

A GENE-BASED RESTORATION OF AKT ACTIVITY IN ENDOTHELIAL PROGENITOR CELLS FROM HUMAN SUBJECTS AT HIGH CARDIOVASCULAR RISK RESCUES VASCULAR REPARATIVE CAPACITY

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Introduction Late-outgrowth endothelial progenitor cells (LEPC) are putative mediators of endogenous vascular repair, and represent an attractive tool for future cell-based cardiovascular repair strategies. However, progenitor function may be impaired in high cardiovascular risk populations, such as those of South Asian (SA) ethnicity, in whom cell based therapies are likely to offer most benefit.

Methods Detailed analysis of in vitro LEPC function (abundance, proliferation, migration, angiogenesis and senescence) was performed in 12 SA men and 12 matched White European (WE) controls. Molecular abnormalities within the Akt/eNOS signalling axis were analysed with PCR and western blotting. LEPC were transfused into immunodeficient mice subsequent to femoral artery injury to assess in vivo reparative function. In vitro and in vivo studies were repeated after lentiviral gene delivery of constitutively active Akt1 (E17K) or control (EGFP) to SA LEPC; augmented Akt activity was confirmed using a Glycogen Synthase Kinase phosphorylation assay. Data are expressed as mean [SEM] and compared with t tests as appropriate; statistical significance is defined as $p < 0.05$ (denoted by *).

Results The two groups were matched for age and cardiovascular risk factors, although the SA group was comparatively insulin resistant (HOMA-IR 1.2 [0.2] vs 0.5 [0.1] au*). SA LEPC exhibited impaired colony formation (0.06 [0.02] vs 0.19 [0.03] colonies/ml blood*), migration to vascular endothelial growth factor (5 [0.7] vs 10 [1.7] cells/microscopic field*) and in vitro angiogenesis (1.9 [0.6] vs 3.8 [0.5] tubular structures/microscopic field*), associated with markedly decreased abundance of the phosphorylated forms of the pro-angiogenic molecules S473-Akt (0.14 [0.05] vs 0.81 [0.2] au*) and S1177-eNOS (0.05 [0.02] vs 0.15 [0.01] au*). Transfusion of WE LEPC into immunodeficient mice after wire-induced femoral artery luminal injury augmented re-endothelialisation; however, neither SA LEPC, nor vehicle, augmented re-endothelialisation (WE: 54.2 [6.4], SA: 36.9 [3.4], vehicle: 31.1 [2.4] % re-endothelialised; WE vs SA*; SA vs vehicle $p = 0.2$). Lentiviral gene delivery of a E17K, but not EGFP control, to SA LEPC was associated with augmented Akt1 activity and rescue of in vivo re-endothelialisation capacity (E17K: 55.2 [4.4] vs EGFP 24.1 [1.3] % re-endothelialised; E17K vs EGFP*; E17K vs WE non-transduced cells $p = 0.9$).

Conclusions These data provide proof of principle for human LEPC based vascular repair therapy, and demonstrate a mechanism by which to rescue marked progenitor dysfunction in a group at high risk of cardiovascular events, whom are likely to benefit from cardiovascular repair therapies.

B ASSESSMENT OF VALVULAR CALCIFICATION AND INFLAMMATION BY POSITRON EMISSION TOMOGRAPHY

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Background The pathophysiology of aortic stenosis is incompletely understood and the relative contributions of valvular calcification and inflammation to disease progression are unknown.

Methods Patients with aortic sclerosis and mild, moderate and severe stenosis were prospectively compared to age and sex-matched control subjects. Aortic valve severity was determined by echocardiography. Calcification and inflammation in the aortic valve were assessed by sodium 18-fluoride (18F-NaF) and 18-fluorodeoxyglucose (18F-FDG) uptake using positron emission tomography. Histological analysis was performed on the valves of five patients who subsequently underwent aortic valve replacement.

Results 121 subjects (20 controls; 20 aortic sclerosis; 25 mild, 33 moderate and 23 severe aortic stenosis) were administered both 18F-NaF and 18F-FDG. Quantification of tracer uptake within the valve demonstrated excellent inter-observer repeatability with no fixed or proportional biases and limits of agreement of ± 0.21 (18F-NaF) and ± 0.13 (18F-FDG) for maximum tissue-to-background ratios. Activity of both tracers was higher in patients with aortic stenosis than control subjects (18F-NaF: 2.87 ± 0.82 vs 1.55 ± 0.17 ; 18F-FDG: 1.58 ± 0.21 vs 1.30 ± 0.13 ; both $p < 0.001$). 18F-NaF uptake displayed a progressive rise with valve severity ($r^2 = 0.540$, $p < 0.001$) and colocalised to osteocalcin staining on histology. Uptake was observed both in the presence and absence of underlying calcium on CT with the latter predominating. 18F-FDG displayed a more modest increase in activity with valve severity ($r^2 = 0.218$; $p < 0.001$) and mapped to areas of macrophage accumulation. Among patients with aortic stenosis, 91% had increased 18F-NaF (> 1.97) and 35% increased 18F-FDG (> 1.63) uptake. A weak correlation between the activities of these tracers was observed ($r^2 = 0.174$, $p < 0.001$) and while 18F-NaF activity was higher in the aortic valve than aortic atheroma (2.68 ± 0.84 vs 2.07 ± 0.30 ; $p < 0.001$) the reverse was true for 18F-FDG (1.56 ± 0.21 vs 1.80 ± 0.25 ; $p < 0.001$).

Conclusions Positron emission tomography is a novel, feasible and repeatable approach to the evaluation of valvular calcification and inflammation in patients with aortic stenosis. Calcification appears to be the predominant process that is particular to the valve and disproportionate to the degree of inflammation, indicating it to be a more attractive target for therapeutic intervention.

C ENDOTHELIAL SPECIFIC NOX2 OVER-EXPRESSION INCREASES SUSCEPTIBILITY TO ANGIOTENSIN II INDUCED AORTIC DISSECTION

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Aortic dissection is a detrimental disease with high mortality. However, the mechanisms regulating the susceptibility to aortic dissection remain unknown. We hypothesise that endothelial oxidative stress due to the activation of the reactive oxygen species (ROS)-generating Nox2 enzyme play an important role in the development of aortic dissection. To investigate this, we generated transgenic mice (C57BL/6J background) with endothelial specific over-expression of Nox2 (Nox2-Tg) under the control of a tie-2 promoter. Expression of the human Nox2 transgene was confirmed by qRT-PCR to be found only in endothelial cells (EC) isolated from transgenic mice, and not in WT EC or vascular smooth muscle cells (VSMC) and macrophages isolated from either genotype. Wild-type (WT) littermates and Nox2-Tg male mice (6 months, $n = 11$) were treated with vehicle or AngII (1 mg/kg/day) via subcutaneous mini-pump for 28 days. There was no significant difference in the pressor responses to AngII between WT and Nox2-Tg mice (WT 121 ± 7 mm Hg vs Nox2-Tg 122 ± 6 mm Hg). However, 5/11 Nox2-Tg mice developed aortic dissections compared to 0/11 WT mice after AngII infusion ($p < 0.001$). Immunohistochemistry revealed significant increases in endothelial VCAM-1 expression, MMP activity and CD45+ inflammatory cell recruitment in the aortas of Nox2-Tg mice after 5 days of AngII infusion. Inflammatory cell recruitment was further confirmed by FACS analysis of cells derived

from explanted aortas ($p < 0.05$). Explanted aortas from Nox2-Tg had significantly higher levels of secreted pro-inflammatory cytokine, cyclophilin A (Cypa) at both baseline and after 5 days of in vivo AngII treatment compared to WT littermates. Compared to primary WT EC and VSMC, Nox2-Tg primary EC, but not primary VSMC, had increased ROS production which was accompanied by increased Cypa secretion and ERK1/2 activation. Furthermore, conditioned media from Nox2-Tg EC induced a greater ERK1/2 phosphorylation compared to the media of WT controls. In conclusion, we demonstrate for the first time that a specific increase in endothelial ROS through the over-expression of Nox2 is sufficient to induce aortic dissection in response to AngII stimulation. Endothelial secreted Cypa could be the signalling mechanism by which increased endothelial ROS regulates the inflammatory response and the susceptibility to aortic dissection.

D ENDOTHELIUM SPECIFIC INSULIN RESISTANCE LEADS TO ACCELERATED ATHEROSCLEROSIS: A ROLE FOR REACTIVE OXYGEN SPECIES

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Background Global insulin resistance and endothelial dysfunction have been identified as predisposing factors for atherosclerosis. However, it is unclear whether selective insulin resistance in endothelial cells alone, is sufficient to promote atherosclerosis. We addressed this question by crossing Endothelial Specific Mutant Insulin Receptor Over-expressing (ESMIRO) mice with ApoE^{-/-} mice. ESMIRO mice over-express a human insulin receptor with an Ala-Thr1134 mutation in the tyrosine kinase domain (which disrupts insulin signalling) selectively in endothelial cells under the control of the tie-2 promoter/enhancer.

Methods Male ApoE^{-/-}/ESMIRO mice were compared with sex-matched littermate ApoE^{-/-} mice (both on a C57Bl6 background) after feeding a Western-style diet for 12 weeks.

Results ApoE^{-/-}/ESMIRO mice were morphologically indistinguishable from ApoE^{-/-} control littermates and showed normal development with no differences between groups in body mass. Heart rate, systolic blood pressure, glucose tolerance, insulin sensitivity and fasting glucose levels were similar in ApoE^{-/-}/ESMIRO and ApoE^{-/-} mice. ApoE^{-/-}/ESMIRO cultured endothelial cells demonstrated insulin resistance through significantly reduced insulin mediated eNOS activity ($p = 0.003$). Aortic lipid deposition along the whole aorta, assessed by en-face oil red O staining, was similar in ApoE^{-/-}/ESMIRO and ApoE^{-/-} mice ($6.4\% \pm 0.5\%$ vs $5.8\% \pm 0.5\%$; $p = 0.39$). Analysis of lipid deposition along the lesser curvature of the aortic arch revealed a significant increase in ApoE^{-/-}/ESMIRO when compared to controls (9.4 ± 0.89 vs $12.43 \pm 1.19\%$ $p = 0.035$). Atherosclerotic lesion area in cross sections of aortic sinus was also significantly increased in ApoE^{-/-}/ESMIRO mice compared to ApoE^{-/-} controls ($24.8\% \pm 2.4\%$ vs $16.6\% \pm 2.4\%$; $p = 0.02$). Vascular function assessed through relaxation responses of aortic rings in response to the endothelial specific vasodilator acetylcholine revealed that aortic rings from ApoE^{-/-}/ESMIRO mice had blunted relaxation responses to acetylcholine (E_{max} ApoE^{-/-} 102.88 ± 6 , E_{max} ApoE^{-/-}/ESMIRO $65 \pm 41\%$, $p = 0.02$), which was restored by the superoxide dismutase mimetic and antioxidant MnTMPyP (E_{max} ApoE^{-/-}/ESMIRO without MnTMPyP $65 \pm 41\%$, with MnTMPyP $112 \pm 15\%$ $p = 0.048$). Endothelial cells from ApoE^{-/-}/ESMIRO mice had significantly increased basal generation of superoxide (1.87-fold increase compared to ApoE^{-/-} $p < 0.05$) which was blunted by the selective NADPH oxidase inhibitor gp91ds-tat (11% reduction ± 0.02 , $p = 0.03$) and the

non-selective NO synthase inhibitor L-NMMA (6% reduction ± 0.01 , $p = 0.03$).

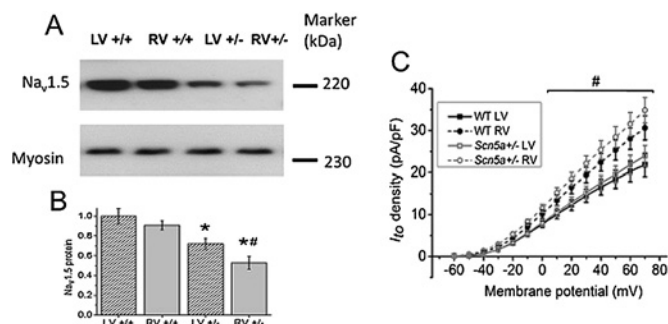
Conclusions Endothelial specific insulin resistance is sufficient to promote atherosclerosis and increase lesion area in ApoE null mice potentially via the increased ROS displayed in this model. This suggests that enhancing endothelial insulin sensitivity may be an appropriate target to prevent atherosclerosis in insulin-resistant conditions.

E RIGHT VENTRICULAR ORIGIN OF ARRHYTHMIAS IN SCN5A+/- MICE IS DUE TO REDUCED NA+ AND HIGHER K+ CHANNEL EXPRESSION AND FUNCTION

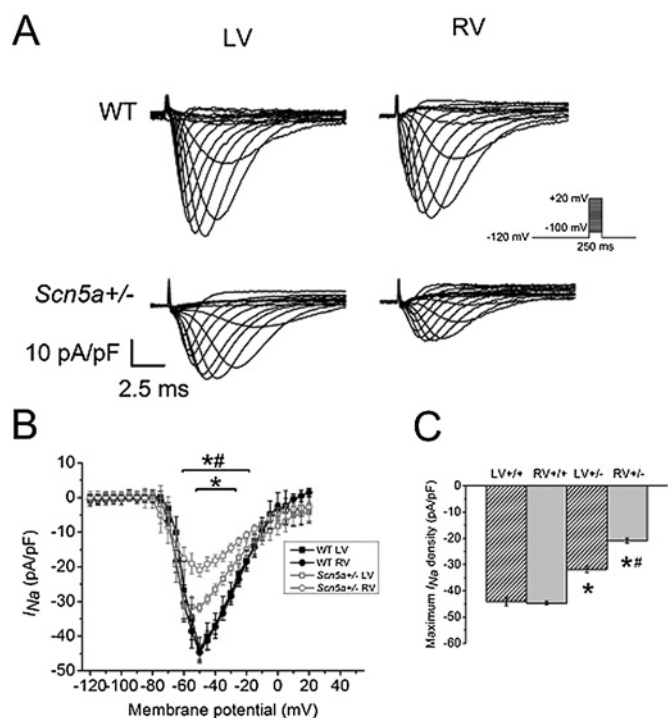
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Introduction Brugada syndrome is associated with ventricular tachycardia originating in the right ventricle (RV); this has been



Abstract E Figure 1



Abstract E Figure 2