METABOLISM

Unravelling metabolic dependencies

Lara Crow/NPG

> cancer cells may selectively rely on PFKFB4 for managing ROS accumulation

Metabolic differences between cancer cells and normal cells have long been realized, but a recent resurgence of interest is driving the molecular characterization of these differences and an exploration of the therapeutic opportunities that they might provide. A new study identifies a cancer-specific dependency on an isoform of the glycolytic enzyme phosphofructokinase 2.

To search for cancer-specific metabolic dependencies, Almut Schulze and colleagues carried out a small interfering RNA (siRNA) screen of 222 metabolic enzymes in four prostate cell lines: untransformed RWPE1 cells, and DU145, LNCaP and PC3 metastatic cancer cells. From the screen, the authors identified 18 genes for which knockdown was selectively lethal to the three cancer cell lines compared with RWPE1 cells. As evidence for an association of these genes with cancer progression, published data sets revealed that these genes are typically overexpressed in prostate cancer metastases compared with primary tumours.

However, the metabolic characteristics of cells heavily depend on the tissue culture conditions. For example, the authors showed that depleting lipids from the cancer cell media increased both the rate of lipid synthesis in the cells and the cytotoxic effects of inhibitors of lipid synthesis. Therefore, because the cancer cells and RWPE1 cells were maintained in different culture media, the screen results needed to be interpreted responsibly.

The authors focused on two of the strongest hits from the screen — 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4 (*PFKFB4*) and protein kinase, AMP-activated, β1 non-catalytic subunit (*PRKAB1*) — as being selectively lethal to the cancer cell lines. Importantly, knockdown of these genes also caused cancer-cell-specific toxicity when the culture conditions were matched between RWPE1 cells and cancer cells. This toxicity was shared across cancer cell lines of additional tissue types, although the toxicity to additional untransformed lines remains to be determined.

To test whether cancer cell dependency on these genes also occurred in physiologically relevant settings, the authors used doxycycline-inducible short hairpin RNAs (shRNAs) to knock down expression of either gene in established PC3 xenograft tumours in mice. Although *PRKAB1* knockdown only had modest effects on tumour growth, *PFKFB4* knockdown almost completely blocked tumour growth and even caused regression.

What is the mechanistic basis of this tumour-cell dependency on PFKFB4? Consistent with its known enzymatic role in regulating levels of fructose-2,6-bisphosphate (Fru-2,6-BP), *PFKFB4* knockdown caused an increase in Fru-2,6-BP levels; interestingly, this effect was specific to the cancer cell lines. Accumulation of Fru-2,6-BP is known to allosterically activate glycolysis, thus diverting metabolites away from the generation of NADPH, a coenzyme that has crucial downstream anabolic and antioxidant functions. Accordingly, *PFKFB4* knockdown in prostate cancer cells led to lower levels of NADPH and a reduced rate of lipid synthesis. Perhaps most importantly, *PFKFB4* knockdown caused the accumulation of reactive oxygen species (ROS) specifically in the cancer cells. This suggests that the cancer cells may selectively rely on PFKFB4 for managing ROS accumulation; in support of this, treatment with a ROS scavenger rescued the viability defect in the *PFKFB4*-knockdown prostate cancer cells.

It will be interesting to see whether PFKFB4 could be a promising therapeutic target for cancer. In this regard, it will be important to determine whether systemic PFKFB4 inhibition, rather than tumour-specific gene silencing, would provide sufficient antitumour efficacy without excessive normal-cell toxicity.

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ORIGINAL RESEARCH PAPER Ros, S. *et al.* Functional screen identifies 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase (PFKFB4) as an important regulator of prostate cancer cell survival. *Cancer Discov.* 22 Mar 2012 (doi:10.1158/2159-8290.CD-11-0234)