Citrullination of pyruvate kinase M2 by PADI1 and PADI3 regulates glycolysis and cancer cell proliferation

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Chromodomain helicase DNA binding protein 4 (CHD4) is an ATPase subunit of the Nucleosome Remodelling and Deacetylation (NuRD) complex. CHD4 regulates expression of PADI1 (Protein Arginine Deiminase 1) and PADI3 in multiple cancer cell types modulating citrullination of arginine residues of the allosterically-regulated glycolytic enzyme pyruvate kinase M2 (PKM2). Citrullination of PKM2 R106 reprograms cross-talk between PKM2 ligands lowering its sensitivity to the inhibitors Tryptophan, Alanine and Phenylalanine and promoting activation by Serine. Citrullination thus bypasses normal physiological regulation by low Serine levels to promote excessive glycolysis and reduced cell proliferation. We further show that PADI1 and PADI3 expression is up-regulated by hypoxia where PKM2 citrullination contributes to increased glycolysis. (Coassolo et al., Nat Commun. 2021 Mar 19;12(1):1718.)

CHD4 regulates PAD1 and PAD3 expression and melanoma cell proliferation

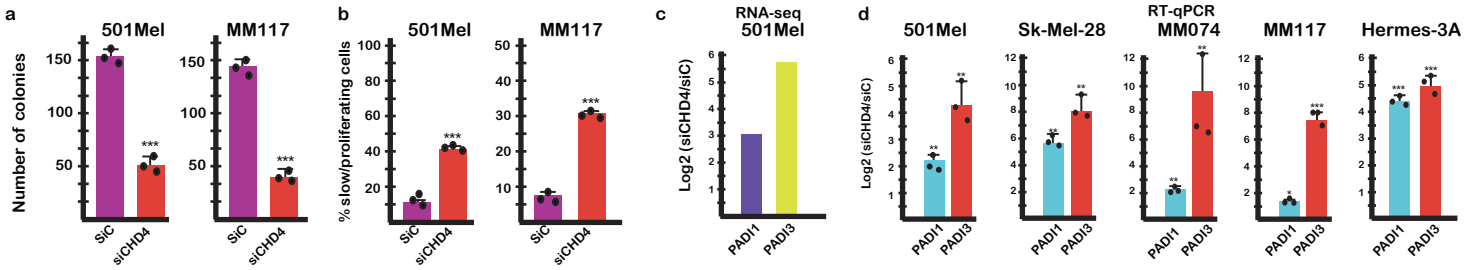


Figure 1. a-b. SiCHD4 silencing in melanoma cells reduced colony forming ability (a) and reduced cell proliferation (b) compated to control siRNA (SiC). c-d. CHD4 silencing induced PADI1 and PADI3 gene expression in a collection of melanoma lines as measured by RNA-seq or RT-qPCR. SiCHD4 silencing also induced PADI1 and PADI3 expression in breast, renal, and cervical carcinoma cells (data not shown).

PAD1 and PAD3 citrullinate PKM2

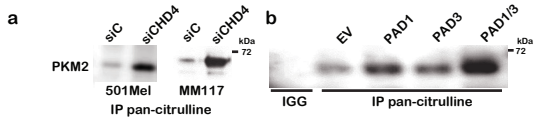


Figure 2. a-b. Protein extracts were prepared from SiC and SiCHD4 melanoma cells and immunoprecipitated with a pan-citrulline antibody. The IP was analysed by masss spectrometry showing that PKM2 was enriched in the SiCHD4 extracts and identifying PKM2 R106 as a modified arginine. PKM2 enrichment was confirmed by analysing the pan-citrulline IP by immunoblot with PKM2 antibody. PKM2 was enriched in the SiCHD4 extracts (a) and extracts from cells ectopically expressing the PAD1 and/or PAD3 enzymes (b).

PAD1/PAD3 stimulates PKM2 enzymatic activity and cellular glycolysis.

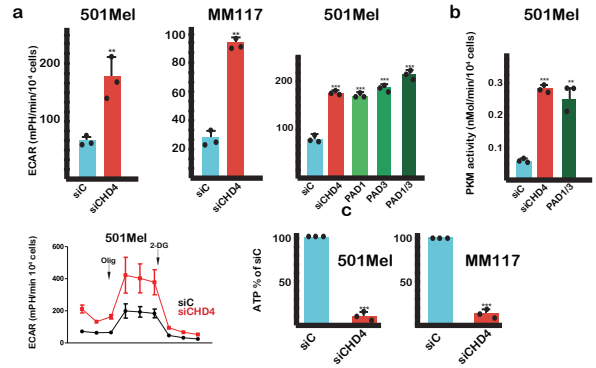


Figure 3. To assess the effect of PKM2 citrullination on glycolysis, we assessed glycolysis in real time in living cells by measuring the extracellular acidification rate (ECAR) using the Seahorse instrument. CHD4 silencing or PAD1 and/or PAD3 expression stimated glycolysis (a) and PKM2 enzymatic activity (b). The excessive glycolysis led to reduced intracellular ATP levels (c). The reduced intracellular ATP likely accounts for the reduced proliferation seen under these conditions.
These observations therefore define a novel link between the epigenetic regulation of PAD1 and PAD3 expression, PKM2 citrullination, glycolysis and cancer cell proliferation.

PAD1 and PAD3 are regulated by hypoxia activating PKM2 and glycolysis under hypoxic conditions

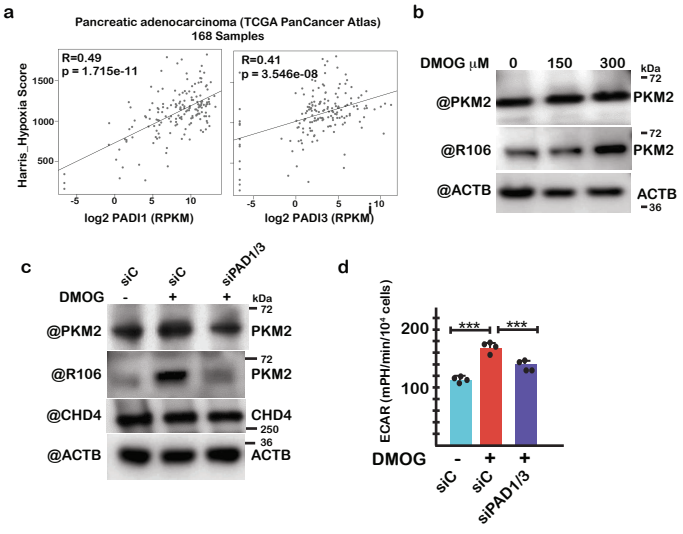


Fig. 5. While PADI1 and PADI3 expression is regulated by CHD4 in cells, in tumours in situ their expression is positively correlated with hypoxia. This is seen in pancreatic adenocarcinoma (a) and in renal and lung carcinomas (data not shown). PKM2 R016 citrullination is increased when melanoma cells are grown under hypoxic or pseudo-hypoxic conditions in presence of DMOG as detected using an antibody specific for R016<Cit (b-c). Under pseudo-hypoxic conditions, R106 citrullination and increased glycolysis are impaired by siPAD1/3 silencing. Hence PAD1 and PAD3 are upregulated by hypoxia in cells and in tumours in situ leading to increased R106 citrulination and glycolysis.

PAD1/PAD3 catalyzed R106 citrullination modulates PKM2 allosteric regulation

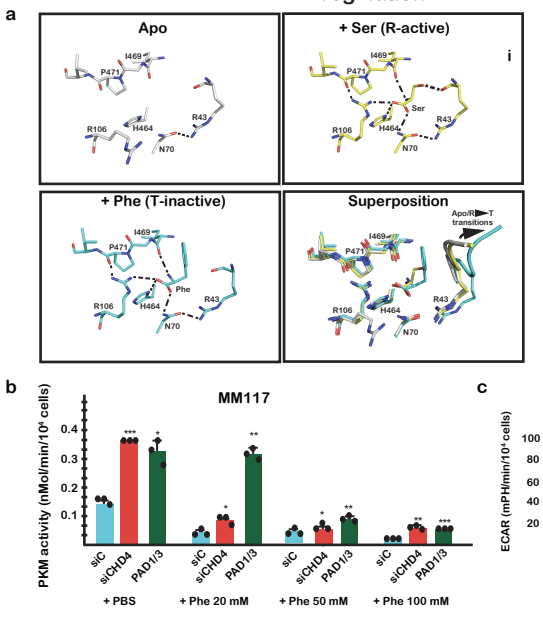
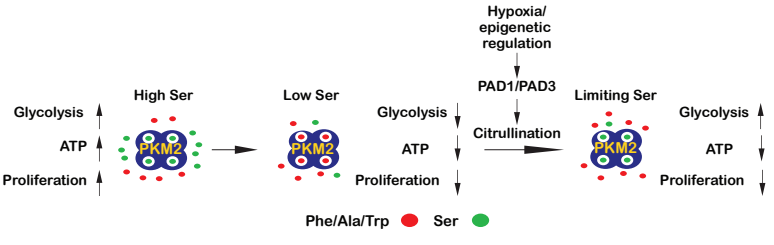


Fig. 4. PKM2 activity is stimulated by Serine (Ser), but inhibited by Phenylalanine (Phe), Tryptophan (Trp) or Alanine (Ala) that all competitively bind to PKM2. Mass spectrometry identified R106 as one of the PKM2 citrullinated arginine residues. The positively charged guanadino group of R106 is located at the entry of the binding pocket and makes strong hydrogen bonds with the carboxyl groups of the bound amino acids (a). Conversion of R106 to Cit leads to loss of positive charge and hence reduced hydrogen bonding to the free carboxyl groups. Consequently, binding of Trp/Phe/Ala are more inhibited than Ser whose binding is stabilized by extensive contacts within the pocket. This altered equilibrium allows PKM2 to remain active even under limiting Ser concentrations leading to excessive glycolysis and reduced anabolic capacity detrimental for cell growth. This was confirmed by assaying PKM2 enzmatic activity (b) and glycolysis in living cells (c) where inhibition under basal conditions by excess Phe or Trp (not shown) was in part restored by siCHD4 silencing or ectopic PAD enzyme expression. Strikingly therefore, this subtle post translational modification of PKM2 R106 leading to diminished hydrogen bonding capacity translated into a major impact on cancer cell metabolism.

A model for regulation of glycolysis by PKM2 citrullination



Conclusion

When present at high levels, Ser activates PKM2 (represented as a tetramer) assuring glycolysis, ATP production and proliferation. When Ser levels are lowered, PKM2 activity is reduced by binding of Trp/Phe/Ala leading to reduced glycolysis. When PAD1 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 ATP levels and the availability of glycolytic metabolites for anabolic processes.

In conclusion, we defined a novel pathway where epigenetic and/or hypoxia-mediated regulation of PAD1 and PAD3 expression modulates PKM2 citrullination, glycolysis and cancer cell proliferation.