Isolated Cardiomyocytes from Transgenic Mouse Hearts Show Tissue-specific mTOR Overexpression

Journal of Health Disparities Research and Practice

Volume 9
Issue 5 *Special Issue - NIDDK STEP UP*

Article 41

2016

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Abstract

Mechanistic target of rapamycin (mTOR) is a key downstream effector of insulin that plays an important role in glucose uptake and cardioprotection. We previously used transgenic (Tg) mice with cardiac-specific overexpression of mTOR driven by the murine alpha-myosin heavy chain promoter to demonstrate that mTOR protects the heart against acute MI by inhibiting necrosis in cardiac cells (cardiomyocyte, CM). Since the heart consists of multiple cell types, including CMs and fibroblasts, protein assays with tissue lysates from the whole heart alone cannot demonstrate tissue specific protein expression in transgenic mice.

To determine whether our hemagglutinin (HA)-tagged rat mTOR transgene is expressed in CMs, we assessed HA-mTOR expression and examined the mTOR signaling pathway in CMs isolated from the heart. Hearts harvested from either wild-type (WT) or mTOR-Tg mice were treated with collagenases using an *ex vivo* perfused heart system for isolating CMs. The isolated CMs and whole hearts from either WT or Tg mice were homogenized for Western blotting.

Western blotting demonstrated a significant increase in mTOR expression in the whole heart and CMs from mTOR-Tg mice. The increase in mTOR expression was more significant in isolated CMs compared to the whole hearts. mTOR-Tg mice showed an increase in phosphorylation of mTOR downstream molecules Akt and S6. HA was only observed in the heart when compared to skeletal muscle, liver, kidney, and spleen samples in Tg mice. We observed cardiac specific mTOR expression and an increase in kinase activity of mTOR in CMs isolated from mTOR-Tg mice.

Keywords

Isolated cardiomyocytes; mTOR; Western blotting

Authors

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Journal of Health Disparities Research and Practice
Volume 9, Special Edition 1, Summer 2016, pp. 63-64

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ABSTRACT

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ACKNOWLEDGEMENTS

The STEP-UP HS program is supported by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health, Grant number: R25DK078386.