PROLIFERATION

Multitasking

Pyruvate kinase catalyses the final step in glycolysis and as such can influence cellular metabolism. Recent studies have also shown that one isoform of this enzyme, PKM2, which is the predominant form expressed by cancer cells, is involved in the regulation of transcription. Two recent papers have examined these apparently unrelated functions in greater detail.

Building on their previous findings showing that activation of the epidermal growth factor (EGF) signalling pathway promotes the nuclear translocation of PKM2 where it binds to β -catenin (which has been phosphorylated on Tyr333) and then to the cyclin D1 (*CCND1*) promoter, Zhimin Lu and colleagues examined why the presence of PKM2 at this promoter is required for the acetylation of histone 3 (H3). Immunoprecipitation studies showed that PKM2 is required for the phosphorylation of H3-Thr11 and that this then enables the acetylation of H3-Lys9. Pull-down analyses showed that PKM2 and H3 interact and that this is increased by EGF signalling. Moreover, in the presence of phosphoenolpyruvate (PEP; the physiological phosphate group donor for PKM2), PKM2 phosphorylates H3-Thr11, and this promotes the dissociation of histone deacetylase 3 (HDAC3) from the *CCND1* and *MYC* promoters. This sequence of events was shown to be essential for the induction of cyclin D1 and *MYC* expression in response to EGF in glioblastoma cell lines, and these events also required the kinase activity of PKM2. Depletion of PKM2 in glioblastoma cells that expressed a constitutively active EGF receptor (EGFRvIII) reduced proliferation *in vitro* and xenograft growth *in vivo*.

Moreover, expression of recombinant H3 in which Thr11 was mutated to alanine in glioblastoma cells that were depleted of endogenous H3 had similar effects. Immunohistochemical analysis of 45 human glioblastoma samples indicated that nuclear localization of PKM2, high levels of H3-Thr11 phosphorylation and EGFR activity correlated with one another. Additionally, high levels of H3-Thr11 phosphorylation significantly correlated with lower median overall survival and were associated with high glioblastoma grade in 85 glioblastoma samples. Thus, the nuclear localization and kinase activity of PKM2 would seem to be required for glioblastoma progression.

Moving from the nucleus to the cytoplasm, Matthew Vander Heiden and colleagues have previously shown that the expression of the physiologically constitutively active PKM1 isoform in H1299 human non-small-cell lung cancer xenografts reduces their growth, possibly owing to the high enzymatic activity of PKM1 compared with PKM2. PKM2 has low activity in cancer cells partly because of its ability to bind Tyr-phosphorylated proteins, which leads to the release of the physiological activator of PKM2, fructose-1,6-bisphosphate (FBP). Having expressed a Flag-tagged PKM1 in H1299 cells, these authors found that it increased pyruvate kinase activity as expected and formed active heterocomplexes with endogenous PKM2. These cells also failed to efficiently form tumours when injected into nude mice, and those that did grow had reduced expression levels of Flag-PKM1 and reduced pyruvate kinase activity, which was consistent with the hypothesis that reduced pyruvate kinase activity supports tumour growth. Given the low activity of PKM2 in cancer cells, could small-molecule activators of PKM2 have therapeutic potential?

Vander Heiden and colleagues recently identified two distinct classes of PKM2 activators. A representative compound from each class increased PKM2 activity by inducing the formation of homotetramers, much like FBP. However, structural analyses showed that the small-molecule compounds bound at sites that were distinct from those bound by FBP. More importantly, the small-molecule-induced PKM2 tetramers were not sensitive to inhibition by Tyr-phosphorylated proteins. Treatment of mice for 7 weeks with one of the molecules, TEPP-44, significantly reduced the growth of H1299 cell xenografts. Thus, activating PKM2 mimics the effects of expressing PKM1 in H1229 cells.

It will be interesting to discover whether such small-molecule activators also affect PKM2-mediated gene transcription.

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ORIGINAL RESEARCH PAPERS Yang, W. *et al.* PKM2 phosphorylates histone H3 and promotes gene transcription and tumorigenesis. *Cell* **150**, 685–696 (2012) | Anastasiou, D. *et al.* Pyruvate Kinase M2 activators promote tetramer formation and suppress tumorigenesis. *Nature Chem. Biol.* 26 Aug 2012 (doi:10.1038/NCHEMBIO.1060)

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