Pyruvate Kinase M2 Mutations in Human Cancers: Effects on Enzyme Function

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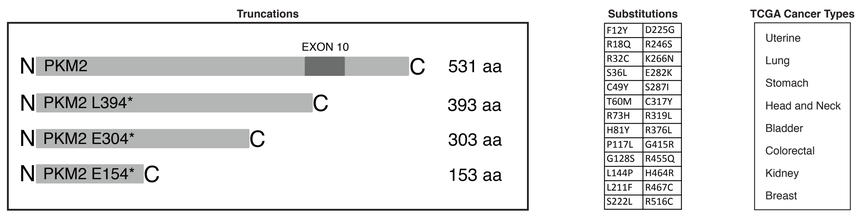
ABSTRACT

Cancer cells must regulate metabolic fluxes to meet both anabolic and catabolic needs. An important point of metabolic regulation is the final step in glycolysis, which is catalyzed in cancer cells by the M2 isoform of pyruvate kinase (PKM2). Mutations in PKM2 arising in human cancers are of special interest because the functional effects of these mutations may provide insight into the selective pressures placed on the regulation of metabolism in human tumors. We therefore sought to characterize the effects of PKM2 mutations from patient tumor samples on the metabolic function of the enzyme. The majority of identified PKM2 mutations were point mutations resulting in changes to highly conserved amino acids, with 13 of 26 mutations occurring in residues that are perfectly conserved from humans to S. cerevisiae. Kinetic characterization of mutant enzymes showed that the mutations generally reduce enzymatic activity by lowering Vmax, increasing Km for substrate (phosphoenolpyruvate), or both. Some amino acid substitutions also reduce binding affinity for the allosteric activator fructose-1,6-bisphosphate (FBP) or completely abolish activation by FBP. These results suggest that tumor growth conditions in vivo tolerate or select for reduced PKM2 activity.

FIGURE 1 PKM2 Mutations Occur in Human Cancers

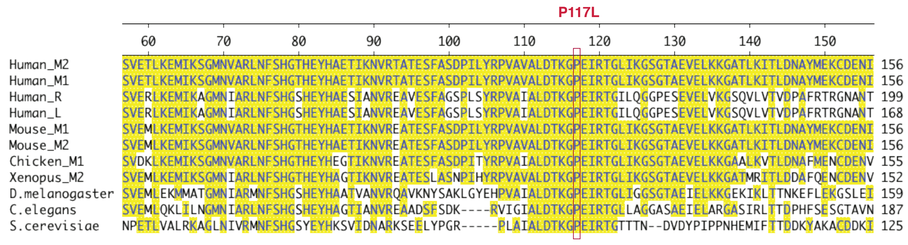
A. Missense Mutations Cause Truncations And Substitutions



Substitutions	
F12Y	D225G
R18Q	R246S
R32C	K266N
S36L	E282K
C49Y	S287I
T60M	C317Y
R73H	R319L
H81Y	R376L
P117L	G415R
G128S	R455Q
L144P	H464R
L211F	R467C
S222L	R516C

TCGA Cancer Types: Uterine, Lung, Stomach, Head and Neck, Bladder, Colorectal, Kidney, Breast

B. Amino Acid Substitutions Occur in Highly Conserved Residues



C. Locations of Select Amino Acid Substitutions are Highlighted in Blue
Kinetics for Numbered Mutants Are Shown in Figure 3.

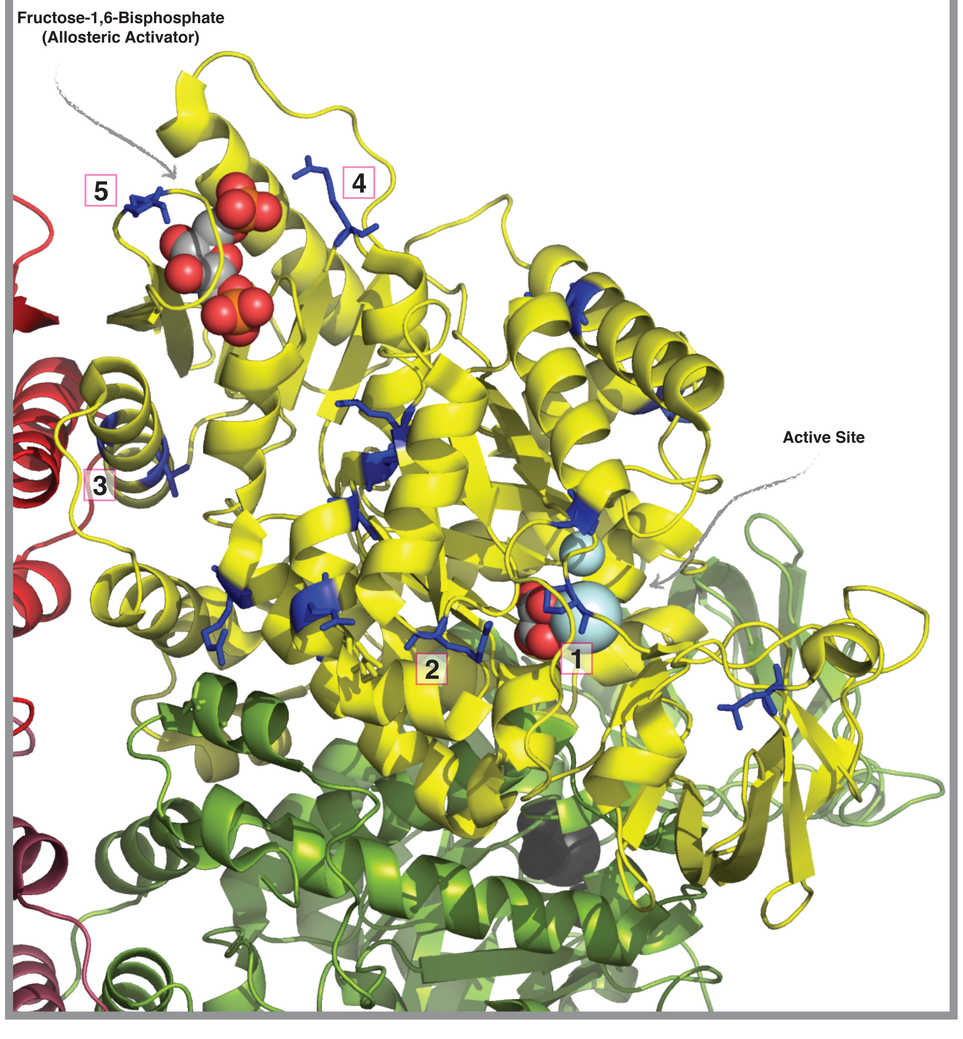
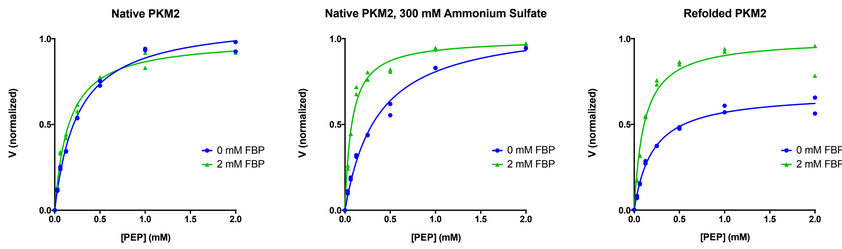


FIGURE 2 PKM2 Copurifies with Bound Fructose-1,6-Bisphosphate (FBP), an Allosteric Activator

A. Single-step IMAC Purification Does Not Remove FBP



B. FBP Activation Can Be Observed After Refolding Without FBP or When Assaying Non-Tetramer Fraction

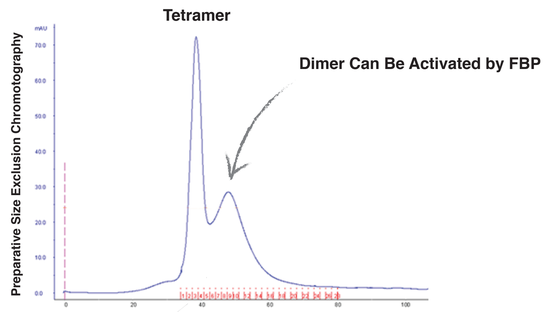
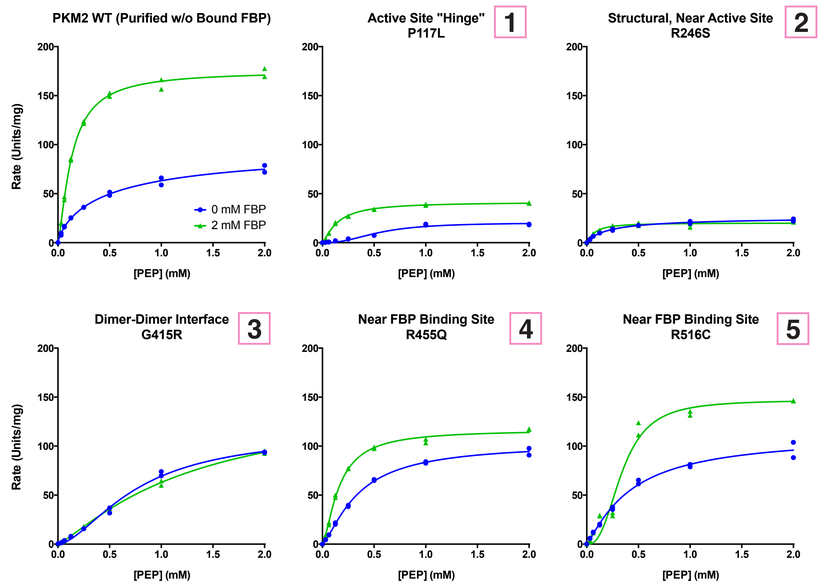


FIGURE 3 Mutations Tend to Reduce PKM2 Activity and Can Affect FBP Binding

A. Enzyme Kinetics With Respect to Phosphoenolpyruvate



B. Functional Assay for FBP Binding

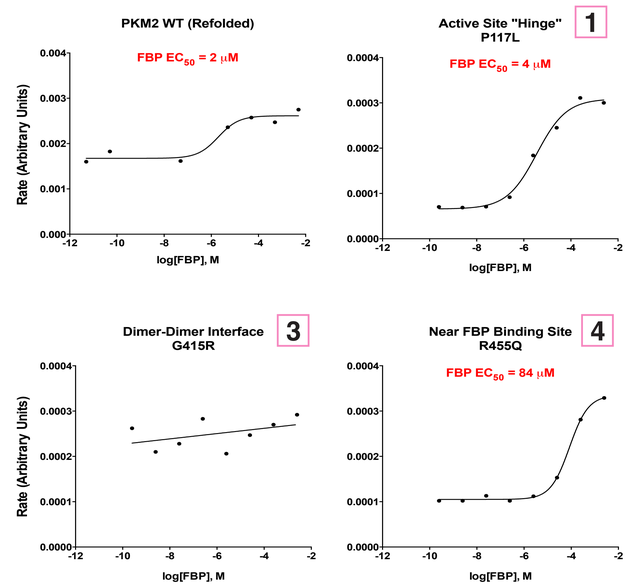
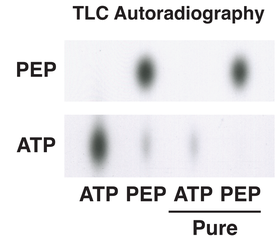
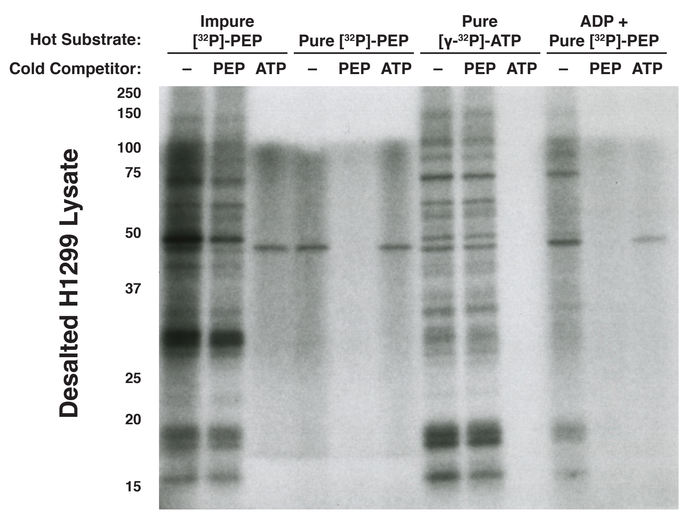


FIGURE 4 Looking for PEP-Dependent Protein Kinase Activity

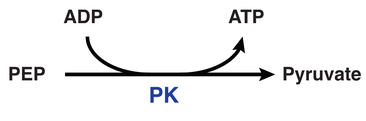
A. Generation of Pure ^{32}P-labeled ATP and PEP



B. PEP-Dependent Protein Phosphorylation Requires ADP



C. The Reaction Catalyzed by Pyruvate Kinase



CONCLUSIONS

- Point Mutations in PKM2 Have Been Found in Human Tumors
- Cancer-Associated Amino Acid Substitutions Affect PKM2 Enzymatic Function
- PEP-Dependent Protein Phosphorylation Requires the Presence of ADP

ACKNOWLEDGEMENTS

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