Role of PKM2 in Tumor Formation

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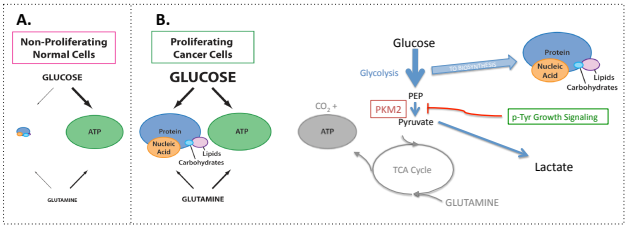
Abstract

Proliferating cells, including cancer cells, regulate their metabolism to meet the demands of rapid growth and division. 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 some differentiated 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 increased availabilty of glycolytic intermediates allows greater flux of carbon into biosynthetic pathways (e.g. nucleotide and amino acid biosynthesis). Consistent with this prediction, loss 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 xenograft models than do PKM2-expressing cells, and treatment of xenograft tumors with a small molecule activator of PKM2 limits tumor size. This suggests that down-regulation of pyruvate kinase activity is important for the metabolic reprogramming seen in cancer cells, and therapies that increase this activity hold potential for limiting tumor growth.

Model

Metabolic Needs

Simplified Metabolic Program

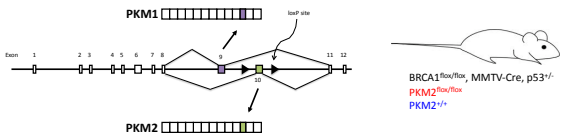


A. Non-proliferating cells efficiently oxidize glucose to CO_2 in the TCA cycle and thereby produce ATP via oxidative phosphorylation. This metabolic program is associated with the constitutively active PKM1 isoform of pyruvate kinase. **B.** Cancer cells convert most glucose to lactate and excrete it 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 increases availability of glycolytic intermediates, which can be used by the cell to meet its biosynthetic needs as it accumulates sufficient biomass to replicate the cell.

Results

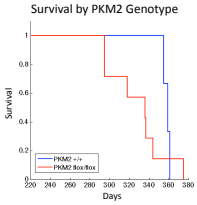
1. Conditional PKM2 Allele and Mouse Model of Breast Cancer



BRCA1$^{flox/flox}$, MMTV-Cre, p53$^{+/-}$
PKM2$^{flox/flox}$
PKM2$^{+/+}$

1. 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 BRCA1$^{flox/flox}$, MMTV-Cre, p53$^{+/-}$ mouse model of breast cancer allows for investigation of the role of PKM2 in tumor formation.

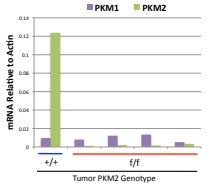
2. Loss of PKM2 Speeds Tumor Formation in BRCA1 Model

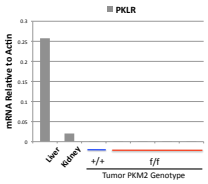


2. PKM2 f/f mice exhibit earlier tumor onset than do PKM2 +/+ mice. This is consistent with the hypothesis that less PK activity is beneficial for tumor growth.

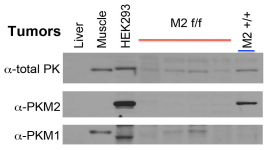
3. BRCA1 Breast Tumors With PKM2 Conditional Alleles Show Loss of PKM2 and No Alternate Isoform Expression

Pyruvate Kinase mRNA by Tumor Genotype

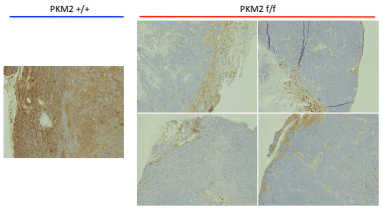




Pyruvate Kinase Protein by Tumor Genotype



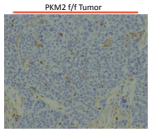
α-PKM2 IHC by Tumor Genotype, 4x

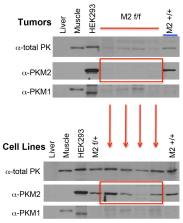


3. Characterization of mammary tumors by qPCR, Western blot, and IHC shows effective knockout of PKM2 in flox/flox tumors, and suggests that PKM2 is not needed for tumor growth. Tumors show no compensatory expression of PKM1, PKL, or PKR.

4. Cell Line Derivation Selects for PKM2 Expression

α-PKM2 IHC, 20x

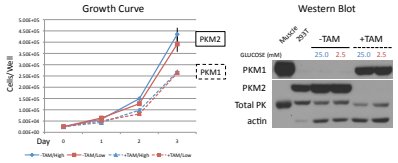




4. Tumors were disaggregated and established as cell lines, which selected for rare, PKM2-expressing tumor cells.

5. Switch From PKM2 to PKM1 Reduces *in vitro* Growth Rate

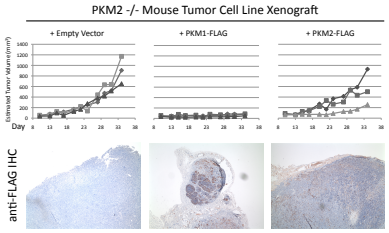
PKM2 f/f CreER^{T2} Tumor Cell Line



5. A PKM2 f/f cell line was stably infected with CreER^{T2}, a tamoxifen-inducible Cre. *In vitro* growth was assayed in 25.0 mM (High) and 2.5 mM (Low) glucose conditions with or without tamoxifen treatment (TAM). Unlike *in vivo*, tumor cells *in vitro* express PKM1 upon PKM2 deletion; however, PKM1 expression reduces growth rate.

6. PKM1 Expression Suppresses Xenograft Tumor Proliferation

PKM2 -/- Mouse Tumor Cell Line Xenograft



5. A PKM2 -/- cell line was stably infected with empty vector or cDNAs for PKM1 or PKM2. PKM1-expressing cells did not proliferate or form tumors but were found at the injection site after 35 days.

Conclusions

- **Deletion of PKM2 in a mouse mammary tumor model accelerates tumor growth, consistent with the reduction in PKM2 activity seen during growth signaling.**
- **Cell line establishment selects for rare PKM2 expressing tumor cells.**
- **PKM1 expression slows growth in vitro and suppresses proliferation in a xenograft model, suggesting that high PK activity from PKM1 is not conducive to proliferation.**

Acknowledgements

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