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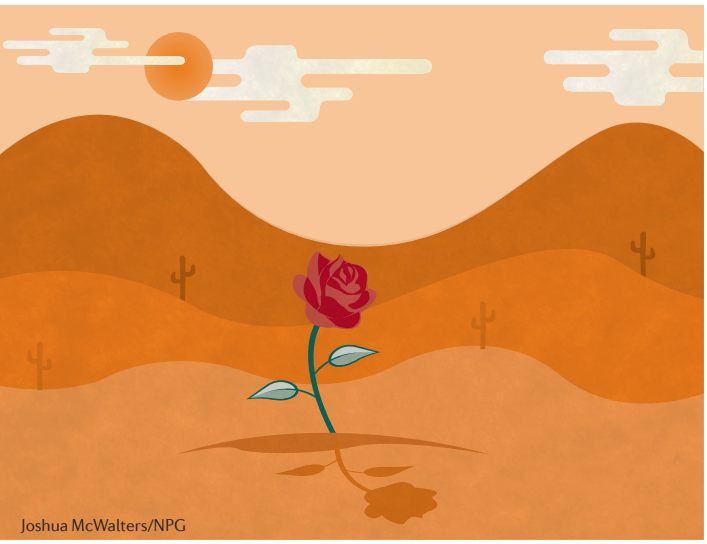
TUMOUR METABOLISM

Adapting to harsh conditions

Metabolic reprogramming can promote tumour cell growth and survival in harsh microenvironmental conditions, such as hypoxia. Pyruvate dehydrogenase kinase (PDK) is known to be induced by hypoxia-inducible factor 1 (HIF1)-dependent transcription to switch tumour metabolism towards glycolysis; however, whether other pathways regulate this hypoxic metabolic adaptation is not known.

Chae *et al.* conducted a phosphoproteomics screen to identify proteins that were differentially phosphorylated in the mitochondria of PC3 prostate cancer cells grown in conditions of hypoxia compared with normoxia. They found increased phosphorylation of several AKT targets in hypoxic mitochondria, and then determined that hypoxia increased the levels of activated AKT in mitochondria, in a process that did not require HIF1-dependent transcription but did require mitochondrial-derived reactive oxygen species (ROS).

> “inhibition of AKT also impaired the switch to glycolysis induced by hypoxia”



Joshua McWalters/NPG

One of the several mitochondrial targets of AKT identified was PDK1, and the authors confirmed the ability of AKT1 and AKT2 to phosphorylate PDK1 (but not PDK2, PDK3 or PDK4) *in vitro* on T346. Phosphorylation of PDK1 by AKT in turn led to phosphorylation of the pyruvate dehydrogenase (PDH) complex E1α catalytic subunit (PDHE1) by PDK1 in PC3 cells. Phosphorylated PDK1 normally inhibits PDH activity; the authors confirmed this in PC3 cells and showed that the phosphorylation of T346 and the kinase activity of AKT were required to suppress PDH activity in hypoxic conditions. Mitochondrial AKT–PDK1 signalling was required for the hypoxia-induced switch of tumour cell metabolism to glycolysis, as PDK1 knockdown cells reconstituted with a PDK1 mutant that cannot be phosphorylated on T346 (PDK1-T346A) did not undergo glycolysis (as measured by glucose consumption) under hypoxia. Furthermore, inhibition of AKT also impaired the switch to glycolysis induced by hypoxia.

Mitochondrial AKT–PDK1 promoted PC3 cell proliferation in hypoxia, as demonstrated using knockdown of AKT1, AKT2 or PDK1. PDK1 knockdown or an inhibitor of AKT also increased aberrant ROS production and induced apoptosis of PC3 cells; cell viability could be restored by re-expression of wild-type PDK1, but not PDK1-T346A, in the PDK1-knockdown cells, indicating the importance of phosphorylation at this site for promoting cell survival in hypoxia. Furthermore, growth of PC3 xenograft tumours was inhibited by PDK1 knockdown, and although growth was restored by re-expression of wild-type PDK1, it was not by PDK1-T346A.

Increased PDHE1 phosphorylation downstream of AKT was also observed in many other (but not all) cancer cell lines grown in hypoxic conditions. To examine a different model, the authors looked at glioblastoma neurospheres, which are hypoxic in their cores. A T346 phospho-specific antibody showed high levels of PDK1-T346 phosphorylation in hypoxic regions in these cultures. Intracranial xenografts of U251 glioblastoma cells were also highly hypoxic and had high levels of PDK1-T346 and PDHE1 phosphorylation. Furthermore, patient-derived glioblastoma samples with high HIF1α staining also had high PDK1-T346 and PDHE1 phosphorylation, and in a cohort of 116 glioma patients, increased phosphorylated PDK1-T346 was highest in the most aggressive tumours and correlated with reduced overall survival.

These data shed light on how tumour cells adapt their metabolic pathways under hypoxic conditions and highlight the importance of mitochondrial AKT signalling in this process, a pathway that might be amenable to therapeutic targeting.

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ORIGINAL ARTICLE Chae, Y. C. *et al.* Mitochondrial Akt regulation of hypoxic tumor reprogramming. *Cancer Cell* **30**, 257–272 (2016)