Clin Transl Oncol (2011) 13:442-445
DOI 10.1007/s12094-011-0680-3

Between *Scylla* and *Charibdis*: eIF2α kinases as targets for cancer chemotherapy

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Received: 25 April 2011 / Accepted: 23 May 2011

Abstract The eIF2α kinases integrate translation initiation rates with nutrient availability, thus allowing cells to adapt to nutrient scarcity. Recent evidence has uncovered new functions of these kinases in tumour cell biology, ranging from regulation of cell cycle progression, maintenance of genome stability, control of apoptosis, and cell survival under nutrient stress and hypoxia. Accordingly, active eIF2α kinases modulate the antineoplasic activity of several antitumour drugs, either by exacerbating their cytotoxic effect or by promoting chemoresistance. Understanding of eIF2α kinases molecular roles may provide mechanistic insights into how tumour cells sense and adapt to nutrient restriction, thus helping to implement more effective approaches for cancer chemotherapy.

Keywords eIF2 alpha phosphorylation · GCN2 · PERK · PKR · Translation

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Introduction

Scylla and *Charybdis* were mythical sea monsters of the Greek tradition which sat on opposite sides of the Strait of Messina between Sicily and mainland Italy. Having to navigate between the two sea hazards posed an inescapable threat to passing sailors. A similar dilemma emerges when we explore the role of eIF2α kinases as molecular targets in cancer cell biology (Fig. 1).

Under nutrient deprivation conditions, eukaryotic cells transiently inhibit protein synthesis to avoid misfolding of proteins which could compromise cell viability. This response forms part of a protective mechanism triggered by several stimuli and is generically known as "integrated stress response" (ISR) [1]. Translation inhibition is achieved by posttranslational modification of the eukaryotic translation initiation factor 2 (eIF2). Nutrient stress activates protein kinases that phosphorylate eIF2 in Ser51 of its alpha subunit (eIF2α) [1]. Phosphorylated eIF2α behaves as a competitive inhibitor of the eIF2B exchanger, thus blocking eIF2 recycling. As the concentration of eIF2 normally exceeds that of eIF2B, very modest increases of eIF2α phosphorylation can completely inhibit protein synthesis initiation and favour selective translation of some mRNAs [1]. Some of these mRNAs encode transcription factors that regulate a gene expression programme that allows cells to adapt to nutrient stress [2].

The ISR pathway is conserved throughout evolution, from yeast to man. In humans, the phosphorylation of eIF2α is controlled by four protein kinases [3]: the double-stranded (ds) RNA-activated protein kinase (PKR), the hemin-regulated inhibitor kinase (HRI), the pancreatic eIF2α or PKR-endoplasmic reticulum (ER)-related kinase (PEK/PERK) and the general control nonderepressible-2 (GCN2) kinase. Each kinase is activated by distinct stress-

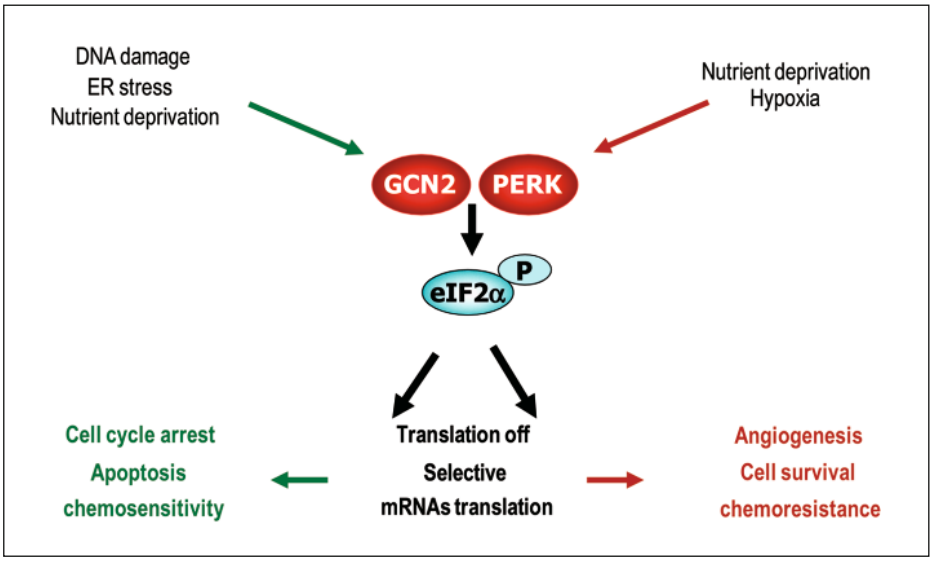

Fig. 1 Schematic representation of the contradictory roles of eIF2 kinases in tumour cell survival/death. Please see the text for full explanation

es that decrease protein synthesis by an appropriate response: PKR by double-stranded RNA (dsRNA), HRI by heme deficiency, PEK/PERK by misfolded proteins in the endoplasmic reticulum (ER) and GCN2 by amino acid deprivation. As the major cellular signalling pathways that regulate cell proliferation also control translation, eIF2α phosphorylation is causally involved in mammalian development and human diseases such as neurodegeneration, ageing and cancer [1].

eIF2 kinases as tumour suppressors

Several lines of evidences suggest that the ISR can be considered a tumour suppressor pathway. For instance, DNA damage efficiently activates eIF2α kinases, which, in turn, trigger a cell cycle checkpoint at the G1/S transition and, in many cases, apoptosis [4, 5]. Interestingly, the checkpoint function of the ISR pathway is conserved from yeast to man, thus reflecting is fundamental nature [6, 7]. In human cells, the major eIF2α kinases regulating cell cycle progression are PERK and GCN2 [8, 9]. Phosphorylation of eIF2α is a well known response to various forms of stress, but how this response causes the specific cell cycle effects is not fully understood [1]. Exposure of cells to ER stress leads to activation of PERK, eIF2α phosphorylation, repression of cyclin D1 translation (CLN3 in yeast) [10] and subsequent cell cycle arrest in the G1 phase [8, 9]. Additionally, exposure to UVB irradiation induces phosphorylation of the alpha subunit of the eukaryotic initiation factor 2 (eIF2alpha) and inhibits global protein synthesis in a PERK- and GCN2-dependent manner [4, 11, 12].

The role of eIF2 kinases as cell cycle regulators is best represented by the role of GCN2 in T-lymphocyte proliferation. T-cell activation requires extracellular nutrients to provide energy for cellular proliferation and effector functions [13, 14]. Inhibition of nutrient transport drastically blocks cell cycle progression at G1, activates GCN2 and increases phosphorylation of eIF2α [14]. Therefore, inhibitors of nutrient transport might provide a novel class of immunosuppressants or antitumour agents. Along these lines, brasilicardin A, an inhibitor of the system L amino acid transporter, appears to be a good candidate agent [15].

Cytotoxicity of some antineoplasic agents, such as doxorubicin, etoposide or vorinostat, requires full activation of PERK and/or GCN2 and subsequent phosphorylation of eIF2 to ensue [16–18]. Indeed, the degree of eIF2α phosphorylation can greatly determine the tumour sensitivity towards chemotherapy. Thus, strategies that maintain eIF2α in a hyperphosphorylated state may be novel therapeutic approaches to maximise antitumour drug-induced apoptosis and reduce residual disease and recurrences in several forms of cancer. This is exemplified by the small molecule salubrinal, an inhibitor of GADD34-PP1C phosphatase complex, and, consequently, eIF2α dephosphorylation [19]. Interestingly, simultaneous or sequential combination therapy with salubrinal greatly increased the effectiveness of bortezomib by delaying eIF2α dephosphorylation in multiple myeloma cells [20]. In addition, the histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA), enhances cisplatin (CDDP)-induced apoptosis in the oral squamous cell carcinoma (OSCC) cell line by sustained phosphorylation of eIF2α [21]. The mechanisms by which treatments that stabilise P-eIF2α levels enhance antitumour drug cytotoxicity remain obscure. They might involve selective translation of specific mRNAs, encoding proapoptotic proteins such as the CHOP protein [22]. Alternatively it could be due to translation inhibition of mRNAs encoding regulators of either cell proliferation, like the ErBb2 oncogene [22] or the D1 cyclin [9], or cell survival, such as the hypoxia inducible factor 1α (HIF-1α) [18].

The role of eIF2 kinases in hypoxia deserves further attention. Hypoxia within the tumour microenvironment

promotes angiogenesis, metabolic reprogramming and tumour progression. These cell responses are mainly mediated by stabilisation of the HIF-1α transcriptional regulator [23]. Due to the importance of HIF-1α in cancer, targeting HIF-1α has become a novel approach in cancer therapy [23]. Tirapazamine (TPZ) represents a class of hypoxia-selective anticancer agent. TPZ acts by inhibiting HIF-1α mRNA translation driven by hypoxia or growth factors in human cancer cells and in HepG2 cell-derived tumours in athymic nude mice [24]. Interestingly, the inhibitory effect of TPZ on HIF-1α mRNA translation is dependent on the phosphorylation of eIF2α [24]. A similar mechanism has been recently reported for other antitumour drugs [18]. Therefore, activation of the eIF2 kinases provides a new and effective strategy to reduce HIF-1α expression and diminish tumour aggressiveness.

eIF2 kinases as tumour promoters

Paradoxically, eIF2 kinases are also critical for proliferation and survival of tumour cells. Reduced blood flow contributes to tumour hypoxia and nutrient deprivation. Cancer cells survive in this limited nutrient environment by activating the GCN2 kinase and its target, the transcription factor ATF4 [25]. Activation of GCN2 leads to a transient block in protein synthesis and a concomitant translation of ATF4 mRNA. ATF4 is a transcription factor that regulates an expression programme involved in nutrient uptake and tolerance to oxidative stress [26]. Accordingly, ATF4 knockdown in human fibrosarcoma and colorectal cancer cells enhanced tumour cell death by cell cycle arrest and apoptosis [25]. Most interestingly, the mechanism by which cell lethality ensues seems to involve asparagine and asparagine synthase (ASNS), as excess asparagine relieved cell death induced by loss of ATF4.

Nutrient stress signals to GCN2 by accumulation of uncharged tRNAs, which bind directly to a His tRNA synthetase-like domain in the GCN2 regulatory region [2]. It is remarkable that the activation of GCN2 by nutrient stress is conserved from yeast to man, highlighting the fundamental role of this pathway in cell biology. PERK is also required for eIF2α phosphorylation in tumour cells by nutrient stress, indicating that protein synthesis and folding in the ER is severely affected by amino acid deprivation. Indeed, PERK has a long and well established role in tumour resistance to hypoxia, by increasing expression of angiogenic genes [27].

The GCN2/PERK and ATF pathway not only increases tumour resistance to nutrient limitation, but also to chemotherapy. Acute lymphoblastic leukaemia is treated by the use of asparaginase, which depletes the circulating plasma of asparagine and deprives malignant lymphoblasts [28]. However, asparaginase treatment activates GCN2, stimulates translation of ATF4 and induces the expression of ASNS, thus promoting resistance to chemotherapy [28]. Furthermore, activation of the ISR pathway, by inducing ER stress, protects gastric cancer cells against apoptosis induced by doxorubicin and cisplatin [29]. In the light of these data, the above-mentioned therapies for cancer treatment should be reevaluated. Effectiveness of chemotherapeutic treatments that induce nutrient limitation or endoplasmic reticulum stress might be enhanced by combination with others that inhibit specific components of the GCN2/PERK-ATF4 signalling pathway.

Conclusions

The central importance of eIF2α kinases in cancer cell biology is emphasised by the expanding and apparently counterintuitive roles that they take on in tumour cell survival/death. However, despite the wealth of information available today, many mechanistic aspects of the ISR pathway remain obscure. How do DNA-damaging agents/anticancer drugs activate PERK/GCN2? Which are the substrates and effectors of cell cycle regulation and/or cell death? What is the actual contribution of each component of the pathway to resistance/sensitivity towards different stresses? Which set of mRNAs is selectively translated by nutrient limitation, hypoxia and DNA-damaging agents? Addressing these issues will help drug discovery research to safely navigate between the *Scylla* of tumour cell chemosensitivity and the *Charibdis* of tumour chemoresistance induced by small-molecule modulators of eIF2α kinases.

Conflict of interest The authors declare that they have no conflict of interest relating to the publication of this manuscript.

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