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# Raf kinase inhibitor protein of the bone marrow contributes to cardiac fibrogenesis in pressure-overloaded myocardium

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**Background:** Raf Kinase Inhibitor Protein (RKIP) regulates myocardial remodeling under conditions of enhanced myocardial oxidative stress in pressure-overloaded left ventricle (LV) modulating myocardial production of reactive oxygen species (ROS). A second mode of action may be the mobilization of circulating fibroblasts (fibrocytes) from the bone-marrow (BM). However the underlying mechanisms are incompletely understood.

**Methods and results:** To further characterize the role of RKIP in BM cells for myocardial remodeling 10-week-old wild-type (WT) C57BL/6N mice were subjected to transplantation of bone marrow (BMT) from 10-week-old C57BL/6-RKIP-deficient (RKIP–/–) N or WT C57BL/6N mice expressing green fluorescent protein (GFP)+ ubiquitously. 28 days later, transverse aortic constriction (TAC, 360 $\mu$m) or SHAM-operation was performed. 5 weeks post TAC, LV systolic pressure (LVSP) and heart weight to tibia length ratio were significantly increased in both types of BMT, compared with corresponding SHAM. Increased afterload elicited myocardial fibrosis as assessed by picrosirius red staining (WT/WT SHAM 15±2.5%, WT/WT TAC 21.3±1.4%, $p<0.05$; RKIP–/–/WT SHAM 17±2%, RKIP–/–/WT TAC 18±3%, p=ns) and significantly increased the number of LV fibroblasts per mm$^2$ estimated by immunostaining for intracellular fibronectin, which were further reduced by transplantation of RKIP–/–N BM (WT/WT SHAM 5499±313, WT/WT TAC 7493±741 per mm$^2$, $p<0.05$; RKIP–/–/WT SHAM 5737±259, RKIP–/–/WT TAC 5282±551, per mm$^2$, p=ns). Moreover, transplantation of RKIP–/–N BM significantly diminished the number of circulating BM-derived GFP+ fibroblasts in the peripheral blood and LV myocardium during pressure overload (WT/WT SHAM 961±129, WT/WT TAC 2326±273 per mm$^2$, $p<0.05$; RKIP–/–/WT SHAM 1041±209, RKIP–/–/WT TAC 1518±107, per mm$^2$, p=ns). The myocardial redox status was assessed by the co-immunostaining for ROS production marker 8-hydroxyguanosin (8-dOHG), cardiomyocyte marker $\alpha$-sarcomeric actin and fibroblast marker intracellular fibronectin. Pressure overload during 5 weeks significantly increased the percentages of 8-dOHG+cardiomyocytes (WT/WT SHAM 34±9%, WT/WT TAC 63±6%, $p<0.05$; RKIP–/–/WT SHAM 29±6%, RKIP–/–/WT TAC 31±8%, p=ns) and 8-dOHG+fibroblasts (WT/WT SHAM 57±6%, WT/WT TAC 73±4%, $p<0.05$; RKIP–/–/WT SHAM 58±2%, RKIP–/–/WT TAC 58±7%, p=ns) in mice transplanted with WT BM but not with RKIP–/–N BM.

**Conclusions:** In pressure-overload induced enhanced myocardial ROS production, deficiency of RKIP-expression in the bone marrow abrogates left ventricular fibrosis by reduction of myocardial ROS production and mobilization of BM-derived fibroblasts. These findings suggest that the function of RKIP in the bone marrow may be important for maladaptive myocardial remodelling.