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Review

A New Perspective on the Adaptation and Proliferation Mechanism of Cancer Cells: Atypical kinase eEF-2K

Kanser Hücrelerinin Adaptasyon ve Proliferasyon Mekanizmasına Yeni Bakış: Atipik kinaz eEF-2K

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Abstract

Eukaryotic elongation factor 2 kinase (eEF-2K), also known as calcium/calmodulin-dependent protein kinase III, is a member of the group of atypical α-kinases. eEF-2K is an essential regulatory molecule in cellular protein synthesis. It can control the protein chain elongation rate by phosphorylating/inactivating eukaryotic elongation factor 2 (eEF-2). The activity of eEF-2K is dependent on calcium ions and calmodulin and is extensively regulated by posttranslational phosphorylation. Recent data show that eEF-2K helps protect cancer cells against nutrient restriction and energy depletion. It also exerts a cytoprotective effect in many conditions, including oxidative stress and hypoxia. In light of the foregoing, eEF-2K may be a new potential pharmacological target for cancer therapy.

Keywords: eEF-2, eEF-2K, Protein synthesis, Cancer, mTORC1, AMPK

ÖZ

Ökaryotik uzama faktörü 2 kinaz (eEF-2K), atipik α-kinazlar grubunun bir üyesidir. Kalsiyum/kalmodüline bağımlı protein kinaz III olarak da bilinen eEF-2K, hücresel protein sentezinde önemli bir düzenleyici moleküldür. eEF-2K, ökaryotik uzama faktörü 2'yi (eEF-2) fosforile/inaktive ederek protein zinciri uzama oranını kontrol etme işlevi görür. eEF-2K'nın aktivitesi kalsiyum iyonlarına ve kalmoduline bağlıdır. Buna ek olarak, posttranslasyonel fosforilasyon ile aktivitesi kapsamlı bir şekilde düzenlenir. Son veriler, eEF-2K'nın kanser hücrelerini besin açlığı ve enerji tükenmesine karşı korumaya yardımcı olduğunu ve ayrıca oksidatif stres, hipoksi dahil pek çok koşulda sitoprotektif etkili olduğunu göstermektedir. eEF-2K'nın kanser tedavisi için yeni bir potansiyel farmakolojik hedef olabileceği düşünülmektedir.

Anahtar Kelimeler: eEF-2, eEF-2K, Protein sentezi, Kanser, mTORC1, AMPK

Introduction

Cells constantly need protein synthesis to perform their metabolic activities. Notably, this synthesis process entails high rates of energy consumption. Protein synthesis is a tightly controlled biochemical process with many control steps (1). The eukaryotic elongation factor-2 kinase (eEF-2K), which functions as modulator of protein synthesis, is a calcium/calmodulin (Ca^{2+}/CaM)-dependent protein kinase. eEF-2K phosphorylates eukaryotic elongation factor-2 (eEF-2) (2). The phosphorylation of eEF-2 by eEF-2K generally reduces protein translation. Especially under stress conditions, such as nutrient and energy deprivation, oxidative stress, and hypoxia, cells increase their eEF-2K activity to slow down protein synthesis, thus saving energy and adapting to new conditions (3). This activation–inactivation mechanism is an important intersection of the cellular adaptation and differentiation processes.

Especially in the uncontrolled proliferation mechanism specific to cancer cells, eEF-2K is selectively activated. In vitro experiments have shown that cell proliferation and eEF-2K activity increase in the presence of epidermal growth factor and insulin-like growth factor 1. When eEF-2K activity is inhibited by Rottlerin (eEF-2K inhibitor), cell proliferation induced by epidermal growth factor and insulin-like growth factor 1 is blocked. It has been shown that decreased expression of eEF-2K causes downregulation of signaling pathways involved in the cell cycle and proliferation (4–6). Inhibition of eEF-2K activity further highlighted the importance of inhibitory mechanisms in chemotherapy. Inhibitors with different chemical origins can be effective in energy metabolism, cell proliferation, signaling pathways and protein synthesis stages. Catalytic inhibition of eEF-2 (competitive inhibition), inhibition of ATP production, downregulation of mRNA levels, induction of protein degradation and inhibition of cell permeability are the prominent inhibition mechanisms of inhibitor substances (6–9).

Increasing evidence indicates that the eEF-2K/eEF-2 pathway is involved in crucial physiological and pathological processes, such as cancer, cardiovascular diseases, and neurodegenerative diseases (10). In this review, we will summarize the main evidence supporting the defined role of eEF-2K activity in cancer.

Structures of eEF-2 and eEF-2K

The eEF-2 molecule is a protein of approximately 95.2 kDa. It is located in the cytoplasm. Protein translation includes initiation, elongation, and termination phases. The eEF-2 molecule is involved in the elongation phase and acts as the translocase responsible for the movement of newly formed peptidyl-tRNAs (11). The addition of newly synthesized amino acids to the peptide chain is an energy-requiring process. The energy required for translation is obtained from the hydrolysis of GTP, the ATP analog, by eEF-2 (12,13). The phosphorylated/dephosphorylated control of eEF-2 activity allows rapid and transient modulation of protein synthesis. The phosphorylation of eEF-2 through the Threonine-56 (Thr-56) residue reduces its affinity for the ribosome, which inhibits protein chain elongation (14). eEF-2 is catalyzed by a highly specific protein kinase, eEF-2K (2).
The eEF-2K enzyme is a member of group called "α-kinases," which differ from the eukaryotic protein kinase superfamily. It contains 725 amino acid residues and has a mass of about 82.1 kDa (15,16). Notably, the catalytic domain and the CaM binding site are found in the N-terminus of the primary structure. Meanwhile, the SEL1 domain can be found in the C-terminal part; protein-protein interactions usually take place in this region. At the end of the C-terminal is a key region for interactions with eEF-2 (Figure 1). The catalytic domain and SEL1 domains are linked by a so-called "binding domain," which contains several phosphorylation sites that can regulate the activity of eEF-2K (2,17,18).

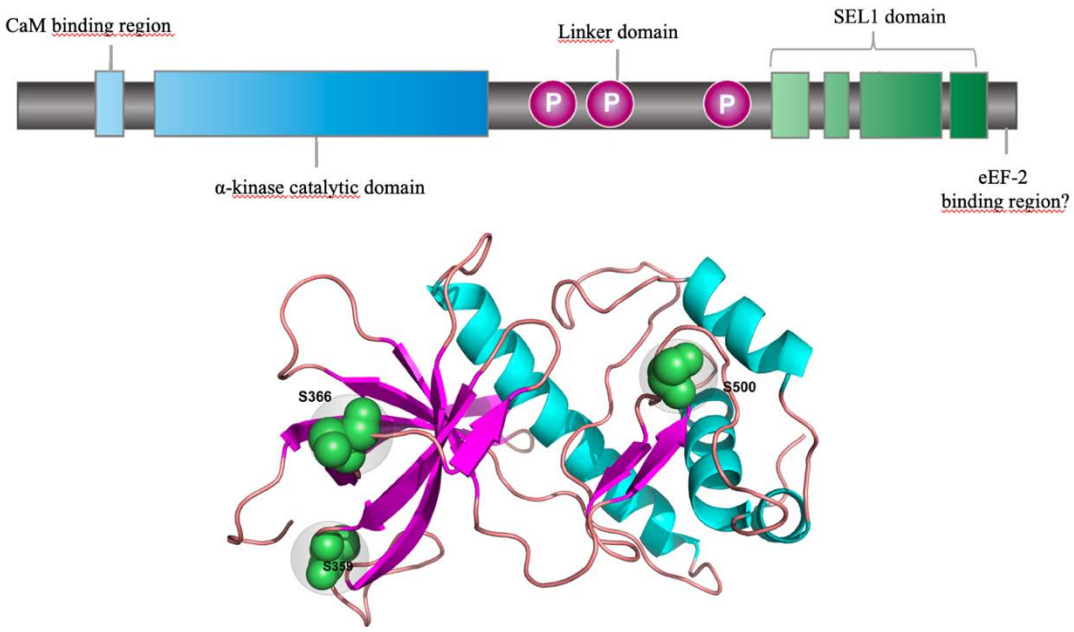

Figure 1: The structure of the eEF-2K. eEF-2K is a protein kinase with a CaM binding site at its N-terminus, α-kinase catalytic domains, and a SEL1 domain at its C-terminal region (2).

Activation of eEF-2K

The activation of eEF-2K using a two-step allosteric activation mechanism is regulated by autophosphorylation in the presence of Ca^{2+} and CaM. In the first step of eEF-2K activation, high-affinity Ca^{2+}/CaM binding results in autophosphorylation at the Thr348 residue, giving rise to a conformational change. The binding of phosphorylated Thr348 to a phosphate-binding site in the kinase domain results in a second conformational change. Consequently, this change increases eEF-2K activity by increasing its affinity for eEF-2 and its intrinsic catalytic activity (19,20).
eEF-2K activity can be controlled by several other signals in addition to autophosphorylation reactions. It is affected by intracellular pH, hypoxia, stress, and nutrient restriction. AMP-activated protein kinase (AMPK) and glycogen synthase kinase 3 have been identified as positive regulators of eEF-2K activity (3,21,22) AMPK is the main sensor of cellular energy levels that is activated when ATP levels change (23). AMPK phosphorylates several substrates to maintain energy balance. The activation of AMPK induces the phosphorylation of eEF-2K and inhibits protein synthesis, potentially conserving energy (22). This probably includes the inhibition of the mammalian target of rapamycin complex 1 (mTORC1), a negative regulator of eEF-2K. AMPK may also contribute to its activation by directly phosphorylating eEF-2K (24).
Hypoxia causes the activation of eEF-2K. eEF-2K undergoes hydroxylation on the Proline-98 residue located between the CaM binding site and its catalytic domain. Proline hydroxylation is catalyzed by the oxygen-dependent enzyme proline

hydroxylase. Hydroxylation under normoxemic conditions partially impairs CaM binding to eEF-2K and significantly limits the CaM-induced activity of eEF-2K. At low oxygen levels, where proline hydroxylase function is impaired, this residue cannot be hydroxylated, and eEF-2K is more strongly activated by Ca^{2+}/CaM. This will slow down protein synthesis and save energy under conditions where oxidative metabolism cannot keep up with cellular energy demands and oxygen is insufficient (15,25).

Another factor affecting the activation of eEF-2K is pH. Low pH values (approximately pH 6.8-6.6) lead to eEF-2K activation and increased phosphorylation of eEF-2. Xie et al. argued that the histidine residues detected in the structure of eEF-2K play a key role in the formation of this effect. Due to their structural properties, histidines can be positively charged by taking protons at acidic pH values. Ultimately, the positive charge at the CaM binding site increases Ca^{2+} binding and positively regulates eEF-2K activation (26).

It has been reported that eEF-2K is negatively regulated by the mTORC1 signaling pathway via direct or indirect phosphorylation (21). The mTORC1 signaling pathway is activated by amino acids, hormones, or growth factors; it has been identified as a key cellular sensor of the nutrient or energy state of cells (27). There are several interactions described between mTORC1 and eEF-2K. By directly phosphorylating eEF-2K via Ser78/Ser396, mTORC1 can disrupt Ca^{2+}/CaM activity. In addition, an indirect phosphorylation reaction occurs when the p70S6 kinase activated by mTORC1 phosphorylates eEF-2K, resulting in the inhibition of eEF-2K activity. ERK signaling, which is an oncogenic signaling pathway, also causes the inactivation of the eEF-2K enzyme by phosphorylating it over serine residues (21,28).

In summary, eEF-2K supports cell survival by providing adaptation to hypoxia, nutrient, and energy deprivation, and by blocking translation. Even more remarkable, cancer cells use eEF-2K activity to adapt to a nutrient-restricted state (2,29).

Cancer and eEF-2K

Cancer cells are typically characterized by uncontrolled cell division and proliferation. In this state of uncontrolled growth and proliferation, protein synthesis is unregulated. The question of how the known role of eEF-2K in protein synthesis changes in cancer cells has drawn the interest of cancer researchers. Studies have reported that eEF-2K plays a critical role in the cell cycle, autophagy, and apoptosis. eEF-2K has been shown to be overexpressed in breast cancer, glioma, esophageal squamous cell carcinoma, and pancreatic cancer (7,12,30–32).

Breast cancer: In a study with MDA-MB-231 and MCF-7 breast cancer cell lines, the silencing of eEF-2K with the help of siRNA has been shown to significantly reduce cell proliferation and colony formation. However, *in vivo* targeting of eEF-2K expression by systemic liposomal siRNA in a mouse breast cancer model showed that it inhibited tumor growth, induced apoptosis, and increased sensitivity to the chemotherapy agent doxorubicin. It was concluded that the disruption of eEF-2K expression in breast cancer cells results in the downregulation of signaling pathways that affect the growth, survival, and resistance to chemotherapy agents (5). Similar results were also found evaluating the effects of silencing eEF-2K on autophagy, cell viability, tumorigenesis, and invasion in the breast cancer cell line. It has been shown that silencing eEF-2K significantly suppresses the tumorigenesis, invasion, and chemotherapy resistance of chemotherapy-resistant cancer cell lines while reducing the accumulation of LC3 protein, an autophagic marker, and suppressing autophagy (33).

Esophageal squamous cell carcinoma: Examining the biological function of eEF-2K in esophageal squamous cell carcinoma, Zhu et al. showed that eEF-2K expression is higher in esophageal squamous cell carcinoma tissues compared with non-tumor tissues. It was also found that the overexpression of eEF-2K had higher rates of migration and invasion and promoted tumor growth. Cells overexpressing eEF-2K had increased proliferation compared with the controls, while silencing eEF-2K decreased cell proliferation (30).

Pancreatic cancer: Pancreatic cancers with low survival rates constitute one of most aggressive types of cancer. Ashour et al. showed that eEF-2K is overexpressed in pancreatic cancer cells (PANC-1 and MIAPaCa-2). It has been shown that the inhibition of eEF-2K induces intrinsic and extrinsic factors in pancreatic cancer cells and contributes to the sensitivity of tumor cells to the chemotherapy agent doxorubicin (7).

Lung cancer: Bircan et al. reported that eEF-2K is overexpressed in lung cancer cell lines (A549, H1299, H322, H292, and H226) and is associated with shorter patient survival in lung cancer. The inhibition of eEF-2K expression by siRNA or a chemical inhibitor (Rottlerin) has been shown to significantly suppress lung cancer cell proliferation, colony formation, cellular survival, and tumorigenesis by inhibiting cyclin D1, Src, and MAPK/ERK signaling. In addition, the *in vivo* targeting of eEF-2K expression systemically by nanoliposomal siRNA in a lung cancer xenograft tumor model was found to inhibit tumor growth. Finally, it has been reported that the expression of eEF-2K promotes proliferation, invasion, and tumor growth of lung cancer (34).

Glioma: In human glioma cells, the inhibition of eEF-2K by siRNA or NH125 down-regulates the expression of the anti-apoptotic protein Bcl-xL. This inhibition reportedly increases TRAIL (tumor the necrosis factor-related apoptosis inducing ligand)-induced apoptosis (31). Since TRAIL has the ability to trigger rapid apoptosis in malignant cells, it is accepted as an anticancer agent candidate (35). It has been found that the inhibition of eEF-2K can sensitize glioma cells to TRAIL treatment, and this effect is mediated through the regulation of Bcl-xL. These results show that eEF-2K can regulate tumor cell apoptosis to protect tumor cells and increase drug resistance in cancer (31).

Nasopharyngeal Carcinoma: Zhao et al. investigated the combined antitumor effects of eEF-2K inhibitor NH125 and AKT inhibitor MK-2206 in nasopharyngeal carcinoma cell lines. eEF-2K-mediated autophagy induced by AKT inhibition played a protective role in nasopharyngeal carcinoma cells. The NH125-MK-2206 inhibitor combination showed a synergistic effect. This effect was found to further enhance the growth inhibitory effect of MK-2206 on nasopharyngeal carcinoma cells in *in vitro* and *in vivo* experiments. Based on these results, it was concluded that the inhibition of eEF-2K-mediated autophagy could increase the effects of chemotherapy agents (36).

Hepatocellular Carcinoma It has been found that eEF-2K is overexpressed, and its activity is high in hepatocellular carcinoma. Researchers have stated that this may be a prognostic marker (37). In another study, the suppression of eEF-2K expression in hepatocellular carcinoma cells led to decreased PI3K/Akt and STAT3 activity, which promoted cancer cell growth, survival, and migration. It has been posited that a high level of eEF-2K expression is associated with a high rate of metastatic and worsening liver cancer type, and eEF-2K promotes angiogenesis (38).

Colon cancer: It has been demonstrated that the atypical kinase eEF-2K does not have the same effect on all cancer cells. Cancer cell proliferation, growth, autophagy, and other related mechanisms were investigated by silencing and overexpressing the eEF-2K gene in HT-29 and HCT-116 colon cancer cell lines. Contrary to its previously described role, siRNA-mediated silencing of the eEF-2K gene was found to promote cancer cell growth and proliferation and result in increased autophagy mediated by the AMPK-ULK1 pathway (39).
It should be noted that although increased eEF-2K expression in many cancer types promotes tumor development by increasing proliferation, invasion, and metastatic activities of cancer cells, there are also studies that prove the opposite (39,40).

eEF-2K-mediated survival strategies of cancer cells

Rapidly growing cancer cells require protein synthesis at high rates for uncontrolled proliferation. However, cancer cells have a limited supply of nutrients (41). Therefore, it seems contradictory that proliferating cells overexpress eEF-2K, which negatively regulates protein synthesis. Several possible mechanisms have been proposed to explain how increased eEF-2K activation and reduced protein synthesis confer a survival advantage for cancer cells (Figure 2). First, considering that protein synthesis consumes a large proportion of cellular energy, eEF-2K may help cells conserve such resources, especially under nutrient-restricted conditions (22). Second, the activation of eEF-2K can inhibit the production of apoptotic and pro-apoptotic proteins that will induce apoptosis in the cell, which may promote the synthesis of proteins that are particularly important for cell survival. In addition, since the inhibition of eEF-2K sensitizes cells to chemotherapy, its overexpression may contribute to increased survival against chemotherapeutic agents (3,5). Another possible mechanism is that eEF-2K may mediate the induction of autophagy, which is one of the cell survival strategies, especially in nutrient deprivation (42). Apart from the above-mentioned mechanisms, there may also be unexplored interactions. The discovery of the eEF-2K molecule's effects on cell survival and proliferation has made it a potential target for cancer therapy. It should not be overlooked that the benefit of targeting eEF-2K in cancer treatment may vary depending on the type and stage of cancer.

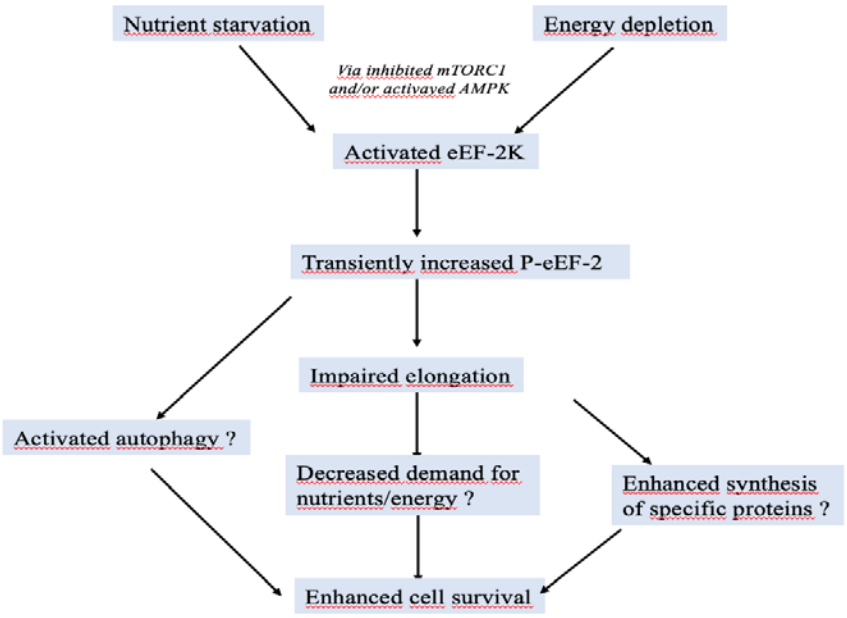

Figure 2: Regulation of eEF-2K activation through nutrient and energy balance (51)

eEF-2K inhibitors

miRNAs and siRNAs carried by nanoliposomes have a very important role in the targeted treatment of cancer for therapeutic purposes. Using these molecules, target genes can be silenced. Moreover, their related protein levels can be controlled by gene-level inhibition. In cancer research, the targeting of eEF-2K by siRNAs or miRNAs is frequently used in *in vivo* and *in vitro* studies (5,39). In addition, there are natural or synthetic compounds that are used to inhibit eEF-2K activity by targeting oncogenic pathways. (Figure 3) (3). Single or dual signal pathway targeted drugs and combination drugs (eEF2K inhibitor NH-125 and Akt inhibitor MK-2206 combination) are compounds used for this purpose (43). There are studies to inhibit eEF2K activity in cancer using re-purposed drugs (e.g. fluoxetine, pemetrexed) (44,45) The pharmacological agents exert their inhibitory effects through different mechanisms, such as cell proliferation inhibition, gene and protein expression inhibition, and ATP/CaM competitive inhibition. Rottlerin (7), NH125 (36,46), TS-2 (4-ethyl-4-hydroxy-2-p-tolyl-5,6-dihydro-4H-1,3-selenazine) and TS-4 (4-hydroxy-6-isopropyl-4-methyl-2-p-tolyl-5,6-dihydro-4H-1,3-selenazine) (9), A-484954 (8), cefatrizine (47), β-phenylalanine derivative inhibitor 211 (48), DFTD (2,6-diamino-4- (2-fluorophenyl) -4H-thiopyran-3,5-dicarbonitrile) (49), Thymoquinone (50) are among the eEF-2K inhibitors identified as a result of studies.

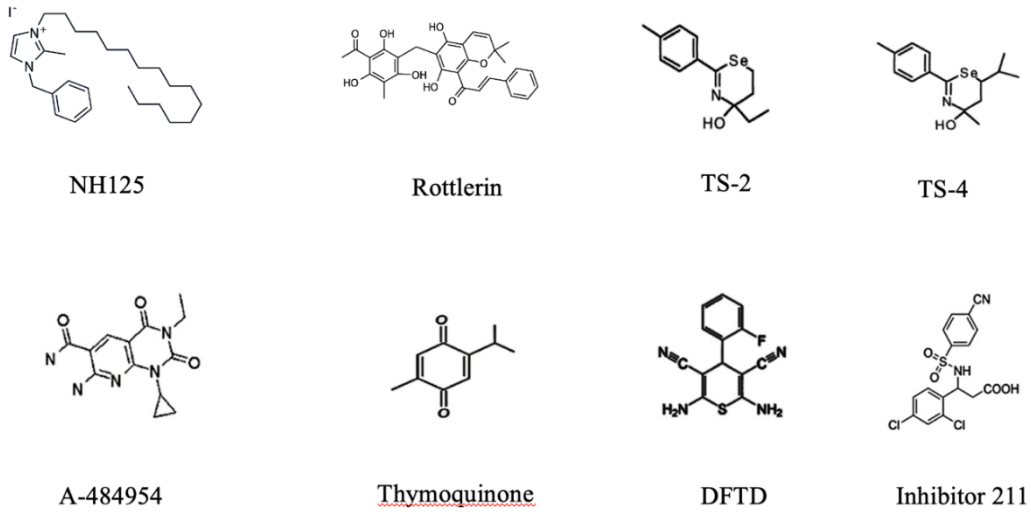

Figure 3: eEF-2K inhibitors. Rottlerin, NH125, TS-2 (4-ethyl-4-hydroxy-2-p-tolyl-5,6-dihydro-4H-1,3-selenazine), TS-4 (4-hydroxy-6-isopropyl-4-methyl-2-p-tolyl-5,6-dihydro-4H-1,3-selenazine), A-484954, β-phenylalanine derivative inhibitor 211, DFTD (2,6-diamino-4- (2-fluorophenyl) -4H-thiopyran-3,5-dicarbonitrile), Thymoquinone are among the eEF-2K inhibitors.

CONCLUSION

In this review, we summarize the main evidence describing the function of eEF-2K, the mechanisms regulating its activity, and its role in cancer cells. It is clear that eEF-2K protects cancer cells under stress conditions, such as nutrient and energy deprivation. In addition, it has been reported that eEF-2K plays a critical role in signaling pathways, such as the cell cycle, proliferation, autophagy, and apoptosis. Notably, it is overexpressed in many cancer types. These findings suggest that eEF-2K may be a new potential pharmacological target for cancer therapy. Moreover, the sensitization of cells to chemotherapy through the inhibition of eEF-2K may provide a new perspective on the combined treatment of chemotherapeutics. More studies are needed to evaluate the usefulness of eEF-2K as a therapeutic target in oncology.

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