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CANCER METABOLISM

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> PKM2 is both activated by and activates hypoxia-inducible factor 1 (HIF1)-dependent transcription

Pyruvate kinase (PK) catalyses a crucial step in glycolysis. The PK isoform PKM2, but not the PKM1 isoform, promotes this pathway in cancer cells, thus facilitating the switch from oxidative phosphorylation to glucose metabolism to generate ATP. However, how PKM2 accomplishes this was not clear based on the known biochemical activities of the enzyme. Gregg Semenza and colleagues have delineated a positive feedback pathway in cancer cell lines through which PKM2 is both activated by and activates hypoxia-inducible factor 1 (HIF1)-dependent transcription.

Both PKM2 and PKM1 result from alternative splicing of the *PKM2* gene. Using wild-type and HIF1α-knockout mouse embryonic fibroblasts, the authors showed that HIF1α is required for the induction of both *Pkm1* and *Pkm2* mRNA under hypoxic conditions. The human *PKM2* gene contains a putative hypoxia response element (HRE), and chromatin immunoprecipitation (ChIP) assays in HeLa cells demonstrated that HIF1α, but not HIF2α, binds to the *PKM2* HRE. Moreover, short hairpin RNA (shRNA) knockdown of HIF1α, but not HIF2α, reduced *PKM2* HRE-dependent transcription.

Interestingly, PKM2 protein levels were increased not only in the cytosol, but also in the nucleus of HeLa cells in hypoxic conditions, indicating that PKM2 might also regulate HIF1 transcriptional activity. Indeed, co-immunoprecipitation experiments demonstrated that HIF1α and PKM2 interact in the nucleus of HeLa cells, and experiments using HIF1 transcriptional reporter constructs indicated that PKM2 increases HIF1-dependent transcription in both HeLa and Hep3B hepatoblastoma cells via the stimulation of the HIF1α transactivation domain. Finally, PKM2 could also promote HIF2-dependent transcription by the same mechanism, but the PKM1 isoform could not stimulate HIF1-dependent transcription.

The alternatively spliced domain of PKM2 that is not present in PKM1 contains a prolyl hydroxylation motif. The authors showed that this domain is hydroxylated on two proline residues by prolyl hydroxylase 3 (PHD3) in HeLa cells, and that PKM2 hydroxylation enhances its interaction with and transactivation of HIF1α. Similar results were observed in RCC4 renal carcinoma cells (which constitutively express HIF1α in non-hypoxic conditions).

Does PKM2 affect the transcription of HIF target genes? ChIP assays in hypoxic HeLa cells expressing *PKM2* shRNA showed reduced HIF1α and HIF2α binding on the HREs of HIF target genes, and additional ChIP assays in hypoxic HeLa cells revealed that both PKM2 and PHD3 occupy HREs of HIF1 target genes. Furthermore, the authors found that *PKM2* or *PHD3* shRNA reduced the transcription of HIF1 target genes that encode glycolytic enzymes and glucose transporters in hypoxic HeLa cells, and a similar effect was evident in RCC4 cells in which PHD3 was knocked down.

If this pathway is confirmed in human tumours it may explain the function of PKM2 in promoting metabolic reprogramming and cancer progression.

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ORIGINAL RESEARCH PAPER Luo, W. *et al.* Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. *Cell* **145**, 732–744 (2011)