

POSTER PRESENTATION

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Protein kinase G I alpha activates an anti-remodeling signaling pathway in the heart via an interaction with the MAPKKK mixed lineage kinase 3

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Background

cGMP signaling inhibits pathologic cardiac hypertrophy and remodeling in vivo. In prior studies, we explored the role of the cGMP-dependent protein kinase I alpha (PKGI α) in regulating cardiac remodeling by studying mice with mutations in the PKGI α leucine zipper (LZ) domain [1], in which PKGI α kinase activity is retained but LZ-mediated protein-protein interactions are abolished. These mice (termed leucine zipper mutant, or LZM, mice) develop accelerated contractile dysfunction, and increased hypertrophy and mortality in response to LV pressure overload induced by Transaortic constriction, (TAC) [2], supporting a critical role for PKGI α in inhibiting remodeling in vivo. The specific myocardial signaling pathways regulated through PKGI α LZ-mediated interactions remain unknown. Cardiac JNK activation is required for normal LV compensation to TAC, and in vitro studies support a role of PKGI α in activating JNK in the cardiac myocyte. We hypothesized that PKGI α inhibits TAC-induced remodeling through activation of JNK in the heart. To test this, we explored JNK activation in LZM mouse hearts in response to various durations of TAC.

Results

JNK activation (phosphorylation) increased from 1.0 ± 0.3 ADUs in WT sham hearts to 3.5 ± 0.6 ADUs in WT 48 hour TAC hearts ($p < 0.001$). However, JNK phosphorylation did not increase significantly in LZM hearts

post TAC (0.5 ± 0.1 ADU in LZM sham hearts vs 1.3 ± 0.2 ADU in LZM 48 hour TAC hearts, $p = \text{ns}$ LZM sham vs LZM TAC; $p < 0.001$ WT TAC vs LZM TAC). Similar trends in JNK phosphorylation were observed at 24 hours post-TAC and 7 days post-TAC. Loss of TAC-induced JNK phosphorylation in LZM mice was accompanied by decreased TAC-induced activation of MAP Kinase Kinase 4 (MKK4) compared with WT controls at 48 hours post-TAC.

We next explored upstream JNK regulators as potential novel PKGI α interacting molecules. In GST pulldown experiments with cardiac lysates from WT or LZM mice, the MAPKKK Mixed Lineage Kinase 3 (MLK3) bound to the WT PKGI α LZ domain, but not the mutant PKGI α LZ domain, supporting complex formation between PKGI α LZ and MLK3. Immunoprecipitation studies with WT cardiac lysates confirmed an interaction between these two signaling molecules. We next tested whether MLK3 is required for cGMP-PKGI-dependent JNK activation in cultured adult mouse cardiac myocytes (CM) following siRNA knockdown of MLK3. MLK3 knockdown blocked 8-Bromo-cGMP-stimulated JNK activation, while control siRNA had no effect on 8-Bromo-cGMP-stimulated JNK activation.

Conclusion

These findings support that the cGMP pathway recruits the JNK signaling pathway by complex formation between PKGI α and MLK3, leading to activation of MKK4 and JNK in the heart. These findings define a novel mechanism by which cGMP and PKGI α inhibit

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pathological remodeling in vivo, and identify MLK3 as a potentially important therapeutic target in heart failure.

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