

MEETING ABSTRACT

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Mixed lineage kinase 3 functions as a cGMP-dependent protein kinase I alpha substrate and regulates blood pressure and cardiac remodeling in vivo

Timothy Calamaras¹, Robert Baumgartner¹, Guang-rong Wang¹, Roger Davis², Mark Aronovitz¹, David Kass³, R Karas¹, Robert M. Blanton^{1*}

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Protein kinase G I alpha (PKG1 α) counteracts hypertension and pathologic cardiac remodeling. These effects require the PKG1 α leucine zipper (LZ) protein binding domain. However, PKG1 α LZ-binding substrates mediating these effects remain incompletely understood. We previously demonstrated that Mixed Lineage Kinase 3 (MLK3) binds the PKG1 α LZ domain in the heart. In the present study we hypothesized that MLK3 functions as a PKG1 α substrate and cardiovascular effector.

We observed that recombinant MLK3 precipitated with affinity purified PKG1 α but not with LZ mutant PKG1 α . When PKG1 α was precipitated with RP-cGMP beads, which inhibit PKG kinase activity, we observed decreased PKG1 α -MLK3 co-precipitation, supporting a requirement of PKG1 α kinase activity for MLK3-PKG1 α interaction. PKG1 α phosphorylated MLK3 in vitro as assayed by Western blot.

We next analysed mice with genetic deletion of MLK3. In the baseline state, MLK3^{-/-} mice display normal cardiac function as assessed by echocardiography and invasive cardiac hemodynamics. MLK3^{-/-} mice develop cardiac hypertrophy by 3 months of age (heart weight/tibia length 64.4 \pm 1.9 mg/cm WT, 73.6 \pm 2.1 mg/cm MLK3^{-/-}; p<0.001; n=11 WT, 14 MLK3^{-/-}). Compared with WT littermates, anesthetized MLK3^{-/-} mice have elevated blood pressure (BP) (94.3 \pm 2.1 mmHg WT, 109.3 \pm 2.5 mmHg MLK3^{-/-}; p<0.001). Conscious male MLK3^{-/-} mice monitored continuously with implantable

arterial radiotelemetry (10-12 weeks of age) had overt hypertension compared with WT littermates (Systolic BP: WT 121.5 \pm 2.0 mmHg, MLK3^{-/-} 161.6 \pm 5.1 mmHg; p<0.01; Diastolic BP: WT 87.0 \pm 2.9 mmHg, MLK3^{-/-} 114.5 \pm 2.7 mmHg; p<0.001; n=4 WT, 3 MLK3^{-/-}). We observed no difference in baseline heart rate between genotypes.

Chronic administration of hydralazine (250 mg/L) normalized BP in MLK3^{-/-} mice, but did not completely inhibit cardiac hypertrophy. Further, in response to LV pressure overload by transaortic constriction (TAC), which equalized left ventricular (LV) systolic pressure between genotypes, MLK3^{-/-} mice had increased LV hypertrophy (LV/Tibia length) as well as elevated LV end diastolic pressure, and worsening of LV ejection fraction, preload recruitable stroke work, and other LV systolic and diastolic indices (n=8-10), indicating advanced cardiac dysfunction.

Together, our findings identify MLK3 as a direct PKGI substrate, and reveal that deletion of MLK3 leads to hypertension and pathologic cardiac hypertrophy. These findings support a model in which, in response to activation by PKGI α , MLK3 inhibits hypertension and cardiac hypertrophy. We conclude that identifying novel PKGI α LZ substrates, like MLK3, may reveal new candidate therapeutic targets for hypertension and heart failure.

Authors' details

¹Molecular Cardiology Research Institute, Tufts Medical Center, Boston, Massachusetts 02111, USA. ²Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, Massachusetts 01605, USA.

* Correspondence: rblanton@tuftsmedicalcenter.org

¹Molecular Cardiology Research Institute, Tufts Medical Center, Boston, Massachusetts 02111, USA

Full list of author information is available at the end of the article

³Department of Cardiology, Johns Hopkins Medical Institutions, Baltimore, Maryland 21205, USA.

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