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PKM2, STAT3 and HIF-1α

The Warburg's vicious circle

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The M2 isoform of pyruvate kinase, highly expressed in tumor cells, is known to engage a feed forward loop with the glycolysis master transcription factor HIF-1α. Gao and co-authors recently showed that dimeric PKM2 localizes to the nucleus in highly proliferating cancer cells, where it regulates in vivo growth by acting as a protein kinase and directly activating STAT3. STAT3 is therefore a novel player of the PKM2/HIF-1α feedback loop, since HIF-induced PKM2 activates STAT3 that in turn induces HIF-1α expression. These findings have profound implications for understanding the complex connections between gene regulation, metabolism, survival and proliferation in cancer.

Keywords: pyruvate kinase isoforms, PKM2, glycolysis, Warburg, cancer, cell proliferation, malignant transformation, growth factors, STAT3

Abbreviations: TCA, tricarboxylic acid cycle; PKM, pyruvate kinase M-type; NADPH, nicotinamide adenine dinucleotide phosphate; IL-3, interleukin-3; EGFR, epidermal growth factor receptor; CCND1, cyclin D1 gene; HIF-1α, hypoxia-inducible factor-1α; MEK5, mitogen-activated protein kinase kinase 5; STAT3, signal transducer and activator of transcription 3; PEP, phosphoenolpyruvic acid; ADP, adenosine diphosphate; IL-6, interleukin-6; NFκB, nuclear factor-κB

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Pyruvate kinase (PK) catalyzes the penultimate step of glycolysis by transferring phosphate from phosphoenolpyruvate (PEP) to ADP to generate ATP and pyruvate, which can then be converted in either lactate or acetyl-CoA to enter the TCA cycle. Of the four known isoforms, PKM1 and PKM2 derive from an alternative splicing of the same *pkm2* gene.[1] PKM2 is highly expressed in embryonal cells and in tumors, and its prevalent expression in cancer cells has been linked to lower O_2 consumption and higher lactate secretion and glucose intake. The switch to the PKM2 isoform, observed in tumors of different origin,[2,3] was shown to be essential for both the "Warburg" effect and proliferation of cancer cells.[4,5]

The main feature distinguishing PKM2 from the PKM1 isoform is the fact that its pyruvate kinase activity is subject to multiple layers of regulation. PKM2 can exist as a tetramer, which is enzymatically active, or as an inactive dimer. Interestingly, tumors show very high levels of dimeric PKM2, which in normal proliferating cells is in contrast mainly tetrameric.[6] Multiple signals such as growth factors, oncogenes or reactive oxygen species (ROS) were shown to destabilize the tetrameric form via a number of mechanisms including tyrosine phosphorylation, association with tyrosine phosphopeptides or oxidation.[7-9] In turn, decreased PKM2 activity correlates with increased proliferation. Although this can be partly explained by the diversion of glucose metabolites from catabolic to anabolic processes[10] and by the increased generation of anti-oxidant NADPH via the pentose phosphate pathway,[9] a consistent body of data points toward an independent role for PKM2 in the nucleus. Indeed, it was shown that both IL-3 and EGFR can induce PKM2 nuclear localization, correlating with cell proliferation.[11,12] Nuclear PKM2 can associate with cSrc-phosphorylated β-catenin, and bind to the *ccnd1* gene promoter activating cyclin D1 transcription.[12] Finally, PKM2 was recently proposed to be part of a "feed forward loop" enhancing the activity of hypoxia-inducible factor (HIF)-1, a key transcriptional regulator of both aerobic and anaerobic glycolysis.[13] Indeed, HIF-1 can activate *pkm2* gene transcription, and PKM2 in turn interacts with the HIF-1α subunit and promotes trans-activation of HIF-1 target genes, thus enhancing cellular responses to oxygen deprivation or oncogene activation.

To investigate the functional significance of nuclear PKM2, Gao et al.[14] have

compared PKM2 nuclear localization in different cancer cell lines characterized by differential proliferative ability. Interestingly, they observed a strong correlation between PKM2 nuclear levels, cell proliferation and *mek5* gene transcription. Upon showing by ChIP analysis that PKM2 associates to the *mek5* promoter, they went on demonstrating that nuclear PKM2 interacts with the transcription factor STAT3 and is able to directly trigger its activation via phosphorylation on tyrosine 705. The authors were also able to demonstrate that *mek5* is a direct STAT3 transcriptional target and that the levels of dimeric PKM2 directly correlate with STAT3-mediated *mek5* transcription, cell proliferation and in vivo growth of tumor cells. Interestingly, PKM2-mediated STAT3 tyrosine phosphorylation can be accomplished in vitro exclusively by dimeric PKM2, which uses PEP as a phosphate donor and likely interacts with its substrate via the ADP binding domain. Incidentally, the reaction will lead to pyruvate production via a yet alternative pathway.

STAT3 is a point of convergence for many oncogenic signals, and its aberrant constitutive activity is crucial for the survival, proliferation and metastatic activity of tumors of different origin.[15] Moreover, STAT3 constitutive activity plays a key role in inflammation-induced cancer, by instating a feed forward loop entailing continuous production of the pro-inflammatory cytokine IL-6 and constitutive NFκB activation.[16] We have recently shown that constitutively active STAT3 induces chronically enhanced *hif-1α* transcription that results in increased HIF-1α protein levels and triggers a metabolic switch toward aerobic glycolysis similar to the Warburg effect.[17,18]

STAT3 activation appears thus to participate in the recently proposed PKM2/HIF-1α positive feedback loop,[13] where oxygen deprivation or oncogenes lead to increased HIF-1α levels, HIF-1 induces *pkm2* transcription, PKM2 enhances HIF-1 trans-activating power and activates STAT3 and finally activated STAT3 enhances HIF-1α expression (**Fig. 1**). On the other hand, we have recently shown that constitutively active STAT3 can act as a first hit during the process of malignant transformation.[19] Aberrantly continuous STAT3 activation like that found in chronic inflammation might therefore sensitize cells to tumor transformation by initiating the above described positive feedback loop, leading to enhanced HIF-1α and PKM2 expression/activity. This would in turn support aerobic glycolysis, anabolic cell metabolism, cell survival and proliferation. Moreover, PKM2-mediated STAT3 phosphorylation may participate in the chronic STAT3 activation observed in highly glycolytic tumors of different origins. Although these findings shed some light on the correlation between the

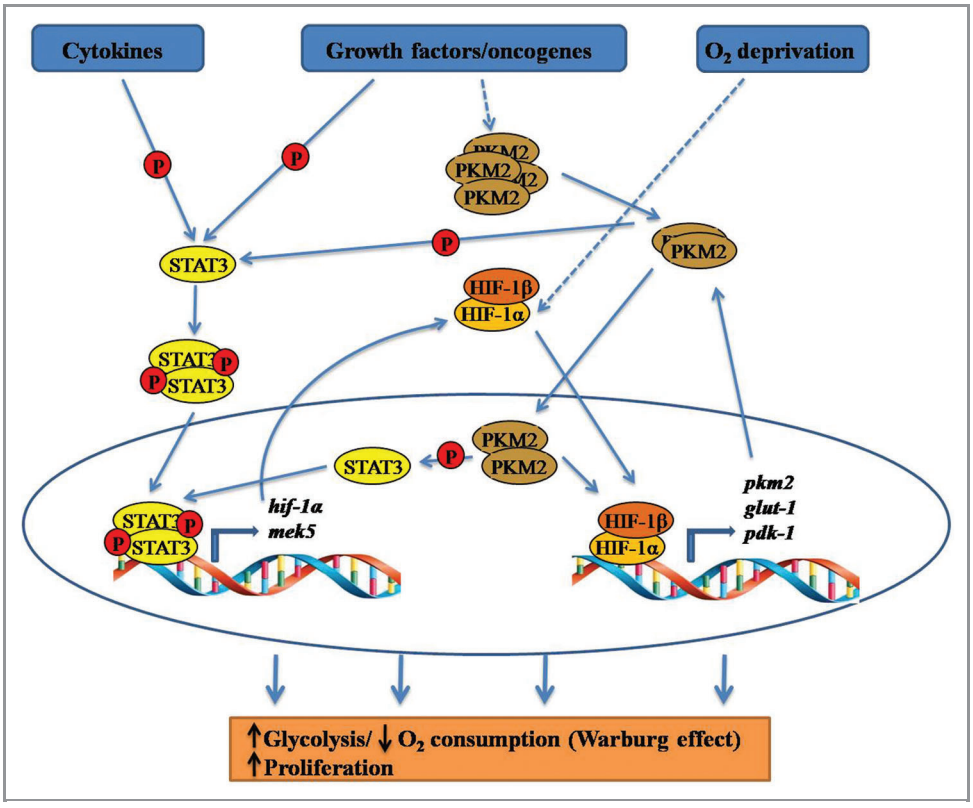

Figure 1. STAT3/PKM2/HIF-1α feed forward loop. Oxygen deprivation, growth factors or oncogenes lead to an increase in HIF-1α levels, HIF-1 induces *pkm2* transcription, PKM2 both enhances HIF-1 trans-activating power and activates STAT3 and activated STAT3 induces HIF-1α expression. On the other hand, cytokines, growth factors or oncogenes activate STAT3 that can induce *hif-1α* transcription and start the positive feedback loop between STAT3, PKM2 and HIF-1α. However initiated, this leads to increased levels of proteins involved in glycolysis, enhancing the anaerobic-like metabolism known as Warburg effect.

tetrameric:dimeric PKM2 ratio, its nuclear activities and enhanced cell proliferation, several questions still remain unanswered. For example, why does PKM2 preferentially localize to the nucleus in more proliferating cells? Is this a special feature of cancer cells? What is the mechanism that drives dimeric PKM2 to the nucleus? An intriguing possibility, adding one more layer of cross-talk, would be that the transcription factors/co-factors known to interact with PKM2, i.e., β-catenin, HIF-1α or STAT3, may mediate its transport to the nucleus. Another interesting implication of the findings by Gao et al. is the idea that STAT3 phosphorylation may take place in the nucleus, opposed to its canonical cytoplasmic activation pathway. This correlates with data sets showing that unphosphorylated STAT3 is continuously shuttling between the cytoplasm and the nucleus generating a reservoir of nuclear unphosphorylated STAT3 available for activation.[20] Additionally, the simultaneous detection of both PKM2 and STAT3 on the *mek5* promoter suggests that nuclear phosphorylation of STAT3 by PKM2 might even occur on the DNA, similar to what has been recently shown for STAT3 serine phosphorylation.[21]

Small molecules enhancing PKM2 tetrameric activity have already been proposed as new therapeutic tools to compromise both the anabolic and the anti-oxidant functions of this protein.[9] The same compounds may also lead to the inhibition of PKM2-mediated STAT3 activation, potentially representing a useful therapeutic tool to help hitting the uncontrolled growth of those highly glycolytic tumors that display both STAT3 activation and dimeric PKM2.

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References

1. Noguchi T, Inoue H, Tanaka T. The M1- and M2-type isozymes of rat pyruvate kinase are produced from the same gene by alternative RNA splicing. J Biol Chem 1986; 261:13807-12; PMID:3020052
2. Brinck U, Eigenbrodt E, Oehmke M, Mazurek S, Fischer G. L- and M2-pyruvate kinase expression in renal cell carcinomas and their metastases. Virchows Arch 1994; 424:177-85; PMID:8180780; http://dx.doi.org/10.1007/BF00193498
3. Steinberg P, Klingelhöffer A, Schäfer A, Wüst G, Weisse G, Oesch F, et al. Expression of pyruvate kinase M2 in preneoplastic hepatic foci of N-nitrosomorpholine-treated rats. Virchows Arch 1999; 434:213-20; PMID:10190300; http://dx.doi.org/10.1007/s004280050330
4. Warburg O. On respiratory impairment in cancer cells. Science 1956; 124:269-70; PMID:13351639
5. Christofk HR, Vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, Wei R, et al. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. Nature 2008; 452:230-3; PMID:18337823; http://dx.doi.org/10.1038/nature06734
6. Mazurek S. Pyruvate kinase type M2: a key regulator of the metabolic budget system in tumor cells. Int J Biochem Cell Biol 2011; 43:969-80; PMID:20156581; http://dx.doi.org/10.1016/j.biocel.2010.02.005
7. Hitosugi T, Kang S, Vander Heiden MG, Chung TW, Elf S, Lythgoe K, et al. Tyrosine phosphorylation inhibits PKM2 to promote the Warburg effect and tumor growth. Sci Signal 2009; 2:ra73; PMID:19920251; http://dx.doi.org/10.1126/scisignal.2000431
8. Christofk HR, Vander Heiden MG, Wu N, Asara JM, Cantley LC. Pyruvate kinase M2 is a phosphotyrosine-binding protein. Nature 2008; 452:181-6; PMID:18337815; http://dx.doi.org/10.1038/nature06667
9. Anastasiou D, Poulogiannis G, Asara JM, Boxer MB, Jiang JK, Shen M, et al. Inhibition of pyruvate kinase M2 by reactive oxygen species contributes to cellular antioxidant responses. Science 2011; 334:1278-83; PMID:22052977; http://dx.doi.org/10.1126/science.1211485
10. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 2009; 324:1029-33; PMID:19460998; http://dx.doi.org/10.1126/science.1160809
11. Hoshino A, Hirst JA, Fujii H. Regulation of cell proliferation by interleukin-3-induced nuclear translocation of pyruvate kinase. J Biol Chem 2007; 282:17706-11; PMID:17446165; http://dx.doi.org/10.1074/jbc.M700094200
12. Yang W, Xia Y, Ji H, Zheng Y, Liang J, Huang W, et al. Nuclear PKM2 regulates β-catenin transactivation upon EGFR activation. Nature 2011; 480:118-22; PMID:22056988; http://dx.doi.org/10.1038/nature10598
13. Luo W, Hu H, Chang R, Zhong J, Knabel M, O'Meally R, et al. Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. Cell 2011; 145:732-44; PMID:21620138; http://dx.doi.org/10.1016/j.cell.2011.03.054
14. Gao X, Wang H, Yang JJ, Liu X, Liu ZR. Pyruvate kinase M2 regulates gene transcription by acting as a protein kinase. Mol Cell 2012; 45:598-609; PMID:22306293; http://dx.doi.org/10.1016/j.molcel.2012.01.001
15. Yu H, Kortylewski M, Pardoll D. Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment. Nat Rev Immunol 2007; 7:41-51; PMID:17186030; http://dx.doi.org/10.1038/nri1995
16. Li N, Grivennikov SI, Karin M. The unholy trinity: inflammation, cytokines, and STAT3 shape the cancer microenvironment. Cancer Cell 2011; 19:429-31; PMID:21481782; http://dx.doi.org/10.1016/j.ccr.2011.03.018
17. Demaria M, Giorgi C, Lebiedzinska M, Esposito G, D'Angeli L, Bartoli A, et al. A STAT3-mediated metabolic switch is involved in tumour transformation and STAT3 addiction. Aging (Albany NY) 2010; 2:823-42; PMID:21084727
18. Demaria M, Poli V. From the nucleus to the mitochondria and back: the odyssey of a multitask STAT3. Cell Cycle 2011; 10:3221-2; PMID:21926478; http://dx.doi.org/10.4161/cc.10.19.17379
19. Demaria M, Misale S, Giorgi C, Miano V, Camporeale A, Campisi J, et al. STAT3 can serve as a hit in the process of malignant transformation of primary cells. Cell Death Differ 2012; 19:1390-7; PMID:22402588; http://dx.doi.org/10.1038/cdd.2012.20
20. Vogt M, Domoszlai T, Kleshchanok D, Lehmann S, Schmitt A, Poli V, et al. The role of the N-terminal domain in dimerization and nucleocytoplasmic shuttling of latent STAT3. J Cell Sci 2011; 124:900-9; PMID:21325026; http://dx.doi.org/10.1242/jcs.072520
21. Yang J, Huang J, Dasgupta M, Sears N, Miyagi M, Wang B, et al. Reversible methylation of promoter-bound STAT3 by histone-modifying enzymes. Proc Natl Acad Sci U S A 2010; 107:21499-504; PMID:21098664; http://dx.doi.org/10.1073/pnas.1016147107