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AUTHOR'S VIEW

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# Linking apoptosis to cancer metabolism: Another missing piece of JuNK

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**ABSTRACT**

Cancer cells become dependent on aerobic glycolysis to sustain rapid proliferation and escape apoptosis. How this metabolic change, also known as the Warburg effect, is linked to apoptosis remains largely unknown. Our new data place c-Jun N-terminal kinase in the center of a hub regulating apoptosis and cancer metabolism.

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The c-Jun N-terminal kinases (JNKs) are members of a larger group of serine/threonine (Ser/Thr) protein kinases known as mitogen-activated protein kinases (MAPKs).[1] Like many other members of the MAPK family that regulate a large variety of cellular functions, the role of JNKs in cancer is complex.[2] For certain type of cancers, activation of JNK signaling has been described as a key tumor promoter factor; in other cancer settings JNK activation is needed for promotion of apoptosis of malignant cells, especially in response to chemotherapeutic drugs inhibiting specific oncogenic signals. This dichotomy is in part explained by the fact that 2 ubiquitously expressed JNK isoforms (JNK1 and JNK2) function differently depending on the cellular context. This complexity could be also due to the increased number of downstream substrates that are activated by JNKs.[1,2]

Originally named JNKs for their ability to phosphorylate and activate the transcription factor c-Jun, the number of JNK substrates has considerably increased since their discovery and characterization.[1] Many JNK substrates are also transcription factors themselves, therefore the JNK-mediated effects are further boosted by these cascades of activation.[1] Recent work from our group has identified the pyruvate kinase M2 isoform (PKM2) as a novel and direct substrate of JNK1, adding another piece to the complexity of the JNK cascade.[3] We have shown that the basal activity of JNK1 is masked by enhanced expression of the antiapoptotic protein poly(ADP-ribose) polymerase family member 14 (PARP14) in a variety of human cancer cells. Remarkably, PARP14 expression is also associated with poor patient prognosis.[3,4] Mechanistically, PARP14 promotes the survival of cancer cells by binding and inhibiting JNK1.[3,4] This inhibition promotes the Warburg effect and glycolytic gene expression by enhancing the activity of pyruvate kinase isozyme type M2 (PKM2). PARP14, therefore, restricts PKM2 activity through inactivation of JNK1 signaling (Fig. 1).[3]

PKM2 is a tumor-specific isoform of the glycolytic enzyme pyruvate kinase (PK), which catalyzes the synthesis of pyruvate and ATP using phosphoenolpyruvate (PEP) and ADP as substrates.[5] Recent research in cellular metabolism has recognized PKM2 as a critical mediator of the Warburg effect.[5-9] Cancer cells, like all highly proliferating cells, facilitate the uptake of glucose and the incorporation of glycolytic metabolites into amino acids, nucleic acids, and lipids (Fig. 1).[6] Moreover, while adapting to these new metabolic needs, the Warburg effect produces increased levels of antioxidant proteins to detoxify reactive oxygen species (ROS), which would result in apoptosis resistance in tumor tissues and therefore contributes to uncontrolled growth.[5,6] Reduced PKM2 activity allows the accumulation of glucose-6-phosphate (G6P), shifting the glucose flux through the pentose phosphate pathway (PPP) to generate reduced nicotinamide adenine dinucleotide phosphate (NADPH) and consequently antioxidant reduced glutathione (GSH). On the contrary, increased PKM2 activity shifts G6P toward pyruvate formation with consequential loss of antioxidant capacities. These losses eventually lead to accumulation of ROS and apoptosis of cancer cells.[5] Therefore, regulation of PKM2 activity is essential in the life and death decision of cancer cells (Fig. 1). The key question is: What makes PKM2 switch from a high- to low-activity state?

Coimmunoprecipitation analyses revealed that PKM2 formed a stable complex with JNK1 in HEK293T cells co-expressing JNK1 and PKM2.[3] Notably, this association was specific for JNK1 as no interaction was detected between JNK2 and PKM2. Endogenous interaction of PKM2 and JNK1 was also detected in different cancer cell types. To establish whether this interaction was direct or mediated by other intermediary proteins, we also demonstrated that bacterial purified PKM2 tightly bound to JNK1. Unlike the well-known interaction between PKM2 and death-associated protein kinase (DAPK),[10] the JNK1/PKM2 interaction requires the presence of "phospho-active" JNK1 to enhance the activity of PKM2, suggesting that the interaction alone is not sufficient to regulate PKM2 activity but phosphoryla-

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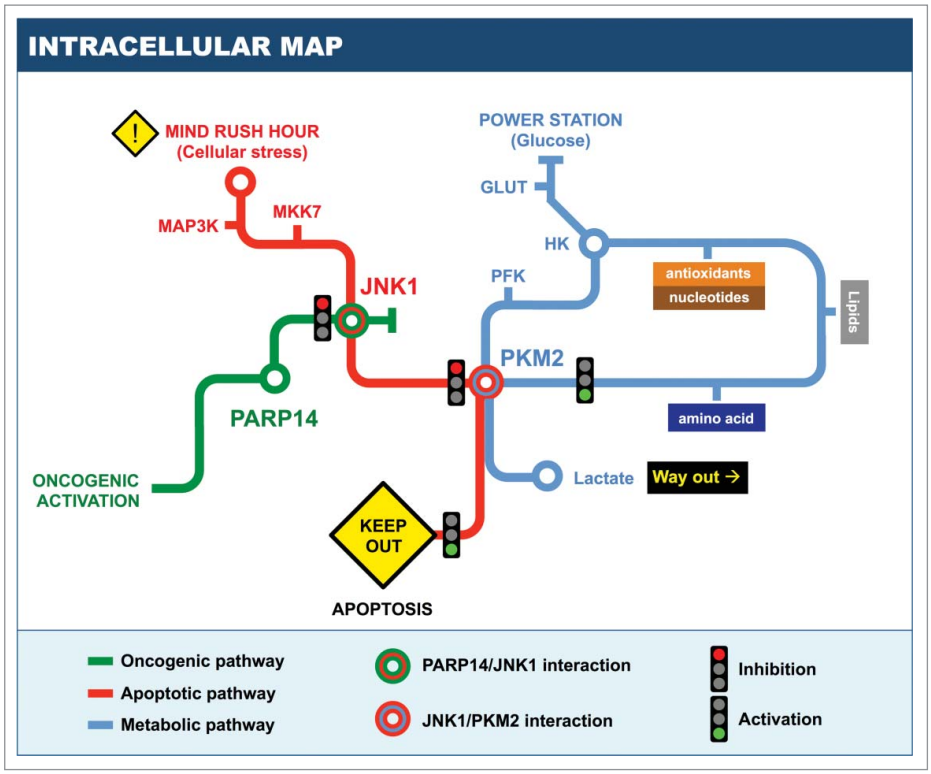


**Figure 1.** Major signaling pathways in glycolytic cancers. Schematic illustration of the intracellular pathways activated in glycolytic cancer cells. Green line: oncogenic stimuli promote the overexpression of tumor initiating molecules (i.e., PARP14). Red line: accumulation of cellular stress activates the JNK cascade that consists of mitogen-activating protein kinase kinase kinase (MAP3K), which in turn phosphorylates and activates mitogen-activated protein kinase kinase 7 (MKK7), a direct activator of JNK. Blue line: cancer cells are fuelled by glucose, which is transported into the cells via glucose transporters (GLUT). Much of the intracellular glucose is converted into lactate by glycolytic enzymes, the most important ones being hexokinase (HK), phosphofructokinase (PFK), and pyruvate kinase isozyme type M2 (PKM2). The high levels of PARP14 observed in cancer cells halt JNK1-mediated phosphorylation of downstream substrates (i.e., PKM2), thus maintaining low PKM2 activity. Consequently, there is an accumulation of upstream glycolytic intermediates that favors the synthesis of cellular building blocks (i.e., amino acid, lipids, nucleotides) that are used to generate daughter cells. During the branching of these synthetic pathways, antioxidants are also produced to counteract the cell-damaging products (i.e., reactive oxygen species [ROS]). In the event of failure to halt JNK1-mediated phosphorylation and activation of PKM2, cancer cells die by apoptosis as a result of enhanced accumulation of intracellular ROS.

tion may be required. *In vitro* kinase assays and mass spectrometry analyses demonstrated the direct phosphorylation of PKM2 Thr365 by JNK1. Consequently, the activity of PKM2 is intensified by JNK1 phosphorylation in a dose-dependent manner.[3] In cancer cells, although basal JNK1 activity is not sufficient to promote PKM2 phosphorylation and drive apoptosis, enhanced JNK1 phosphorylation induced by the absence (or inhibition) of PARP14 greatly enhanced PKM2 activity and inhibited the Warburg effect with consequential cell death. Therefore, we demonstrated that the PARP14-JNK1-PKM2 regulatory axis is an important determinant for the Warburg effect in tumor cells and provides a mechanistic link between apoptosis and metabolism.[3]

The notion that PKM2 is subject to post-translational modification to change its catalytic activity or cellular function is not novel.[7] Direct phosphorylation of PKM2 Tyr105 by fibroblast growth factor receptor type 1 (FGFR1) inhibits its catalytic activity, providing a metabolic advantage to tumor cells.[8] Phosphorylation of Tyr105 uncouples the binding of fructose-1,6-biphosphate preventing the formation of active tetrameric PKM2. Additional phosphorylation sites have been also described. For instance, ERK2-dependent phosphorylation of PKM2 Ser37 helps to recruit peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (PIN1) for cis-trans isomerization of PKM2, which serves to translocate PKM2 to the nucleus.[9] Although phosphorylation of PKM2 Ser37 has no effect on the activity of PKM2 itself, nuclear PKM2 acts as a co-activator of $\beta$-catenin to induce *c-MYC* and glycolytic gene expression, therefore stimulating tumor growth through indirect mechanisms.[9] In addition to PKM2 phosphorylation, amino acid acetylation, glycosylation, and oxidation, as well as protein-protein interactions with regulatory components have been also largely characterized, further validating the importance of PKM2 in tumor metabolism.[7]

Accumulating evidence suggests that cancer cells rely on a variety of metabolic reprograming activities.[6] One of these phenomena is the Warburg effect, in which cancer cells take up more glucose than normal cells and use it to generate reducing equivalents (such as NADPH, GSH) and macromolecules (such as nucleotides, proteins, and lipids) required to suppress apoptosis and generate biomass, respectively.[6] Therefore, understanding the molecular mechanism regulating this metabolic shift may provide ways to improve cancer therapeutics. Our new study offers a novel pathway whereby the PARP14-JNK1-PKM2 axis might be used as target to induce cell death through the inhibition of the Warburg effect in multiple cancer types without causing side effects in normal cells that use alternative processes of energy.[3]

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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