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Regulation of glycolysis and cancer cell proliferation by PKM2 citrullination.

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Running Title : PKM2 citrullination regulates glycolysis

Key words : NuRD complex, chromatin, melanoma, protein arginine deiminase, allosteric regulation.

The Authors declare no competing interests.

Abstract

Conversion of peptidyl-arginine to peptidyl citrulline, known as citrullination, is a post-translational protein modification catalysed by the PADI (Protein Arginine Deiminase) family of enzymes. PADI1 and PADI3 catalyse citrullination of arginine 106 in the glycolytic enzyme pyruvate kinase M2 modulating its allosteric regulation, glycolysis and cancer cell proliferation.

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2 Cancer cells are characterized by glycolysis and lactic acid production under aerobic
3
4 conditions, a phenomenon known as the Warburg effect ¹. While this is an inefficient pathway
5
6 to generate Adenosine TriPhosphate (ATP), glycolytic intermediates are used for *de novo*
7
8 synthesis of nucleotides or amino acids like serine required for cancer cell proliferation. The
9
10 catabolic and anabolic processes are integrated by an alternatively spliced isoform of the
11
12 pyruvate kinase (PK) enzyme designated PKM2 expressed in most types of cancer cells. PKM2
13
14 activity is positively regulated by serine (Ser), fructose 1,6-biphosphate an intermediate of the
15
16 glycolytic pathway and succinylaminoimidazole-carboxamide riboside, an intermediate in *de*
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18 *nov*o purine nucleotide synthesis ². When levels of these molecules become limiting PKM2
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20 activity is inhibited by amino acids such as tryptophan (Trp), alanine (Ala) and phenylalanine
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22 (Phe), coupling glycolytic flux to the level of intermediate metabolites used in anabolic
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24 processes.
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31 The activator Ser and the Trp/Phe/Ala repressors all target the same amino acid binding
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33 pocket in PKM2 in a competitive manner. Binding of Ser stabilizes the active state, whereas
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35 the hydrophobic side chains of Trp/Phe/Ala induce a conformational change in the pocket that
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37 is propagated as an allosteric switch to the inactive state ³. PKM2 R106 is located at the entrance
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39 of the amino-acid binding pocket where its positively charged guanidino group forms strong
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41 interactions with the carboxyl group of the bound amino acids to stabilize their interaction.
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46 In a recent publication ⁴, we showed that PKM2 arginine (R)106 is subject to post-
47
48 translational modification by the PADI1 (Protein Arginine Deiminase) and PADI3 enzymes,
49
50 (better known as PAD1 and PAD3) undergoing conversion of peptidyl-arginine to peptidyl
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52 citrulline, known as citrullination ⁵. Loss of R106 positive side chain charge upon citrullination
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54 reduces its interaction with the free amino acid carboxyl group. We predicted this diminished
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56 interaction would have a stronger impact on binding of Trp/Phe/Ala than on Ser that makes an
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1 extended network of hydrogen bonds within the pocket. Consequently, R106 citrullination
2 should reduce inhibition by Trp/Phe/Ala allowing PKM2 to remain active even under limiting
3 Ser concentrations leading to excessive glycolysis and reduced anabolic capacity detrimental
4 for cell growth. Indeed, synthetic small molecules that activate PKM2 stimulate glycolysis
5 resulting in Ser auxotrophy and reduced cell proliferation ⁶. We confirmed this idea by real
6 time measure of glycolysis in living cells and of PKM2 enzymatic activity in presence of
7 increasing exogenous Trp/Phe/Ala. Ectopic PAD1 and PAD3 expression partially overcame
8 Trp/Phe/Ala-induced repression of PKM2 activity leading to increased glycolysis, diminished
9 ATP levels and reduced cell proliferation (Fig. 1). Strikingly therefore, this subtle post
10 translational modification of PKM2 R106 leading to diminished hydrogen bonding capacity
11 translated into a major impact on cellular metabolism and proliferation of melanoma, renal cell
12 carcinoma, breast and cervical carcinoma cells.

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29 The key step in this pathway is regulation of expression of the *PADI1* and *PADI3* genes
30 that encode the PAD1 and PAD3 enzymes, respectively. In our recent study, we found that
31 *PADI1* and *PADI3* were repressed by Chromodomain helicase DNA binding protein 4 (CHD4);
32 the catalytic ATPase subunit of the Nucleosome Remodelling and Deacetylation (NuRD)
33 complex, an epigenetic regulator of gene expression, acting mainly as a co-repressor ⁷. SiRNA-
34 mediated CHD4 knockdown in multiple cancer cell types de-repressed *PADI1* and *PADI3*
35 expression, stimulating glycolysis and PKM2 enzymatic activity analogous to ectopic PAD1
36 and PAD3 expression. These observations therefore define a novel link between the epigenetic
37 regulation of PAD1 and PAD3 expression, PKM2 citrullination, glycolysis and cancer cell
38 proliferation.

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As described above, the near stoichiometric conversion of glucose to lactate is a
hallmark of cancer cells grown in culture that is impacted by PKM2 citrullination. In tumours
in vivo however, mitochondrial metabolism involving the tricarboxylic acid cycle (TCA) cycle

1 and oxidative phosphorylation are also essential for production of ATP and important
2 metabolites ⁸. To address the role PKM2 citrullination may play *in vivo*, we analysed *PADI1*
3 and *PADI3* expression in the TCGA (The Cancer Genome Atlas) database showing they were
4 most expressed in solid tumours like bladder, pancreatic, cervical, head and neck and clear cell
5 renal cancers with known hypoxic character. Moreover, *PADI1* and *PADI3* expression
6 positively correlated with the patient hypoxic signature of pancreatic adenocarcinoma, clear
7 cell renal cancers, and lung adenocarcinoma. Regulation by hypoxia was experimentally
8 confirmed by growth of melanoma cells in hypoxic or pseudo-hypoxic conditions *in vitro* where
9 increased PAD1 and PAD3 expression catalyzed PKM2 R106 citrullination contributing to the
10 increased glycolysis. Hypoxia therefore seems to regulate PKM2 activity by proline
11 hydroxylation ⁹ and by citrullination both of which contribute to increased glycolysis. PAD
12 enzyme expression is also de-regulated in other pathological situations characterised by
13 hypoxia such as rheumatoid arthritis (RA). Thus, PAD1 and PAD3 expression and PKM2
14 citrullination seen in RA ¹⁰ may account for the increased glycolysis seen in activated RA-
15 associated fibroblast-like synoviocytes.
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36 In conclusion, we defined a novel pathway where epigenetic and/or hypoxia-mediated
37 regulation of PAD1 and PAD3 expression modulates PKM2 citrullination, glycolysis and
38 cancer cell proliferation.
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48 **Disclosure of potential conflicts of interest**

49 The Authors declare no competing interests.
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Legend to Figure

Regulation of glycolysis by PKM2 citrullination. When present at high levels, Serine (Ser) activates pyruvate kinase M2 (PKM2; represented as a tetramer) assuring glycolysis, Adenosine TriPhosphate (ATP) production and proliferation. When Ser levels are lowered, PKM2 activity is reduced by binding of Tryptophan/Phenylalanine/Alanine (Trp/Phe/Ala) leading to reduced glycolysis. When PAD1 (protein arginine deiminase) and PAD3 are up-regulated by hypoxia or other epigenetic mechanisms, PKM2 citrullination reduces the inhibitory potential of Trp/Phe/Ala maintaining glycolysis under limiting Ser conditions and reducing the availability of glycolytic metabolites for anabolic processes. In addition, excessive pyruvate conversion to lactate limits the tricarboxylic acid cycle cycle leading to reduced ATP levels and diminished proliferation.

Figure

