic mice were isolated and applied to the adventitial side of injured femoral arteries resulting in migration of both types of cells to the intima. Upon migration the cells displayed beta-gal positivity, indicating SMC differentiation.

Further *in vitro* studies revealed that DKK3 can induce differentiation of Sca1+ vascular progenitors and fibroblasts into SMCs via activation of the TGFβ/ATF6 and Wnt signalling pathways. Finally, we assessed the therapeutic potential of DKK3 in mouse and rabbit models and found that DKK3 increases atherosclerotic plaque stability via an increase in SMC numbers and reduced vascular inflammation. Cumulatively, we provide the first evidence that DKK3 is a potent SMC differentiation factor, which may have a therapeutic effect in reducing acute haemorrhagic conditions through promotion of atherosclerotic plaque stability.

C NOX4-DEPENDENT REPROGRAMMING OF GLUCOSE METABOLISM AND FATTY ACID OXIDATION FACILITATES CARDIAC ADAPTION TO CHRONIC PRESSURE-OVERLOAD

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Introduction Increased reactive oxygen species (ROS) production is involved in the pathophysiology of cardiac hypertrophy and failure. Interestingly, a specialised ROS-generating enzyme NADPH oxidase-4 (Nox4) was previously found to have beneficial effects by promoting adaptive remodelling during pressure-overload cardiac hypertrophy. Nox4 modulates intracellular signalling cascades but how it achieves beneficial effects in the chronically overloaded heart remains unclear.

Methods and results To obtain an unbiased global overview of putative Nox4-mediated changes, the proteome of cardiac-specific Nox4 transgenic (TG) and wild-type (WT) mouse hearts was first characterised through a 2D-DIGE approach. TG hearts had a significant over-representation of changes in protein levels of enzymes involved in glucose and fatty acid utilisation. We therefore analysed the metabolome using 1H-NMR and targeted LC-MS approaches. This identified a differential accumulation of glycolytic intermediates in the proximal part of glycolysis both in unstressed and pressure-overloaded TG hearts, as well as an increase in alanine levels (1.4 fold, $p = 0.05$), confirming significant alterations to metabolism. To specifically quantify glucose uptake, glycolysis, glucose oxidation and fatty acid oxidation rates, *ex vivo* working heart studies were conducted. TG hearts had a marked increase cf. WT in palmitate oxidation rate in the unstressed as well as pressure-overloaded heart (3.6 fold increase; $n = 6$/group; $p = 0.01$). Glucose uptake was unaltered but glycolysis and oxidation rates were decreased, suggesting diversion of glucose away from oxidation. Importantly, an increase in palmitate oxidation was not detrimental either for *in vivo* cardiac energetics (31P-NMR) or contractile function during pressure-overload hypertrophy. We found that activity of the hexosamine biosynthesis pathway (HBP), an alternative route for glucose metabolism, was increased in TG hearts as assessed by the O-GlcNAc post-translational modification of cardiac proteins by N-acetylglucosamine, the end-product of HBP. O-GlcNAc levels were 2.4 fold higher in TG cf. WT ($n = 4$/group; $p = 0.02$). In cultured cardiomyocytes, endogenous Nox4 induced similar changes in HBP and palmitate oxidation (extracellular flux analysis), and it was found that changes in O-GlcNAcylation regulated fatty acid oxidation.

Discussion These results show that Nox4 reprograms substrate utilisation in the heart by directing glucose towards the HBP and inducing a linked increase in fatty acid oxidation. These changes appear to enable the heart to better adapt to chronic pressure overload and may be important in the beneficial effects of Nox4 on cardiac remodelling. These data identify a novel redox mechanism that drives beneficial metabolic reprogramming in the heart and suggest potential new therapeutic approaches to promote adaptation to chronic overload stress.

D IMPACT OF HIGH-FLOW OXYGEN ON PERFUSION, MICROVASCULAR AND CAPILLIARY FUNCTION IN NORMAL VOLUNTEERS AND PATIENTS WITH CORONARY ARTERY DISEASE: A CARDIOVASCULAR MAGNETIC RESONANCE AND INVASIVE CORONARY PHYSIOLOGY STUDY

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