in these mice. We studied mutant actin by an in-vitro motility assay. Thin filaments were reconstituted with purified mouse f-actin and human heart tropomyosin and troponin. The E99K thin filaments had higher Ca^{2+} sensitivity than non-transgenic thin filaments. E99K actin thin filaments did not respond to troponin dephosphorylation. The ACTC E99K mouse reproduces many features of HCM, as observed in patients. The basic effect of the ACTC E99K mutation is increased Ca^{2+} sensitivity together with a blunted response to troponin I phosphorylation. The increased myofibrilar Ca^{2+} sensitivity may be sufficient to provoke arrhythmia and account for the high mortality at early ages. Hypertrophy may be a chronic response to Ca^{2+} overloading or due to energy depletion.

022 HYPOXIC PRECONDITIONING OF CARDIOSPHERE-DERIVED CELLS TO INCREASE RETENTION IN THE INFARCTED HEART

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Myocardial infarction results in the formation of a hypoxic scar region. Resident stem cells have been discovered in the adult heart that may be expanded in vitro via the formation of cardiospheres. Administration of these cardiosphere-derived cells (CDC) to the infarcted heart has been shown to improve cardiac function; however, levels of stem cell retention are low. Preconditioning of CDC to a hypoxic environment may increase cell retention, promote proliferation within the scar and further improve cardiac function. CDC were cultured under 2% oxygen for 1 week. Proliferation rates were calculated and hypoxic inducible factor (HIF1α) protein expression and oxygen consumption were measured in intact cells over 1 week. CDC culture under hypoxia for 24 h increased HIF1α by 214% compared with control cells cultured under normoxia. After 1 week in hypoxia, however, there was no difference in HIF1α levels compared with controls. CDC proliferation was increased fivefold under hypoxia. CDC cultured under hypoxia had decreased oxygen consumption compared with control cells cultured under normoxia, with oxygen consumption decreased by 22% with both ADP and FCCP after 24 h. After 1 week of hypoxia, oxygen consumption was decreased by 92% with ADP and 94% with FCCP. Culture under hypoxia generated sufficient CDC for therapy more rapidly than under normoxia. The resulting CDC had reduced oxygen consumption and thus may be better adapted to survive within the hypoxic scar.

023 TRANSCRIPTIONAL REGULATION OF P40PHOX AND P47PHOX EXPRESSION VIA HBP1 IN ENDOTHELIAL CELLS

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Transcription factor HMG-box protein 1 (HBP1) is a member of the HMG-box family of transcription factors and has been found to play an important role in the transcriptional repression of the p47phox gene. The promoter region of p40phox also has a HBP1 binding site, which makes p40phox a possible candidate for HBP1. In this study, we examined the role of HBP1 in the regulation of p47phox and p40phox in endothelial cells. Knockdown of p47phox in a mouse lymphoid endothelial cell line (SVEC4-10) resulted an ~50% increase of HBP1 protein expression, and this was accompanied with a significant increase in p40phox protein expression as detected by Western blot. The levels of HBP1 expression were significantly higher (~2.3-fold) in coronary microvascular endothelial cells isolated from p47phox knockout mice compared with cells isolated from wild-type mice. The role of HBP1 in the transcriptional regulation of p40phox and p47phox expression was further examined by transient in-vitro knockdown of HBP1 using shRNA in human microvascular endothelial cell (HMEC1). Knockdown of HBP1, as shown by Western blot, resulted in a significant increase in p47phox expression and this was accompanied with a significant reduction in p40phox expression. In conclusion, HBP1 plays dual roles in the regulation of NADPH oxidase: it represses p47phox expression and in the mean time promotes p40phox expression. HBP1 may represent an important transcriptional mechanism involved in the regulation of endothelial reactive oxygen species production by NADPH oxidase.

024 ENDOTHELIAL NOX4 NADPH OXIDASE ENHANCES VASODILATATION VIA HYDROGEN PEROXIDE-INDUCED HYPERPOLARISATION AND REDUCES BLOOD PRESSURE

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Introduction NADPH oxidases (Noxs) are reactive oxygen species-generating enzymes implicated in cardiovascular disease. Nox4 is the most abundantly expressed isoform in endothelial cells but its function remains unknown. We investigated the role of endothelial Nox4 on vascular function and blood pressure (BP) in vivo.

Methods and Results We generated transgenic mice with endothelium-specific overexpression of Nox4 (Nox4TG) and studied the effects on endothelial function (aortic rings ex vivo) and blood pressure (telemetry). Nox4 protein levels were twofold higher in Nox4TG aorta compared with wild-type (wt) littermates, with no changes in the expression of other Nox isoforms. Nox4TG had enhanced relaxation to acetylcholine (ACh) compared with wt mice (−log EC50 7.76Â±0.07 vs 7.20Â±0.05; n=12; p<0.001) but similar relaxation to sodium nitroprusside. The ACh response in Nox4TG and wt mice was dentical in the presence of catalase (1500 U/ml) or with high extracellular potassium (30 mM) pre-contraction, but remained greater in Nox4TG in the presence of inhibitors of nitric oxide synthesis (L-NMMA, 100Â μM), soluble guanylate cyclase (ODQ, 5Â μM) or protein kinase G (KT5823, 2Â μM). Ox4TG also had significantly lower BP than wt mice (mean BP 102.5Â±1.8 vs 109.5Â±2.0 mm Hg; n=10; p=0.05), which was abolished after chronic treatment with N-acetylcysteine or an OD/catalase imetic, EUK-8. Plasma nitrite/nitrate levels and aortic levels of phosphorylated VASP were identical and acute intravenous treatment with L-NMMA (10 mg/kg) increased BP to a similar extent in Nox4TG and wt mice. The hypertensive response to chronic 14-day angiotensin II infusion (1.1 mg/kg per day) was lower in ox4TG compared with wt mice (mean BP 116.7Â±4.7 vs 129.4Â±3.5 mm Hg; n=10; p<0.05).

Conclusions Nox4TG had significantly enhanced ACh-induced vasodilatation compared with wt mice as a result of hydrogen peroxide-induced hyperpolarisation. Nox4TG also had a lower BP, which was not attributable to altered nitric oxide bioactivity but was normalised by chronic antioxidant treatment. These results suggest that endothelial Nox4 has potentially beneficial effects on vascular tone and BP.

025 IS DEPRESSED MYOCYTE CONTRACTILITY AN EARLY EVENT IN THE NATURAL HISTORY OF HEART FAILURE?

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It is generally accepted that abnormal intracellular Ca^{2+} handling accounts for the depressed left ventricular (LV) systolic and diastolic