The Role of Pyruvate Kinase Regulation for Tumor Growth

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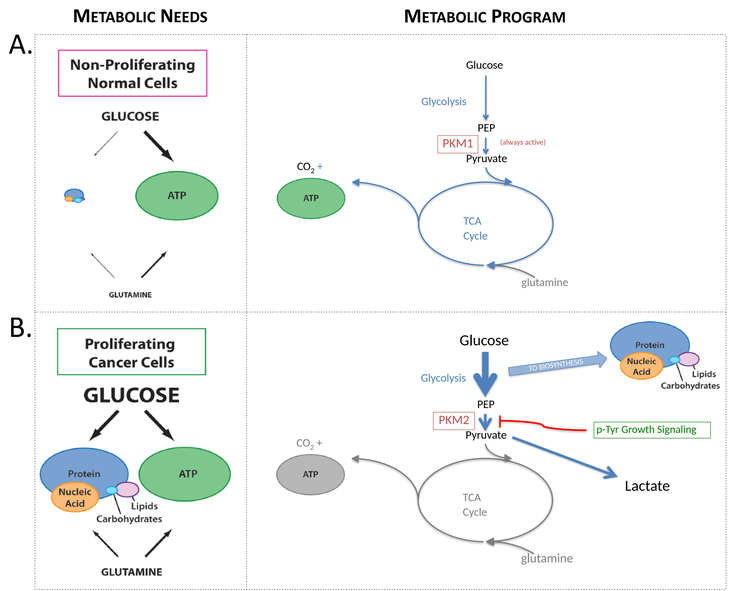
Abstract

Proliferating cells, including cancer cells, regulate their metabolism to meet the demands of rapid growth and division. A hallmark of cancer metabolism is aerobic glycolysis, the increased consumption of glucose and its conversion to lactate even in the presence of oxygen. The metabolic enzyme pyruvate kinase catalyzes the final step of glycolysis, and expression of the M2 isoform (PKM2) in cancer cells is an integral part of their metabolic reprogramming. Unlike the PKM1 isoform found in most adult tissue, PKM2 activity is regulated, and is inhibited by phosphotyrosine-mediated growth signaling. We hypothesize that the ability of cancer cells to down-regulate PKM2 activity is beneficial for cell biomass accumulation and proliferation, because the resulting increase in glycolytic intermediates allows greater flux of carbon into biosynthetic pathways (e.g. nucleotide and amino acid biosynthesis). Consistent with this prediction, loss of one or both alleles of PKM2 speeds tumor onset in a transgenic mouse model of breast cancer. The resulting tumors exhibit loss of PKM2 protein and reduced pyruvate kinase activity. Conversely, cancer cells engineered to express high-activity PKM1 form fewer, smaller tumors in a xenograft model than do PKM2-expressing cells. Preliminary results suggest that treatment of xenograft tumors with a small molecule activator of PKM2 limits tumor size. In conclusion, down-regulation of pyruvate kinase activity is important for the metabolic reprogramming seen in cancer cells, and therapies which increase this activity hold great potential for limiting tumor growth.

Introduction: Cancer Metabolism

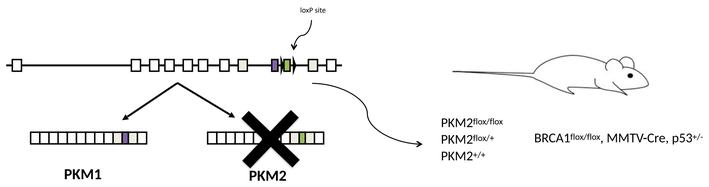


A. Non-proliferating cells efficiently oxidize glucose to CO_2 in the TCA cycle and thereby produce ATP via oxidative phosphorylation. This metabolic program is associated with the constitutively active PKM1 isoform of pyruvate kinase. **B.** Cancer cells convert most glucose to lactate and excrete it from the cell, even in the presence of oxygen. This phenomenon, aerobic glycolysis, is associated with the PKM2 isoform of pyruvate kinase. PKM2 activity is inhibited by phosphotyrosine growth signaling.

We hypothesize that PKM2 is important for cancer proliferation because its inhibition allows accumulation of upstream intermediates in glycolysis. This pool of intermediates can then be used by the cell to meet its biosynthetic needs as it accumulates sufficient biomass to replicate the cell.

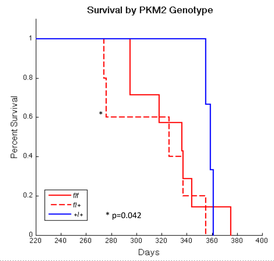
Results

A. PKM Gene and Mouse Model



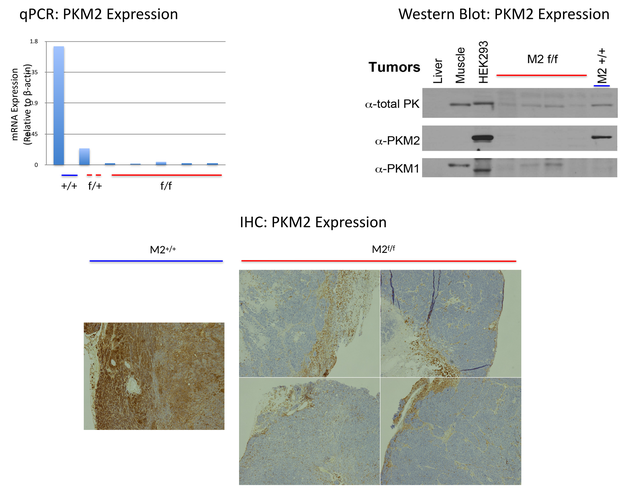
A. PKM1 and PKM2 are spliced from the PKM gene in a mutually exclusive exon inclusion event. Flanking exon 10 with lox P sites allows tissue specific deletion of the "M2" exon by Cre recombinase without disrupting the entire gene. Combining this allele with the $BRCA^{flox/flox}$, MMTV-Cre, $p53^{+/-}$ mouse model of breast cancer allows for investigation of the role of PKM2 in tumor formation.

B. Loss of PKM2 Speeds Tumor Formation



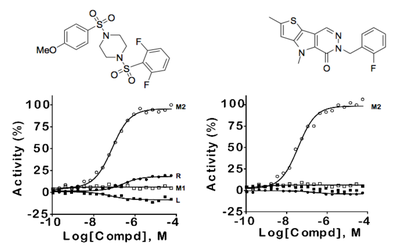
B. $PKM2^{flox/flox}$ and $PKM2^{flox/+}$ mice exhibit earlier tumor onset than do control mice. This is consistent with the hypothesis that less PK activity is beneficial for tumor growth.

C. Tumor Characterization



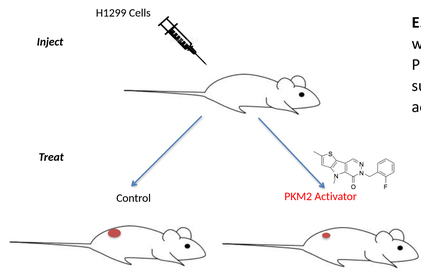
C. Characterization by qPCR, Western blot, and IHC shows effective knockout of PKM2 in flox/flox tumors.

D. Small Molecule Activators of PKM2



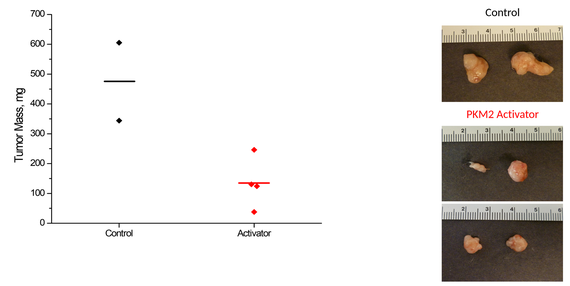
D. Collaborating groups have developed small molecule activators of PKM2. These activators are specific for the PKM2 isoform and have no effect on PKM1, PKL (liver isoform) or PKR (red blood cell isoform). The activator depicted on the right has properties allowing for *in vivo* administration.

E. Xenograft Experiment: Treatment of Tumors with PKM2 Activator



E. Mice were injected subcutaneously with H1299 cells and dosed orally with PKM2 activator. Preliminary results suggest that the forced constitutive activation of PKM2 limits tumor growth.

Prelimary Results: Tumor Masses . . . . . and Tumor Sizes



Conclusions

1) Deletion of PKM2 in a mouse model of breast cancer accelerates tumor onset. This is consistent with the model that inactivation of PKM2 activity via phospho-tyrosine growth signaling is beneficial for rapid cell growth and proliferation.
2) Pharmacological activation of PKM2 results in reduced tumor size in a xenograft model. We suggest that forced activation of PKM2 starves the cells of glycolytic intermediate needed for cell growth and proliferation.

References:

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Jiang J., et al. Evaluation of thieno[3,2-b]pyrrole[3,2-d]pyridazinones as activators of the tumor cell specific M2 isoform of pyruvate kinase. *Bioorg. Medicinal Chem. Lett.* 20 (**2010**) 3387–3393