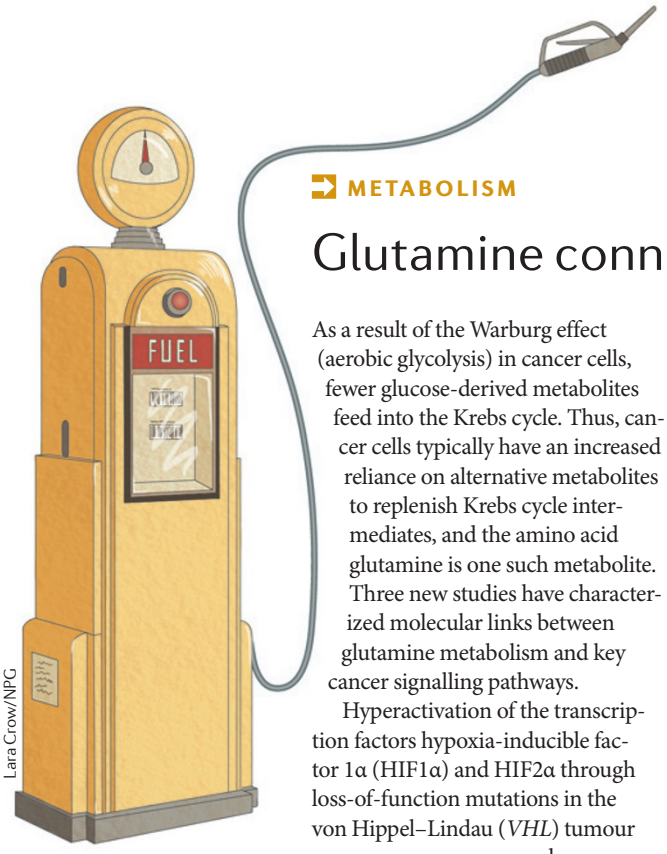
Nature Reviews Cancer | AOP, published online 12 April 2013; doi:10.1038/nrc3515



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METABOLISM

Glutamine connections

As a result of the Warburg effect (aerobic glycolysis) in cancer cells, fewer glucose-derived metabolites feed into the Krebs cycle. Thus, cancer cells typically have an increased reliance on alternative metabolites to replenish Krebs cycle intermediates, and the amino acid glutamine is one such metabolite. Three new studies have characterized molecular links between glutamine metabolism and key cancer signalling pathways.

Hyperactivation of the transcription factors hypoxia-inducible factor 1α (HIF1α) and HIF2α through loss-of-function mutations in the von Hippel–Lindau (*VHL*) tumour suppressor gene commonly occurs in renal cell carcinoma (RCC). The authors of the first new study had previously shown that *VHL*-mutant RCC cell lines use glutamine to generate citrate and lipids through the reductive carboxylation of glutamine-derived α-ketoglutarate, and that HIF activity in hypoxic cells promotes the conversion of glucose to lactate, thus preventing the use of glucose in the Krebs cycle. To determine whether these findings are linked in RCCs with mutant VHL, Gameiro *et al.* carried out manipulations in RCC cells — such as by expressing mutant VHL or VHL-insensitive HIF subunits — and used metabolic profiling *in vitro* and in tumour-bearing mice to show that loss of HIF regulation by VHL is sufficient to switch the Krebs cycle inputs from mostly glucose-derived inputs to mostly glutamine-derived inputs. They also found that replenishing citrate levels could block the switch to glutamine usage, thus intracellular citrate deficiency might promote the switch to glutamine use. Finally, the authors highlighted the therapeutic relevance of this reliance on glutamine metabolism by showing that VHL deficiency sensitizes RCC cells and xenografts to inhibitors of glutaminase, the enzyme that catalyses the first step of glutamine metabolism.

In a separate study, Son *et al.* analysed the contribution of activated KRAS to metabolism in pancreatic ductal adenocarcinoma (PDAC) cells that are dependent on glutamine. Using genetic and pharmacological interventions the authors found that the growth of KRAS-mutant PDAC cells and tumour xenografts does not rely on glutamate dehydrogenase 1 (GLUD1; also known as GDH1) in the conversion of glutamine to α-ketoglutarate for use in the Krebs cycle, and instead requires a non-canonical route involving the GOT1 aspartate transaminase to convert glutamine-derived aspartate to oxaloacetate, which can be used to generate malate and pyruvate. This series of reactions increases NADPH levels and helps to maintain the cellular redox state through the generation of reduced glutathione. Indeed, glutamine deprivation results in increased reactive oxygen species (ROS) levels in PDAC cells, and inhibitors of glutaminase synergized with H_2O_2 to kill PDAC cells. Knockdown of KRAS resulted in GOT1 downregulation and reduced metabolic flux through this non-canonical route, thus demonstrating the role of mutant KRAS in specifying the use of this pathway.

As glutamine deprivation can occur naturally through the increased use of glutamine reserves by tumours, and is a goal of various therapeutic approaches, it is important to understand how cells respond to glutamine deprivation. Reid *et al.* tested whether α4 (also known as IGBP1) has a role in the response to glutamine deprivation, because an orthologue of this protein, Tap42, is known to have this function in yeast. Indeed, expression of α4 protected mouse embryo fibroblasts (MEFs) and human fibrosarcoma cells from the cytotoxic effects of glutamine deprivation. Using various genetic, biochemical and proteomic approaches, the authors showed that glutamine deprivation triggers an increase in ROS levels, which leads to the α4-mediated assembly of a B55α-subunit-containing protein phosphatase 2A (PP2A) complex. This results in the activation of p53 and the induction of pro-survival p53 target genes, such as *Cdkn1a* (which encodes p21) and *Gadd45a*. Consistent with this, the growth of fibrosarcoma xenograft tumours (the cores of which were shown to have low levels of glutamine) was inhibited by B55α knockdown, and p53-deficient HCT116 cells *in vitro* were more sensitive to glutamine deprivation than p53-wild-type controls. Reid *et al.* concluded that glutamine is required to generate reduced glutathione, again to maintain the cellular redox state.

Blocking metabolic functions as a therapeutic approach raises the challenge of avoiding toxicity in normal tissues. However, these molecular links between cancer signalling pathways and altered glutamine metabolism support the applicability of anticancer therapeutic approaches that are based on glutamine deprivation and suggest that their efficacy might be enhanced in p53-deficient tumours or in combination with other ROS-generating stresses.

Darren J. Burgess

> “glutamine is required to generate reduced glutathione”

ORIGINAL RESEARCH PAPERS Gameiro, P. A. *et al.* In vivo HIF-mediated reductive carboxylation is regulated by citrate levels and sensitizes VHL-deficient cells to glutamine deprivation. *Cell Metab.* **17**, 372–385 (2013) | Son, J. *et al.* Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. *Nature* 27 Mar 2013 (doi:10.1038/nature12040) | Reid, M. A. *et al.* The B55α subunit of PP2A drives a p53-dependent metabolic adaptation to glutamine deprivation. *Mol. Cell* 13 Mar 2013 (doi:10.1016/j.molcel.2013.02.008)