


 METABOLISM

Warburg effect revisited

STOCKBYTE

It was Otto Warburg who first noted the difference between metabolism in cancer cells and that in normal adult tissues: cancer cells take up glucose at higher rates than normal tissue but use a smaller fraction of this glucose for oxidative phosphorylation. This effect is known as aerobic glycolysis or the Warburg effect. Lewis Cantley and colleagues now report that the human M2 (fetal) isoform of pyruvate kinase (*PKM2*), an enzyme that is involved in glycolysis, is a phosphotyrosine-binding protein and promotes the Warburg effect.

The authors identified PKM2 in a proteomic screen for phosphotyrosine-binding proteins. Although four pyruvate kinase isoforms exist in mammals, phosphotyrosine-peptide binding is specific to the M2 isoform. PKM2 contains a 56-amino-acid stretch, which forms an allosteric pocket unique to PKM2 that allows binding of its activator, fructose-1,6-bisphosphate (FBP). Binding of phosphotyrosine peptides to PKM2 results in release of the allosteric activator FBP and

subsequent inhibition of enzymatic activity.

To assess whether tyrosine phosphorylation can regulate the activity of PKM2 in cells, the authors tested the effects of tyrosine kinase overexpression (by expressing constitutively active SRC) and stimulation of the insulin-like growth factor receptor tyrosine kinase. Both experiments resulted in inhibition of PKM2 activity. Analysis of cell lines expressing the different pyruvate kinase isoforms confirmed that this effect is specific to the M2 isoform and requires the phosphotyrosine-peptide binding capability.

What is the biological role of this novel regulation? Cancer cell lines exclusively express PKM2, and knockdown of PKM2 expression in cancer cells results in reduced glycolysis and decreased cell proliferation. Both a wild-type mouse M2 and a phosphotyrosine-binding mutant M2KE can rescue pyruvate kinase activity and glycolysis in knockdown cells. However, unlike the wild-type mutant, M2KE is unable to rescue

the proliferation phenotype. Further analysis of M2KE-mutant cells revealed reduced lactate production and increased oxygen consumption compared with wild-type cells. This finding indicates that tyrosine kinase regulation of PKM2 activity is involved in mediating the Warburg effect in tumour cells.

But how do tumour cells achieve this altered metabolic phenotype? The authors reasoned that tumour tissue switches pyruvate kinase expression from an adult isoform to the embryonic M2 isoform. Immunoblotting and immunohistochemistry analysis of a breast cancer tumour model showed that PKM1 is the primary isoform before tumour development, whereas PKM2 is the primary isoform in four independent tumours. The authors then knocked down PKM2 expression in human cancer cell lines and expressed PKM1 instead. This switch — from the fetal (M2) to the adult (M1) isoform — leads to reduced lactate production and increased oxygen consumption; however, the mechanism by which this occurs is unclear. *In vivo*, PKM2 expression was found to provide a selective growth advantage for tumour cells in nude mouse xenografts.

The observation that expression of the M2 isoform is advantageous for tumour cell growth *in vivo* demonstrates that the unique metabolism of tumour cells is crucial for tumorigenesis.

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ORIGINAL RESEARCH PAPERS Christofk, H. R. et al. Pyruvate kinase M2 is a phosphotyrosine-binding protein. *Nature* 13 Mar 2008 (doi:10.1038/nature06667) | Christofk, H. R. et al. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature* 13 Mar 2008 (doi:10.1038/nature06734)