Regulation of Pyruvate Kinase M2 (PKM2) Expression and Activity in Cardiac Hypertrophy

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Introduction

Cardiac hypertrophy is characterized by robust structural, metabolic, and signaling changes that include increased myocyte size, increased glycolytic flux, aerobic glycolysis, and induction of transcriptional programs governed by such factors as c-Myc, Fos, and Jun.[1] We have noted that this phenotypic profile exhibits similarities to cancer development, where c-Myc, HIF-1α and PKM2 contribute to tumorigenesis and enhanced cancer cell survival in the setting of oxidative stress.[2,3] PKM2 is thought to participate in shifts between anabolic and catabolic flux in glycolysis.[3,5] Experiments were conducted to assess the importance of PKM2 in neonatal rat ventricular myocytes exposed to hypertrophy-inducing agonists or hypoxia.

Pyruvate Kinase M

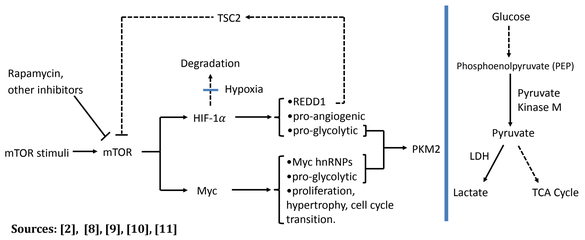
In cardiomyocytes, pyruvate kinase M (PKM) is the enzyme responsible for conversion of phosphoenolpyruvate (PEP) to pyruvate in the final step of glycolysis. PKM has two splice variants: PKM1 and PKM2.[3] The ratio of PKM1/PKM2 is dictated by hnRNPs, and the activity of each isoform differs.[2] In the setting of oxidative stress, the activity of PKM1 is unaffected, while the activity of PKM2 is reduced due to subunit dissociation resulting from oxidation at Cys-358.[3,4] Oxidation of PKM2 increases flux to anabolic pathways (pro-growth) and to the pentose phosphate pathway (producing reducing equivalents for protection against oxidative stress).[3] Upstream regulators of PKM2 are HIF-1α and c-Myc hnRNPs.[2] PKM2 exhibits importance in cancer cells, and a switch to the M2 isoform is necessary to cause the Warburg effect. The M2 isoform is the sole PKM isoform expressed in a variety of tumors.[5]

Background

Hypothesis: Increased PKM2 protein levels and oxidation contribute to cardiac hypertrophy.

The treatment conditions analyzed are hypoxia and α-adrenergic signaling. As indicated in the figure below, hypoxia is expected to increase HIF-1α and PKM2 downstream. *In vivo* hypertrophy conditions have also been shown to increase c-Myc mRNA expression.[6] Both α-adrenergic signaling and hypoxia generate reactive oxygen species (ROS). The former generates ROS through an NADPH oxidase 2 mediated mechanism.[3,7]

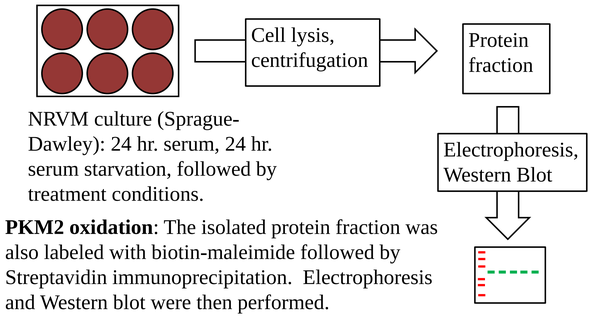
Pathways Involved



Sources: [2], [8], [9], [10], [11]

Figure 1a (left), 1b (right): Figure 1a: In cancer cells, mTOR hyperactivity has been shown to increase PKM2 protein levels via two downstream regulators: HIF-1α and Myc.[2] HIF-1α increases transcription of glycolytic enzymes, including PKM2. Myc also has pro-glycolytic effects, as well as increasing Myc hnRNPs which cause selective splicing of PKM2.[2] Figure 1b: A simplified glycolytic pathway showing the role of PKM.

Methods



NRVM culture (Sprague-Dawley): 24 hr. serum, 24 hr. serum starvation, followed by treatment conditions.

PKM2 oxidation: The isolated protein fraction was also labeled with biotin-maleimide followed by Streptavidin immunoprecipitation. Electrophoresis and Western blot were then performed.

Phenylephrine Treatment

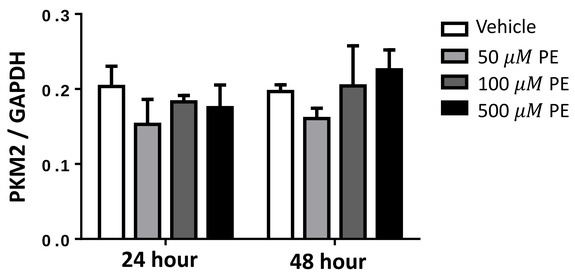
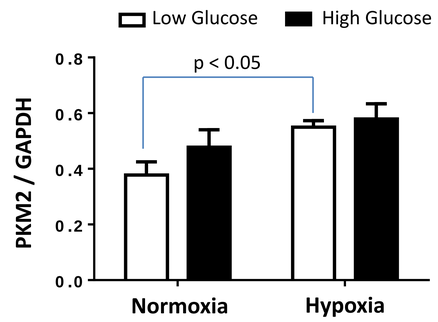
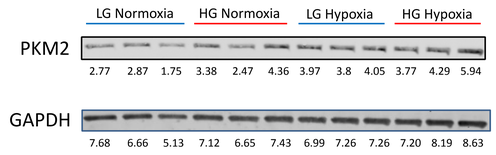


Figure 2: 24-hour and 48-hour phenylephrine (PE) treatment of NRVMs in low glucose media exhibited no significant increase in PKM2 protein levels when normalized to GAPDH protein signal. Three PE concentrations were tested: 50 μM, 100 μM, and 500 μM. Previous tests (not shown) suggested no difference in PE effects on NRVMs in either low or high glucose media. Analysis was performed via quantitation of western blot signal.

Hypoxia Treatment



Figures 3 (above) & 4 (below): Figure 3: 16-hour hypoxia treatment (1% O_2) of NRVMs exhibited an increase in PKM2 protein levels when normalized to GAPDH protein signal. The increase in PKM2/GAPDH was larger in low glucose media than high glucose media. Figure 4: Western blot and band quantitation used to obtain the results shown above.



Oxidized PKM2: Sham and TAC surgery

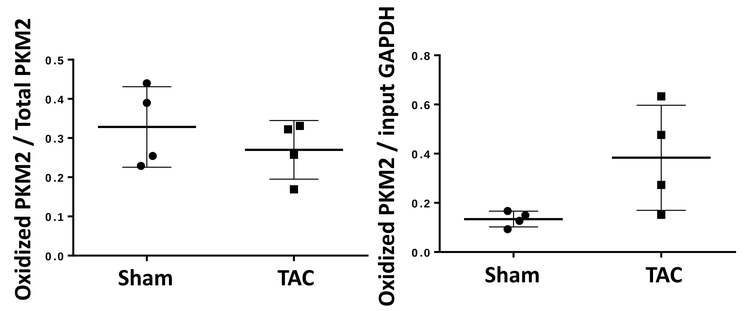


Figure 5 (left) and 6 (right): Measurement of oxidized PKM2 in one week sham surgery and transverse aortic constriction (TAC) mice. Fractional oxidized PKM2 did not increase with one week TAC; however, total oxidized PKM2 normalized to input GAPDH increased with one week TAC. Total PKM2 protein levels are elevated in 1 week TAC as well (results not shown).

GPCR Agonist Treatment

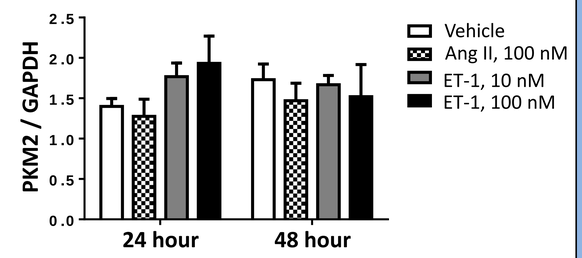


Figure 7: 24-hour and 48-hour GPCR agonist treatment of NRVMs in low glucose media. Angiotensin II (100 nM) and Endothelin-1 (10 nM, 100 nM) were tested. Angiotensin II exhibited no increase in PKM2/GAPDH, while ET-1 exhibited marginal increases in PKM2/GAPDH in 24-hour treatment; however, not in 48-hour treatment. Analysis was performed via quantitation of western blot.

Oxidized PKM2: GPCR Agonists

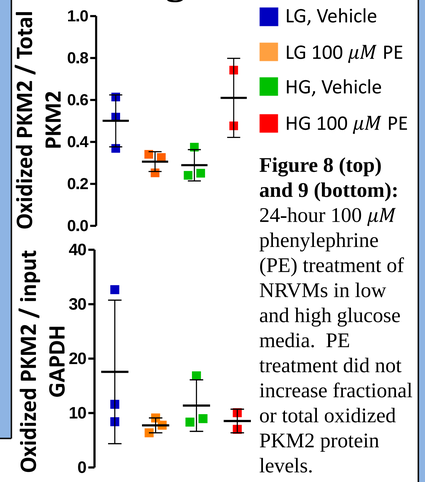


Figure 8 (top) and 9 (bottom): 24-hour 100 μM phenylephrine (PE) treatment of NRVMs in low and high glucose media. PE treatment did not increase fractional or total oxidized PKM2 protein levels.

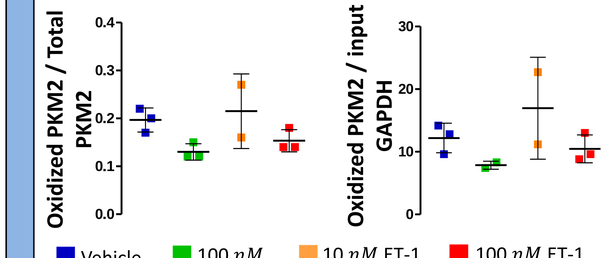
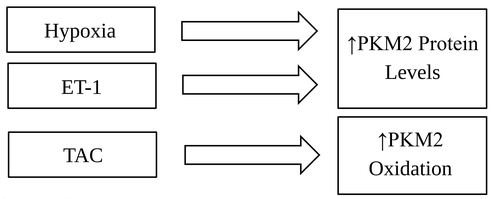


Figure 10 (left) and 11 (right): 24-hour GPCR agonist treatment of NRVMs with Angiotensin II (100 nM) and Endothelin-1 (10 nM, 100 nM) in low glucose media. Angiotensin II and Endothelin-1 treatment did not increase fractional or total oxidized PKM2 protein levels.

Conclusions



PKM2 Protein Levels:

• Phenylephrine and Angiotensin II (low glucose, 100 nM) treatment alone are insufficient to increase normalized PKM2 protein levels in cultured NRVMs.

PKM2 Oxidation:

• PKM2 protein fractional oxidation is not increased by Ang II (100 nM), PE, or ET-1 (10 nM, 100 nM) in cultured NRVMs under the time frame and media conditions tested.

Future Work

PKM2 Protein Levels:

• Hypoxic conditions (Oxygen concentrations: 1%, 10% O_2) can be tested further with and without agonist treatment (PE, ET-1, etc.)
• Test agonists for c-Myc (norepinephrine, mechanical stretch).
• Measure c-Myc and HIF-1α mRNA or protein levels with agonist treatment to narrow the pertinent pathway for PKM2 upregulation.
• Test treatment conditions in an adult cardiomyocyte model.

PKM2 Oxidation:

• Test PKM2 oxidation under conditions of hypoxia, H_2O_2, and other ROS producing treatment conditions in cultured NRVMs.
• Apply Biotin-labeling and immunoprecipitation protocol to other pertinent proteins. Additional positive controls should be identified.
• Apply chemicals that prevent PKM2 oxidation (e.g DASA-10) in the presence of hypertrophy-inducing stimuli in NRVMs. Measure extent of hypertrophy using leucine incorporation.

References

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