Eukaryotic elongation factor 2 kinase confers tolerance to stress conditions in cancer cells

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Received: 19 July 2014 / Revised: 23 August 2014 / Accepted: 11 September 2014 / Published online: 24 September 2014
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Abstract Eukaryotic elongation factor 2 (eEF2) is a member of the GTP-binding translation elongation factor family that is essential for protein synthesis. eEF2 kinase (eEF2K) is a structurally and functionally unique protein kinase in the calmodulin-mediated signaling pathway. eEF2K phosphorylates eEF2, thereby inhibiting eEF2 function under stressful conditions. eEF2K regulates numerous processes, such as protein synthesis, cell cycle progression, and induction of autophagy and apoptosis in cancer cells. This review will demonstrate the mechanisms underlying eEF2K activity in cancer cells under different stresses, such as nutrient deprivation, hypoxia, and DNA damage via eEF2 regulation. In vivo, in vitro, and clinical studies indicated that eEF2K may be a novel biomarker and therapeutic target for cancer.

Keywords Eukaryotic elongation factor 2 kinase · eEF2K · eEF2 · Cancer · Stress

Introduction

Eukaryotic elongation factor 2 kinase (eEF2K) is a structurally and functionally unique protein kinase in the calmodulin-

mediated signaling pathway; eEF2K phosphorylates and inhibits the function of eukaryotic elongation factor 2 (eEF2) (Chen et al. 2011a, b; Devkota et al. 2012). eEF2K regulates many cellular processes, such as protein synthesis, cellular differentiation and malignant transformation. Several types of cancer cell lines and human malignancies show increased eEF2K activity. Thus, eEF2K may be a valid target for anti-cancer treatment.

eEF2 and eEF2K

eEF2 is a member of the GTP-binding translation elongation factor family. This member is essential for protein synthesis and can be completely inactivated by EF2K phosphorylation. The eEF2 gene is located in chromosome 19 in humans and has a size of 9407 bases (Wullner et al. 2008). eEF2 is a cytoplasm-located monomeric protein with a mass of 95,207 Da (95.2 kDa). It comprises 857 amino acid residues. The eEF2 molecule contains three structural blocks (domains I–II, domain III, and domains IV to V), which can move relative to each other. The amino acid sequence of eEF2 is highly conserved and has a high degree of identity (>99 %) among mammals; the sequence around the diphthamide residue represents the most conserved region in the molecule (Zhang et al. 2007).

eEF2K is a unique calcium/calmodulin-dependent enzyme that inhibits protein synthesis by phosphorylating and inactivating eEF2, which is approximately 95–103 kDa in polypeptide size. Its gene is located in chromosome 16p12.2 in humans and has a size of 107 kbp. eEF2k undergoes extensive autophosphorylation, which enables the phosphorylation of eEF2 in the absence of added Ca^{2+} ions and calmodulin (Niu et al. 2013). The C-terminus alone can bind eEF2. The loss of the last 19 amino acids results in an enzyme that fails to phosphorylate eEF2, but undergoes

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autophosphorylation. Thus, the extreme C-terminus contains a key site for eEF2 interaction (Kurata et al. 2013). The increased phosphorylation of eEF2 and inhibition of protein synthesis, which were observed in neurons in response to excitotoxic activation of glutamate receptors, may serve a cytoprotective function (Chen et al. 2011a; Kurata et al. 2013).

eEF2K phosphorylation and protein synthesis

eEF2K phosphorylates eEF2 at Thr56, thereby disabling the function of eEF2 in mediating ribosomal elongation during messenger RNA (mRNA) translation. Phosphorylation of eEF2 terminates peptide elongation by decreasing affinity for the ribosome. Protein synthesis is highly regulated by both initiation and elongation. Phosphorylation by cyclin A–cyclin-dependent kinase 2 (CDK2) on a novel site, serine 595 (S595), directly regulates Thr56 phosphorylation by eEF2K. S595 phosphorylation varies during the cell cycle and is required for efficient Thr56 phosphorylation in vivo (Yang et al. 2013; Monteggia et al. 2013). S595 phosphorylation by cyclin A–CDK2 directly stimulates eEF2. eEF2K phosphorylates Thr56 in vitro, whereas S595 phosphorylation facilitates Thr56 phosphorylation by recruiting eEF2K to eEF2. The first known eEF2 modification that regulates eEF2 inhibition by eEF2K is S595 phosphorylation, which provides a novel mechanism linking the cell cycle machinery to translational control. Given that all known eEF2 regulations are exerted via eEF2K, S595 phosphorylation may globally couple the cell cycle machinery to regulatory pathways that affect eEF2K activity (Yang et al. 2013; Pyr Dit Ruys et al. 2012). eEF2K undergoes extensive regulatory phosphorylation, allowing diverse pathways to affect elongation. eEF2K phosphorylates a peptide substrate through a sequential mechanism. The peptide substrate inhibits the binding of ATP and must bind after ATP binds to form a productive ternary complex. “Inhibitor” of eEF2K, NH125 failed to show any inhibition of eEF2 phosphorylation in a variety of cancer cell lines, supporting the argument that NH125 is not a cellular inhibitor of eEF2K (Devkota et al. 2012).

eEF2K and cell cycling

Calcium-dependent protein kinases (PKCs) function in a myriad of cellular processes, including cell cycle regulation, proliferation, apoptosis, malignant transformation, etc. PKC inhibitors, when targeted to these pathways, have demonstrated efficacy against several types of cancer. PKCs coordinate several signaling pathways that are crucial to cellular malignant transformation. Experimental and clinical evidence suggests that pharmacological approaches using PKC inhibitors

may be effective in the treatment of cancer, such as chronic myeloid leukemia (Mencalha et al. 2014).

eEF2K is a unique and representative calcium/calmodulin-dependent enzyme, which has critical role in cell cycling. The mechanism of protein elongation is closely related to cell cycle progression, whereas eEF2 and eEF2K follow a prescribed pattern. Dysregulation of eEF2/eEF2K can alter the cell cycle and vice versa. And eEF2 or eEF2K may be overexpressed in cancer cells partly to modulate their effects over cell cycling. Overexpression would allow the cellular pools of eEF2 and eEF2K to buffer some metabolic stressors, such as hypoxia and free radical generation, which inevitably affect cancer cells. The protein synthesis modification machinery may indicate the importance of this system in carcinogenesis. As of this writing, information is lacking on the interactions between these enzymes and the machinery of the cell cycle. Possible relationships with Rb, ubiquitin ligases, and the majority of the cyclin family remain uncharacterized (Smith and Proud 2008; Hizli et al. 2013). Future studies on the relationship between eEF2 and eEF2K and the cell cycle are encouraged.

eEF2K, autophagy, and apoptosis

eEF2K is a critical controller of endoplasmic reticulum (ER) stress-induced autophagy and apoptosis in tumor cells. DNA-damage-inducible transcript 4-mediated activation of eEF2K has an important regulatory function in controlling the switch between autophagy and apoptosis in cancer cells under ER stress, and phosphorylation of eEF2K at Ser398 is necessary for autophagy induction. Silencing of eEF2K results in a blunted autophagic response. eEF2 is linked to apoptosis, and apoptosis is in part regulated by Bcl-2, both of which have been found upregulated after chemoradiotherapy in patients with rectal cancer (Casado et al. 2012). Moreover, both of the two genes are linked with Ca^{++} regulation (Ferdek et al. 2012).

Therefore, eEF2K-mediated autophagy has a protective function in cancer cells under metabolic stress conditions. Suppression of eEF2K-regulated autophagy impedes cell growth in serum/nutrient-deprived cultures, inhibits cell survival and enhances the efficacy of the growth factor inhibitors, such as trastuzumab, gefitinib, and lapatinib. Activation of eEF2K-mediated autophagy has protective function in cancer cells under metabolic stress conditions, and targeting autophagic survival by eEF2K may be a novel approach to enhance the effectiveness of growth factor inhibitors (Cheng et al. 2011; Gills et al. 2008). eEF2K also dictates cross-talk between autophagy and apoptosis induced by Akt inhibition, thereby modulating the cytotoxicity of a novel Akt inhibitor MK-2206 (Cheng et al. 2011).

eEF2K and 1-benzyl-3-cetyl-2-methylimidazolium iodide (NH125)

NH125 is a widely accepted inhibitor of mammalian eEF2K, and an efficacious anti-proliferative agent against different cancer cells. The anticancer activity of NH125 is more correlated with the induction of eEF2 phosphorylation than the inhibition of eEF2K. The induction of eEF2 phosphorylation is reportedly correlated with the inhibition of cancer cell growth. In the comparison of several known inducers of eEF2 phosphorylation, including AMPK activators and an mTOR inhibitor, stronger induction of eEF2 phosphorylation is correlated with more effective growth inhibition than the other inducers. Preliminary data suggested that NH125-induced eEF2 phosphorylation is likely mediated by multiple pathways, although the results still with argue. However, the aforementioned observations helped to identify a new multi-pathway approach to anticancer therapies (Chen et al. 2011a, b; Devkota et al. 2012).

eEF2K in cancer therapeutics

Breast cancer Disruption of eEF2K expression in vivo and in vitro results in the downregulation of signaling pathways that affect growth, survival, and resistance of breast cancer. Systemic administration of liposomal eEF2K small interfering RNA (siRNA) demonstrated the downregulated expression of eEF2K in an orthotopic xenograft mouse model of a highly aggressive triple-negative (breast cancer that does not express the genes for estrogen receptor (ER), progesterone receptor (PR) and Her2/neu) MDA-MB-231 tumor. The targeting resulted in a substantial decrease in eEF2 phosphorylation in the tumors and led to growth inhibition, apoptosis induction, and sensitization of tumors to the chemotherapy agent doxorubicin (Tekedereli et al. 2012). Similar results were observed in ER-positive MCF-7 cells and an invasive phenotype of the MDA-MB-231 cells (Niu et al. 2013). Moreover, a clinical study involving the collection of primary tumors from 190 patients with stages I to III hormone receptor-positive breast cancer and a median 96-month follow-up for surviving patients showed that increased eEF2K activity is associated with poor prognosis in hormone receptor-positive breast cancer, as well as other players of translational aberrations showing comprehensive results in the combined analysis (Meric-Bernstam et al. 2012). These data support the notion that eEF2K has potential as a prognostic marker and therapeutic target to treat breast cancer.

Glioma The expression and activity of eEF2K are significantly upregulated in gliomas and contribute to the migration and invasion of tumor cells. Tumor treatment with the necrosis factor-related apoptosis-inducing ligand (TRAIL), a targeted

therapy that induces apoptosis in cancer cells but is not cytotoxic to most normal cells, results in the downregulation of the anti-apoptotic protein Bcl-xL. Overexpression of Bcl-xL can abrogate the sensitizing effect of eEF2K inhibition on TRAIL. Inhibition of eEF2K by RNA interference or pharmacological inhibitor NH125 enhances TRAIL-induced apoptosis in human glioma cells, as shown by an increase in apoptosis in tumor cells treated with eEF2K siRNA or eEF2K inhibitor (Zhang et al. 2011). Akt inhibition by MK-2206 or siRNA-mediated attenuation strongly activates autophagy, whereas silencing of eEF2K blunts this autophagic response in glioma cells. Suppression of MK-2206-induced autophagy by eEF2 silencing promotes apoptotic cell death (Cheng et al. 2011). Treatment of human glioma cells with 2-deoxyglucose (2-DG)-activated autophagy is also associated with the activation of eEF2K. Energy stress and cytotoxicity caused by 2-DG can be accelerated by eEF2K inhibition, and targeting the eEF2K-regulated autophagic survival pathway may represent a novel approach to sensitizing cancer cells to glycolytic inhibitors (Wu et al. 2006). These findings indicated the therapeutic evaluation of eEF2K in gliomas.

Pancreatic cancer eEF2K is overexpressed in pancreatic cancer (PaCa) cells, and its downregulation induces apoptotic cell death. Rottlerin (ROT), a polyphenolic compound initially identified as a PKC- δ inhibitor, induces apoptosis and autophagy in various cancer cells, including PaCa cells. ROT downregulates eEF2K at the mRNA level, and induces eEF2K protein degradation through the ubiquitin-proteasome pathway. The downregulation of eEF2K recapitulates the events observed after ROT treatment, whereas its overexpression suppresses ROT-induced apoptosis. The downregulation of eEF2K leads to the induction of intrinsic, extrinsic, and apoptosis-inducing factor-dependent apoptosis in PaCa cells, suggesting that eEF2K may be an attractive therapeutic target for the development of future anti-PaCa agents (Ashour et al. 2014).

Conclusion

eEF2K expression is upregulated in cancer cells. eEF2K regulates many cellular processes, such as protein synthesis, cell cycle progression, and induction of autophagy and apoptosis in cancer cells. eEF2K has a critical function during stressful conditions, such as nutrient-deprived condition, hypoxia, and DNA damage (Gills et al. 2008; Lepruvier et al. 2013; Kenney et al. 2014; Zheng et al. 2014). In vivo and in vitro studies indicated that eEF2K is a novel therapeutic target for cancer. Moreover, clinical studies of patients showed that eEF2K may serve as a biomarker for prognosis and personalized cancer treatment (White-Gilbertson et al. 2009; Grandjean et al.

2013; Sun et al. 2013). Future studies are necessary to further investigate the mechanism and clinical application of this kinase.

Acknowledgments This work was supported by the Natural Science Foundation of China (No. 81272504), Research and Innovation Project for College Graduates of Jiangsu Province (No.CXZZ12_0588), Innovation Team (No. LJ201123 (EH11)), A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD) (JX10231801), grants from Key Academic Discipline of Jiangsu Province “Medical Aspects of Specific Environments,” and Six Major Talent Peak Project of Jiangsu Province (2013-WSN-040).

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