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Raf kinase inhibitor protein mediates myocardial fibrosis in pressure overload through the regulation of the nuclear expression of nuclear factor erythroid 2-related factor 2

**Authors:**
A Kazakov¹, R A Hall², C Werner¹, T Meier¹, A Trouvain¹, S Rodionycheva³, F Lammert², C Maack⁴, M Bohm¹, U Laufs⁵, ¹Saarland University Hospital, Department of Internal Medicine III, Cardiology - Homburg - Germany, ²Saarland University Hospital, Department of Internal Medicine II, Gastroenterology - Homburg - Germany, ³Saarland University Hospital, Department of Thoracic and Cardiovascular Surgery - Homburg - Germany, ⁴Deutsches Zentrum für Herzinsuffizienz Würzburg - Würzburg - Germany, ⁵University of Leipzig, Klinik und Poliklinik für Kardiologie, Universitätsklinikum Leipzig - Leipzig - Germany.

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Background: Pro-fibrotic signalling is a key mediator during maladaptive remodelling in the heart. For genome-wide Quantitative trait locus analysis (QTL), 26 BXD mouse lines representing a genetically mosaic but homozygous for all loci genetic reference population were treated with carbon tetrachloride (CCl4) to induce fibrosis. The cardiac QTLs linked to collagen accumulation were screened for potential candidates by expression QTL analyses and revealed Raf Kinase Inhibitor Protein (RKIP) as novel regulator of cardiac fibrogenesis.

Purpose: To investigate the role of RKIP in pressure-overload induced cardiac remodeling.

Methods & Results: C57BL/6N-RKIP-deficient mice demonstrated diminished interstitial and replacement fibrosis induced by transverse aortic constriction (TAC) or CCl4 treatment compared with wild-type controls. TAC-induced expression of collagen Ia2 mRNA, Ki67+ fibroblasts and markers of oxidative stress such as 8-hydroxy-guanosine (8-dOHG)+ fibroblasts as well as the number of fibrocytes in the peripheral blood and bone marrow were markedly reduced in C57BL/6N-RKIP-/- mice post TAC. The cardiac fibroblasts demonstrated decreased migration and fibronectin production. This was accompanied by a two-fold increase of the nuclear expression of nuclear factor erythroid 2-related factor 2 (Nrf2), the main transcriptional activator of antioxidative proteins, and reduced expression of its inactivators.

To test the importance of oxidative stress for this signaling, C57BL/6J mice were studied. C57BL/6J, but not the C57BL/6N-strain, is protected from TAC-induced oxidative stress due to mutation of the nicotinamide nucleotide transhydrogenase gene (Nnt). After TAC surgery, the hearts of Nnt-deficient C57BL/6J RKIP-/- mice revealed diminished oxidative stress, increased left ventricular fibrosis and collagen Ia2 as well as enhanced basal nuclear expression of Nrf2. In human left ventricular myocardium from both non-failing and failing hearts, RKIP-protein correlated negatively with the nuclear expression of Nrf2.

Conclusions: Under conditions of Nnt-dependent enhanced myocardial oxidative stress induced by pressure overload, RKIP plays a maladaptive role for fibrotic myocardial remodeling by suppressing the Nrf2-related beneficial effects.
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