How do tumours adapt to nutrient stress?

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Reduced blood flow contributes to tumour hypoxia and nutrient deprivation forcing cancer cells to adapt to ensure survival. In this issue of *The EMBO Journal*, Ye *et al* show that a stress response pathway including the eIF2 kinase GCN2 and its downstream target, the transcriptional activator ATF4, is critical for proliferation and survival of tumour cells after starvation for amino acids or glucose and is essential for growth *in vivo* in a xenograft model. This study provides new mechanistic insight into how tumour cells sense and adapt to nutrient restriction and suggests new approaches for cancer chemotherapy.

Rapidly growing tumour cells outgrow their blood supply resulting in a microenvironment with reduced oxygen and nutrients. Tumour cells can adapt to this stressful environment by inducing angiogenesis and altering metabolic strategies, thus ensuring survival and proliferation.

A study by Ye *et al* (2010) published in this issue of *The EMBO Journal* describes a mechanism for how tumour cells

sense and adapt to a limited nutrient environment. The key findings are that a stress response pathway featuring the protein kinase GCN2 and its downstream target, the transcription activator ATF4, is critical for survival and proliferation of cultured tumour cells after starvation for amino acids or glucose and for tumour growth *in vivo*.

In response to nutrient deprivation, GCN2 phosphorylates the α subunit of the translation initiation factor, eIF2, thus allowing cells to conserve resources as they reconfigure gene expression to overcome the nutrient stress (Figure 1) (Hinnebusch, 2005; Wek *et al*, 2006). Concurrent with lowered protein synthesis, eIF2 α phosphorylation enhances the translation of select mRNAs, such as that encoding ATF4, a transcription activator of genes involved in metabolism, nutrient uptake, and alleviation of oxidative stress (Harding *et al*, 2003; Wek *et al*, 2006).

The activation signal for the GCN2/eIF2 α ~P/ATF4 pathway is uncharged tRNAs that accumulate during nutrient stress and

Figure 1 A stressful tumour microenvironment activates GCN2 and PERK and the integrated stress response. A model is shown depicting the activation of GCN2 and PERK eIF2 kinases in response to reduced availability of oxygen, glucose, and amino acids in the tumour microenvironment. Nutrients and oxygen, depicted by the blue gradient, are provided by blood vessels. Activation of PERK and GCN2 phosphorylates eIF2 α leading to lowered global translation, coincident with preferential translation of ATF4 mRNA. Phosphorylation of eIF2 α allows for scanning ribosomes to bypass an inhibitory upstream open reading frame in the 5'-leader of the ATF4 mRNA and instead initiate translation at the ATF4-coding region. Elevated levels of ATF4 enhance transcription of genes subject to the ISR including those involved in metabolism, such as asparagine synthetase (ASNS), nutrient uptake, and anti-oxidation. ATF4 also enhances transcription of GADD34, which participates in the feedback dephosphorylation of eIF2 α .

bind directly to GCN2 (Hinnebusch, 2005). The GCN2-directed stress response is conserved from yeast to humans, emphasizing its central importance for adapting to nutrient deprivation. In mammals, GCN2 functions in conjunction with additional eIF2 kinases, which are each activated by different environmental stresses. For example, the eIF2 kinase PERK is activated by accumulation of unfolded protein in the endoplasmic reticulum (Figure 1) (Marciniak and Ron, 2006). Thus, mammals have expanded the scope of stress arrangements activating eIF2 kinases, which has led to the pathway being referred to as the integrated stress response (ISR) (Harding *et al*, 2003).

Building on these underlying concepts, Ye *et al* found that knockdown of ATF4 in human fibrosarcoma or colorectal tumour cells significantly lowered survival due to decreased proliferation and increased apoptosis. Interestingly, these effects were reversed by the addition of non-essential amino acids. A systematic determination of the underlying amino acids responsible for these findings pointed to the importance of asparagine and asparagine synthetase (ASNS), a well-characterized target gene of ATF4 (Kilberg *et al*, 2005). Asparagine synthetase catalyses the formation of asparagine from aspartic acid, and the addition of asparagine, or the expression of ASNS, reversed the lethality associated with depletion of ATF4 in the tumour cells. These results suggest that loss of the ATF4 component of the GCN2 pathway reduces tumour survival in large part due to asparagine deprivation, a finding that was confirmed using a mouse xenograft model.

The theme that the GCN2/eIF2 α ~P/ATF4 pathway can enhance tumour resistance to nutrient depletion is supported by the use of asparaginase in the treatment of acute lymphoblastic leukaemia (Richards and Kilberg, 2006). The anti-cancer properties of asparaginase involve depletion of asparagine from circulating plasma, which is thought to deprive the malignant lymphoblasts. Asparaginase treatment induces GCN2 phosphorylation of eIF2 α and its downstream target ASNS, and resistance to this chemotherapy is suggested to result from overexpression of ASNS (Bunpo *et al*, 2009). In the light of these new findings, asparaginase therapy for the treatment of solid tumours warrants further investigation.

Ye *et al* also reported that starvation for glucose reduced tumour cell viability and GCN2^{-/-} cells were sensitive to this nutrient stress. Activation of GCN2 by glucose deprivation seems

to be indirect, as amino acids are consumed as secondary energy sources and therefore become limiting. It is curious that PERK was also required for eIF2 α phosphorylation in response to glucose depletion, suggesting that protein processing and assembly in the ER are adversely affected. The combined activation of GCN2 and PERK may affect the kinetics and duration of eIF2 α phosphorylation and can modulate the transcription and translation of distinct mRNAs (Dang Do *et al*, 2009).

PERK has a well-established role in tumour resistance to hypoxic stress. Bi *et al* (2005) were the first to show that hypoxic stress activated PERK and the ISR, and loss of PERK reduced the size of tumours in xenograft models and triggered apoptosis in hypoxic areas within tumour sections. PERK is suggested to facilitate tumour cell adaption to hypoxia by increasing tumour vascularization through enhanced translational and transcriptional expression of angiogenic genes (Blais *et al*, 2006).

Activation of GCN2 and PERK, and their downstream target ATF4, are central for tumours to adapt to different stresses in the microenvironment, eliciting gene expression that alters metabolism and enhances vascularization, which favours tumour survival and proliferation (Figure 1). Measurements of phosphorylation and activation of GCN2 in serial sections of primary tumours suggests that the ISR is not uniformly induced among tumour cells (Ye *et al*, 2010). This is consistent with the dynamic nature of tumour hypoxia and suggests that nutrient stress in the tumour microenvironment activates GCN2, eliciting the ISR gene expression. Repressed translation accompanying eIF2 α phosphorylation would be transient, as ATF4 activates GADD34, a regulatory subunit of type 1 protein phosphatase that serves to dephosphorylate eIF2 α (Figure 1) (Marciniak and Ron, 2006).

The results reported by Ye *et al* (2010) have important implications for new cancer therapies, and understanding these stress pathways and their regulation may provide insight into the combinations of drugs that are the most effective as anti-cancer treatments. Chemotherapeutic regimens that induce nutrient limitation or endoplasmic reticulum stress, combined with others that inhibit specific components within the ISR, or their downstream adaptive processes, may prove to be the most effective.

Conflict of interest

The authors declare that they have no conflict of interest.

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