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Surviving Metabolic Stress: Of Mice (Squirrels) and Men
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Summary: Understanding how cancer cells survive harsh environmental conditions may be fundamental to eradicating malignancies proven to be impervious to treatment. Nutrient and growth factor deprivation, hypoxia, and low pH create metabolic demands that require cellular adaptations to sustain energy levels. Protein synthesis is one of the most notable consumers of energy. Mounting evidence implicates exquisite control of protein synthesis as a survival mechanism for both normal and malignant cells. In this commentary, we discuss the role of protein synthesis in energy conservation in cancer and focus on elongation factor-2 kinase, a downstream component of the PI3K–AKT pathway that behaves as a critical checkpoint in energy consumption. Cancer Discov; 4(6):646–9. © 2014 AACR.

Harsh conditions create stress for all living things. Collections of malignant cells would seem to be especially vulnerable, as they manage to survive under conditions of deprivation, such as lack of adequate blood supply, low pH, and both oxygen and nutrient shortages. Recent evidence provides further insights into how nature deploys a parsimonious approach to address this problem, from mice, to squirrels, to man. The PI3K pathway and autophagy are sensitive to nutrient and growth factor availability and are fundamental mechanisms for cellular adaptation to stress. Although several components of these pathways have been carefully elucidated and reviewed (1, 2), the role of peptide elongation has received less attention.

While studying Ca2+/calmodulin signaling, we identified a previously unappreciated enzymatic activity in glioblastoma cells and specimens (3). A 100-kDa phosphoprotein was present in malignant glial cells but not in normal white matter, a tissue rich in normal glia. Ultimately, we isolated both the phosphoprotein [elongation factor-2 (eEF2)] and the kinase [elongation factor-2 kinase (eEF2K; ref. 4), also known as calmodulin-dependent protein kinase 3, originally described by Nairn and colleagues (5)], cloned the gene, and characterized the enzyme as a unique protein kinase represented by relatively few enzymes in the kinome (6). It had been shown that this kinase terminated protein elongation (7), one of the most avaricious consumers of cellular energy, through phosphorylation of eEF2 at Thr56, thereby decreasing the affinity of the elongating peptide chain for the ribosome. eEF2K, the only known substrate for eEF2K, is an elongation factor that mediates the translocation of peptidyl tRNA from the ribosomal A site to the P site.

At the time, the conspicuously increased activity of eEF2K in glioblastoma and other types of cancer cells compared with adjacent normal tissue was perplexing: why was this enzymatic inhibitor of peptide elongation increased in certain malignancies? Insights came from studies of hibernating squirrels and weanling mice.

Hibernation, a survival strategy used by mammals to combat the threat of severe nutrient deprivation, requires marked metabolic suppression. In hibernating ground squirrels, Chen and colleagues (8) observed increased phosphorylation of eEF2 in the liver and brain due to increased activity of eEF2K. They surmised that increased phosphorylation of eEF2 was a mechanism by which hibernating animals tolerate marked reductions in cerebral blood flow. Since then, several studies have demonstrated that environmental stress, including nutrient and growth factor deprivation as well as oxidative and chemical insults, is a potent activator of this kinase (9).

The regulation of eEF2K activity provided further insights. Proud and others (reviewed in ref. 10) found that control of eEF2K is mediated by key enzymatic activities linked to metabolic homeostasis (Fig. 1). For example, eEF2K is completely dependent on Ca2+/calmodulin, and is activated by AMP kinase (AMPK) through phosphorylation at Ser398. AMPK is a sensor of cellular energy, sensitive to changes in the ratio of AMP to ATP. When the ratio is high, that is, when energy is depleted, eEF2K is activated by AMPK and peptide elongation is terminated. Conversely, when the ratio is low, that is, when energy stores are replete, AMPK is less active, eEF2K is inhibited, and elongation is restored. Furthermore, eEF2K is also regulated by insulin and mTORC1 through phosphorylation at Ser78 and Ser361, respectively (11, 12). Thus, in the presence of adequate nutrients, protein synthesis is favored through inactivation of AMPK and activation of mTORC1, both converging to inhibit eEF2K. In contrast, under conditions of metabolic stress, protein synthesis is undesirable, AMPK is activated, mTORC1 is inhibited, and the increased activity of eEF2K inhibits peptide chain elongation.

It is now appreciated that cancer cells use these same pathways to rapidly terminate protein synthesis to conserve energy. As mentioned above, a variety of stressful conditions increased the activity of eEF2K including hypoxia and nutrient and growth factor deprivation (9). Furthermore, when the kinase was depleted by RNA interference (RNAi) or blocked by chemical inhibition, the ability to survive stress...
was markedly reduced (9). Similarly, Cantley’s laboratory found that failure to inhibit mTORC1 and block the initiation phase of protein synthesis also sensitized cells to the detrimental effects of starvation (13). An unanswered question was, how do these kinases sense nutrient deprivation, and what survival mechanisms does the activity induce?

This leads to weanling mice. Nutrient and growth factor deprivation is a potent inducer of autophagy (from the Greek “self-eating”). Autophagy is stimulated in yeast through a complex containing Arg1, a serine/threonine protein kinase that can form a complex with Atg13 and Atg17. Autophagy protects against severe energy depletion by digesting organelles and cytoplasm and recycling the components for energy production. When energy is abundant, autophagy is suppressed by mTORC1 through direct interactions with the Atg1/13/17 complex. Initially, differences in interpretation of data led to uncertainty about the precise role of autophagy in cancer; was it a mechanism of cell death or a conserved mechanism of survival, or both? An elegant set of experiments highlighted the possibility that autophagy could serve as a potent survival mechanism in malignancies. Like hibernating squirrels, newborns are forced to find ways to survive during the period of starvation between birth and the onset of lactation. Kuma and colleagues (14) studied survival in weanling mice. At birth and before lactation begins, pups unable to use autophagy due to lack of Atg5, an essential component of the murine autophagosome, do not survive the abrupt lack of nutrients preceding lactation. Survival was not impaired in wild-type littermates or in newborn Atg5 mutants who were force fed during the critical prelactation period.

Therefore, several laboratories considered a possible link among nutrient deprivation, inhibition of protein elongation, and autophagy. In the face of nutrient depletion, eEF2K was activated, autophagy increased, and cell survival improved (9). In contrast, when eEF2K was knocked down using RNAi, nutrient deprivation did not induce autophagy and cell survival decreased (9, 15). In certain cells, knockdown

**Figure 1.** A, nutrient availability favors protein synthesis. Adequate supply of glucose and amino acids triggers a series of reactions that stimulate protein synthesis. When the ratio of AMP to ATP is low, AMPK is inhibited and eEF2K is activated, thereby favoring peptide chain elongation. In addition, nutrient availability activates mTORC1, leading to initiation of protein synthesis and inhibition of autophagy. B, nutrient deprivation favors energy conservation. Inadequate glucose and amino acids trigger a series of reactions that diminish protein synthesis. Energy depletion increases the ratio of AMP to ATP, thereby activating AMPK, resulting in the activation of eEF2K, thereby inhibiting elongation through ribosomal stalling. In addition, starvation blocks the activity of mTORC1, leading to inhibition of initiation of protein synthesis and activation of autophagy.
of eEF2K increased protein synthesis and decreased cellular ATP, presumably through the inability to conserve energy by terminating protein synthesis and activating autophagy (15, 16). Thus, in the presence of environmental stress, malignant cells can conserve ATP by activating pathways that transiently stall protein synthesis and, in some cases, activate autophagy.

Recent work by Leprivier and colleagues (16) confirmed and extended several of these observations. By studying adaptation of transformed fibroblasts to nutrient deprivation, they found that adaptation was mediated through the activation of eEF2K by AMPK, and that the ability to adapt was severely compromised in cells where eEF2K was knocked out or depleted by RNAi. The importance of eEF2K activation was also seen in nontransformed fibroblasts, and both these and adapted transformed cells acutely blocked elongation during starvation. These and other data suggest that by limiting peptide elongation, cells are able to decrease the rate of ATP depletion, as suggested by earlier studies (15). Interestingly, when RASV12-transformed NIH 3T3 cells overexpressing eEF2K were transplanted into nu/nu mice, they were resistant to the growth-inhibitory effects of caloric restriction. Finally, this group also found that human gliomas with increased eEF2K transcripts were of high grade and poor prognosis.

If starvation simultaneously inhibits protein synthesis and activates autophagy, how are these processes connected? AMPK seems to provide a critical link. Activation of AMPK by energy depletion promotes autophagy, activates eEF2K, and inhibits mTORC1. Furthermore, loss of AMPK or ULK1 (the mammalian ortholog of ATG1) led to the accumulation of an autophagy adaptor protein, p62, which itself is degraded through autophagy (17). AMPK promotes autophagy not only through direct phosphorylation and activation of ULK1, but also through phosphorylation of beclin-1, a critical adaptor molecule for the class III lipid kinase VPS34, which is essential for autophagosome formation. We recently discovered potent and selective inhibitors of eEF2K and of VPS34. VPS34 inhibitors completely impaired autophagy flux induced by metabolic stress (as indicated by accumulation of p62), whereas eEF2K inhibitors did not, raising questions about earlier data using RNAi of a direct involvement of eEF2K in autophagy regulation. However, the selective eEF2K inhibitors, when used as affinity probes, bound an eEF2K–beclin-1–VPS34 complex present in cellular extracts. The availability of VPS34- and eEF2K-specific inhibitors should allow careful dissection of the regulation of elongation rate and autophagy under stress.

Like other members of the PI3K pathway, inhibition of eEF2K may represent an attractive target for depriving cancer cells of an energy-conserving mechanism. In fact, there have been several attempts at identifying potent and selective eEF2K inhibitors (18, 19). We hypothesized that inhibitors of prokaryotic histidine kinases might also inhibit the activity of eEF2K based on the enzyme’s predicted amino acid sequence deduced from the cloned cDNA. A family of histidine kinase inhibitors contained a small molecule that nonspecifically blocked the kinase at low micromolar concentrations (19). As mentioned above, we recently identified extremely potent and selective inhibitors of both eEF2K and VPS34. Recently, it was shown that MK-2206, the first allosteric inhibitor of AKT to enter the clinic, increased the expression of eEF2K, apoptosis, and autophagy. Inhibition of eEF2K decreased the autophagic response and increased the apoptotic effects of AKT inhibition (20).

In summary, several lines of evidence suggest that cancer cells have adapted mechanisms to survive stressful conditions by conserving energy through rapidly terminating protein synthesis. In the presence of adequate nutrients, protein synthesis proceeds through pathways that inhibit AMPK, activate mTORC1 to initiate protein synthesis, and inhibit eEF2K to promote peptide elongation. In contrast, under conditions of metabolic stress where protein synthesis is metabolically detrimental, AMPK inactivates mTORC1 and activates eEF2K, thereby inhibiting protein synthesis at both the initiation and elongation stages of protein translation. Drugs that disrupt this important survival mechanism may help produce better results in treating previously refractory malignancies.

**Disclosure of Potential Conflicts of Interest**

W.N. Hait and M. Versele are employees of Janssen, the pharmaceutical companies of Johnson & Johnson. No potential conflicts of interest were disclosed by the other author.

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