The role of eukaryotic translation initiation factor 6 in tumors (Review)

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Abstract. Eukaryotic translation initiation factor 6 (eIF6) affects the maturation of 60S ribosomal subunits. Found in yeast and mammalian cells, eIF6 is primarily located in the cytoplasm of mammalian cells. Emerging evidence has demonstrated that the dysregulated expression of eIF6 is important in several types of human cancer, including head and neck carcinoma, colorectal cancer, non-small cell lung cancer and ovarian serous adenocarcinoma. However, the molecular mechanisms by which eIF6 functions during tumor formation and progression remain elusive. The present review focuses on recent progress in terms of the mechanisms and functions of eIF6 in human tumorigenesis or cancer cell lines, along with the signal transduction pathways in which this novel translation initiation factor may participate. Oncogenic Ras activates Notch-1 and promotes transcription of eIF6 via a recombining binding protein suppressor of Hairless-dependent mechanism. In addition, overexpression of eIF6 results in aberrant activation of the Wnt/β-catenin signaling pathway. Similarly, overexpressed eIF6 regulates its downstream modulator, cell division control protein 42, which in turn affects oncogenesis. Finally, the potential of eIF6 as a biomarker for diagnosis of cancer is also discussed in the present review.

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1. Introduction

More than 30 years ago, eukaryotic translation initiation factor 6 (eIF6) was first identified as a protein in wheat germ (1). This protein functions as an anti-association factor to interact with the 60S ribosome, and prevent the assembly of the 60S and 40S subunits in the cytoplasm (2-5). eIF6 is found in yeast and mammalian cells, and the majority of eIF6 is located in the cytoplasm of mammalian cells (4,5). Originally, eIF6 was first observed in the proliferating compartment of the colonic epithelium and stem cells (6), and is also highly expressed in epithelial and embryonic tissues (7-9). Furthermore, an increasing number of studies have demonstrated that eIF6 is overexpressed in human cancer (6,10-15). Accumulating evidence suggests that eIF6 is a useful biomarker in cancer diagnosis, and that it serves as an anti-cancer molecular target. However, the specific role of eIF6 in tumorigenesis remains to be elucidated.

The present review focuses on eIF6-associated studies, particularly those pertaining to its subcellular location, phosphorylation and dephosphorylation, roles in cancer and molecular mechanisms in oncogenesis.

2. Subcellular localization of eIF6

eIF6, also known as integrin β4 binding protein, p27BBP or β4 integrin interactor, is a remarkably conserved protein from yeast to mammals (7,13-14). In yeast, eIF6 is primarily localized in the nucleolus (9-10). By contrast, in mammalian cells, the majority of eIF6 is present in the cytoplasm, with a smaller but significant fraction (~30%) located in the nucleus (4,16-18). Notably, eIF6 is located in the nucleolus of certain cell lines, such as HeLa, A431, NIH/3T3 fibroblasts and Jurkat T cells (8), in addition to neoplastic tissues, including colonic adenoma and carcinoma (6). Previous studies have demonstrated that eIF6 functions as a component of the preribosomal particles in the nucleolus, thus serving an important role in 60S ribosome biogenesis (8,18). In the cytoplasm, eIF6 functions as a translation factor (9), therefore, subcellular localization is crucial for the functional regulation of eIF6.

3. Phosphorylation and dephosphorylation of eIF6

In mammalian cells, eIF6 regulates ribosomal assembly and biogenesis, thus controlling the binding of 40S and 60S ribosomal subunits and participating in 80S assembly (7-9,16). As described above, eIF6 is present in the nucleus and cytoplasm (9,17). Notably, it is reported that nucleocytoplasmic shuttling is caused by the phosphorylation of eIF6, in line with the well-established hypothesis that phosphorylation is able to regulate the biological activity of numerous proteins (9). Therefore, phosphorylation is likely to modulate eIF6 activity, and three potential phosphorylation sites have been identified (18). For nuclear export in mammalian cells, eIF6 is phosphorylated *in vitro* at Ser-175 and Ser-174 by the nuclear isoforms of casein kinase (CK) CK1α or CK1δ, thereby promoting the formation of pre-60S ribosomal particles in the cytoplasm. In addition, the Ca^{2+}/calmodulin-dependent protein phosphatase calcineurin mediates dephosphorylation, which facilitates migration of eIF6 back to the nucleolus and continues 60S ribosome biogenesis (18). Such evidence implies that CK1 controls the subcellular distribution of eIF6.

Although CK1 is widely found in the nucleus, cytoplasm, cell membrane and cytoskeleton of mammalian and yeast cells (19-21), it is unclear whether extranuclear CK1 enters the nucleus to regulate the export of eIF6. It should be noted that cytoplasmic eIF6 in mammalian cells is also phosphorylated by receptors for activated C kinase 1 (RACK1)/protein kinase C (PKC) signaling at positions Ser-174, Ser-175 and Ser-235 (Fig. 1) (16,22). These procedures result in dissociation of eIF6 from the 60S subunit, thus aiding its maturation (18). Recent research demonstrates that GTPase elongation factor-like 1 (EFL1) is involved in the cytoplasmic maturation of the ribosomal 60S subunit (3). SBDS, the protein mutated in Shwachman-Bodian-Diamond syndrome, and EFL1 release the anti-association factor eIF6 from the surface of the 60S subunit (2,5). In addition, the Ser235 PKC phosphorylation site has also been identified in the *Xenopus* eIF6 protein (23).

However, there is little or no evidence to verify whether CK1 and the RACK1-PKC complex phosphorylate the Ser-174 and Ser-175 sites of eIF6 at the same time. Moreover, an increasing number of studies have demonstrated that eIF6 is highly overexpressed in tumor cells (8-11). The C-terminal of eIF6 is subject to RACK1-PKCβII complex phosphorylation at Ser-235, which modulates the protumorigenic activity of eIF6 (16), whereas mutation of the phosphorylation site at Ser235 of eIF6 in mouse models reduces translation and lymphomagenesis (4). A previous study demonstrated that the Ras cascade, which regulates phosphorylation of eIF6, is triggered by agonists of phorbol esters (16). Therefore, it may be speculated that the Ras cascade recruits PKCβII and phosphorylates eIF6 at Ser235, and the activity of eIF6 leads to increased translation and tumorigenesis.

4. Overexpression of eIF6 in human carcinoma

Numerous studies have demonstrated highly aberrant expression of eIF6 in human cancer (10-15). Although the function of eIF6 is not fully understood, differential expression of eIF6 is correlated with cancer pathogenesis, and eIF6 functions as a regulator in cancer development (6,10-15). The cancer tissues and cell lines in which eIF6 is overexpressed are presented in Table I. In this section, the potential of eIF6 as a cancer biomarker is discussed.

Colorectal cancer. eIF6 is regarded as a nuclear matrix protein that accumulates in nucleoli (8), and is found in the cytoplasm of glandular crypt cells in the human colonic epithelium (6). However, higher levels of eIF6 are observed in colorectal carcinoma compared with colorectal precancer and normal mucosa (6-7,16). Consequently, there is a progressive increase of eIF6 from normal tissue to dysplastic adenoma and carcinoma. This raises the question of which mechanisms are involved in the increased expression of eIF6 protein. It is hypothesized that eIF6 is upregulated at the transcriptional level, such that the mRNA coding for eIF6 is highly concentrated in tumors relative to normal colorectal tissues (6). mRNA translation controls distinct cellular processes, including tumorigenesis, cell migration, adhesion and growth, and cell-cycle control (24). Notably, gross gene expression of eIF6 is less well known. Therefore, further research is required to understand the underlying reasons for this.

As a marker of cell proliferation, the distribution of argyrophilic nucleolar organizer region (AgNOR)-associated proteins in the nucleolus and cell correspond to proteins located in the nucleolar organizer regions. Previously, nucleolar staining by AgNORs was considered to be a prognostic marker of malignancy (25). Moreover, the value of AgNORs as proliferation markers has been reported in various forms of cancer, such as breast, ovarian, cervical, prostate, hepatocellular, papillary thyroid, gastric and bladder cancer (25-31). Certain studies have demonstrated a correlation between AgNOR count in tumors and various clinical parameters, including tumor size and staging, and distant metastasis (28-32). Therefore, eIF6 may be used as a diagnostic tool on the basis of the function of AgNORs. In addition, differentially-expressed eIF6 may serve a critical function in colon carcinogenesis and provide a novel marker in surgical pathology.

Head and neck carcinoma. eIF6 is overexpressed in colorectal cancer (6). Similarly, in head and neck carcinoma, the expression of eIF6 is also higher than that observed in normal mucosa (13). Additionally, nucleolar overexpression of eIF6 has been detected in head and neck metastatic carcinoma (13). Head and neck cancer has previously been reported as the sixth leading type of cancer worldwide, accounting for ~6% of all tumors, of which >90% are head and neck squamous cell carcinoma (33,34). Despite advances in treatment, the prognosis remains poor. Therefore, the discovery of molecular markers is not only important for understanding the pathogenesis of head and neck cancer, but may also provide further insight into tumor biology, diagnosis, therapeutic perspectives and prognosis (34,35).

eIF6 is highly concentrated in nucleoli, is easily observed and its overexpression is not difficult to measure. eIF6 may function as a molecular marker for use in surgical pathological diagnosis. Notably, a larger 52-kDa protein, detected by eIF6 antibody, is also observed in lymph node metastases (13). This larger protein has tissue specificity due to its absence in
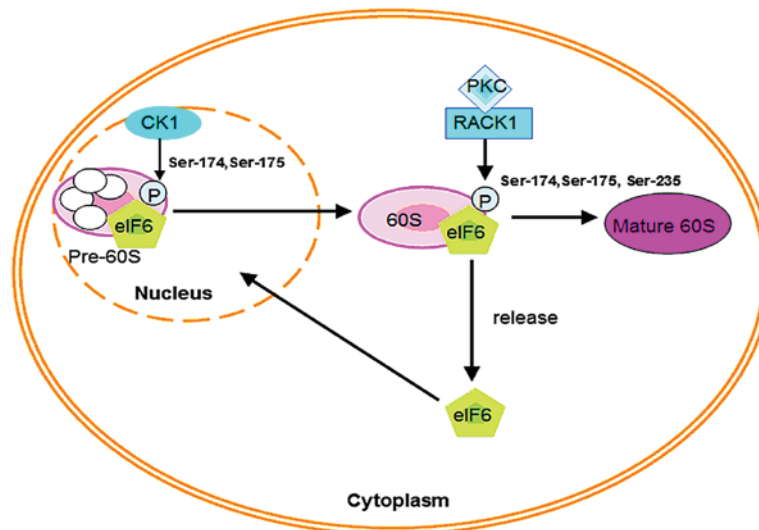
Figure 1. Nucleocytoplasmic shuttling of eIF6 and its release from the 60S ribosomal subunit in a normal cell. In the nucleus, CK1-catalzyed phosphorylation at Ser-174 and Ser-175 promotes eIF6 to associate with the immature large ribosomal subunits (pre-60S) to export to the cytoplasm. In the cytoplasm, the RACK1/PKC complex phosphorylates eIF6 at Ser-174, Ser-175 and Ser-235, leading to eIF6 release from 60S and mature 60S ribosome biogenesis. In the cytoplasm, the Ca^{2+}/calmodulin-dependent protein phosphatase calcineurin dephosphorylates eIF6 to enter the nucleus. eIF6, eukaryotic translation initiation factor 6; CK1, casein kinase 1; RACK1, receptors for activated C kinase 1; PKC, protein kinase C.

samples of colorectal carcinoma, parotid gland adenocarcinoma and leiomyosarcoma of the larynx (13). Consequently, this 52-kDa band is able to be utilized by head and neck surgeons and surgical pathologists during diagnosis.

Non-small cell lung cancer (NSCLC). Lung cancer is an extraordinarily malignant tumor with the highest morbidity and mortality, of which the most common variant is NSCLC (36,37). The primary features of this cancer are invasion and metastasis (36-39). eIF6 interacts with the cytoplasmic integrin β4 subunit, and in a previous study, positive eIF6 staining was observed in 82.5% (66/80) of NSCLC specimens (37). Therefore, eIF6 is likely to be present at a high concentration in NSCLC (6,13). Integrin β4 subunit, α6β4, the receptor for the basement membrane protein laminin-5, is an important cellular adhesion molecule, and is closely associated with tumor invasion and metastasis (6,40). α6β4 integrin is expressed in invasive breast carcinomas and is a potential indicator of poor prognosis (40). Taken together, a large increase in eIF6 is apparent in NSCLC, which may promote the migration of NSCLC cells; however, further study is required to confirm this.

Ovarian serous adenocarcinoma. Ovarian serous adenocarcinoma is the most prevalent form of epithelial ovarian cancer and a fatal type of gynecological malignancy (41,42). Human eIF6 is located on chromosome 20q12, which is an amplified chromosomal region (20q12-12) in ovarian cancer (43). This suggests that increased eIF6 may be a consequence of increased protein turnover in rapidly proliferating malignant cells based upon its role in ribosome assembly. Notably, eIF6, Dicer and RNaseIII endonuclease, which are essential components of miRNA machinery, are overexpressed in ovarian serous adenocarcinoma and associated with its clinicopathological features (15). miRNAs are a class of small, noncoding RNAs that affect the post-transcriptional control of mRNA and contribute to human tumorigenesis (44-46). Low eIF6

expression has increasingly been associated with reduced the probability of disease-free survival (15). Therefore, it is not inconceivable that downregulated expression of miRNAs and eIF6 could be useful biomarkers for the prediction of ovarian serous adenocarcinoma. Additionally, eIF6 and proteins of the miRNA machinery are closely related to future RNA interference-based therapy.

5. Upstream modulator of eIF6

As aforementioned, eIF6 is overexpressed in human colorectal cancer (6), head and neck cancer (13), lung cancer (14) and ovarian serous adenocarcinoma (15). Therefore, it is necessary to determine which oncogenes in the transcriptional network control eIF6 expression during tumorigenesis. Previous studies have established that the transcription factor complex GA-binding protein (GABP) regulates eIF6 expression, as the eIF6 promoter region contains GABP-binding sites (47). GABP is an E26 transformation-specific sequence (ETS) transcription factor, which contains an unrelated GABP protein, an ETS DNA-binding domain and a nuclear localization signal (48). The transcription of nuclear genes involved in mitochondrial respiration is controlled by the GABP complex (48). Moreover, certain ribosomal proteins are also GABP targets (49,50). For these reasons, GABP may be essential in regulating the transcription of ribosomal genes. The activity of the eIF6 promoter could be inhibited through blocking endogenous GABP activity. To date, a possible function for GABP in tumorigenesis remains to be described. Accounting for the fact that GABP could be vital in mediating the proliferative response, it may be useful to determine whether certain oncogenes directly affect GABP expression.

It is worth noting that the Notch-1 receptor has been demonstrated to directly regulate transcription of the eIF6 gene (12). The Notch-1 receptor belongs to the Notch

Table I. Overexpression of eIF6 in various cancer tissues and cell lines.

Type	Overexpression of eIF6
Cancer tissues	Colorectal cancer, head and neck carcinoma, NSCLC, ovarian serous adenocarcinoma
Cancer cell lines	A2780 ovarian cancer cells, WM793 primary melanoma cells, SW480 colorectal cancer cells

eIF6, eukaryotic translation initiation factor 6; NSCLC, non small cell lung cancer.
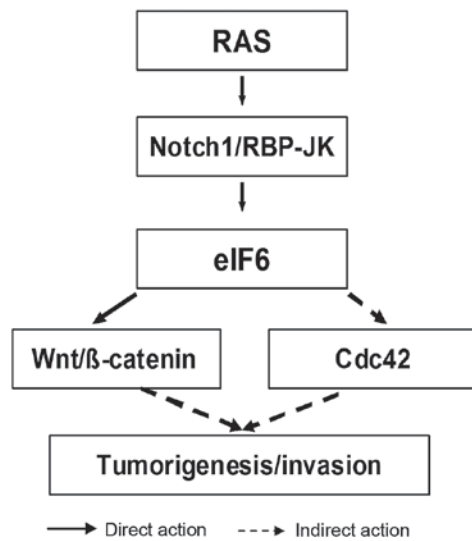

Figure 2. Schematic illustration depicting several cross-cellular pathways present in cancer cells overexpressing eIF6. eIF6, eukaryotic translation initiation factor 6; RBP-Jκ, recombining binding protein suppressor of Hairless; Cdc42, cell division control protein 42.

family of transmembrane proteins in mammals (51). The highly-conserved Notch signaling pathway is essential in the regulation of various physiological processes, including cell development, differentiation and proliferation (52-55). In particular, activation of the canonical Notch-1 pathway is of major significance in human tumorigenesis (12,51,56). A high level of Notch-1 expression has been observed in salivary adenoid cystic carcinoma (56) and breast cancer (57). Notably, it was reported that Notch-1 activation resulted in a 2 to 3-fold overexpression of eIF6, thus enhancing the invasiveness of A2780 cells (12,57). Therefore, it is conceivable that the Notch-1 signal is key to control the expression of eIF6. Notch-1 functions as an upstream regulator of eIF6, which directly regulates eIF6 expression via a recombinant binding protein suppressor of Hairless (RBP-Jκ)-dependent mechanism. In other words, the Notch-1/RBP-Jκ signaling pathway stimulates eIF6 promoter activity, resulting in abnormal expression of eIF6 (57,12). Overexpression of eIF6 enhances cell migration and invasiveness, but it is noteworthy that it does not affect proliferation (12).

6. Downstream regulation of eIF6

eIF6 and the canonical Wnt/β-catenin signaling pathway. In previous studies, eIF6 has primarily been used to control translation through regulation of ribosome biogenesis and assembly (3,4,9,18). Further research using yeast two-hybrid assays has demonstrated that eIF6 interacts with the C terminus of β-catenin, functioning as a transcriptional activation domain (7,55). In addition, the Wnt signal transduction cascade, with β-catenin as a major transducer, is a canonical cellular pathway in cell adhesion and proliferation during embryogenesis in animals (58-61). In general, the Wnt signal is absent in normal cells or tissues. However, the aberrant activation of Wnt/β-catenin signaling leads to the dysregulation of cellular growth and development, and contributes to human tumorigenesis (58,60). The targets of β-catenin transcription are also overexpressed in various types of carcinoma (59,63). Although the molecular mechanism remains to be clarified, previous research has demonstrated that dysregulation of Wnt/β-catenin signaling results in large accumulation of β-catenin in the nucleus (62,63). Subsequently, combined with T cell factor/lymphoid enhancing factor (TCF/LEF), transcription of target genes, including c-Myc (64) and cyclin D1 (65), may be activated resulting in carcinogenesis. Previous research has demonstrated that eIF6 serves as a factor participating in Wnt/β-catenin signaling and the distribution of eIF6 and β4 is altered in colonic adenoma and carcinoma (6). Furthermore, in SW480 cells transfected with full-length eIF6, the level of activated β-catenin was reduced compared with controls (66). The question may therefore be raised as to whether eIF6 has the same effect as Dickkopf antagonists on the Wnt/β-catenin signaling pathway. However, this is problematic to answer as eIF6 is overexpressed in colorectal carcinoma (6). Moreover, MG132, a proteasome-specific inhibitor, fails to inhibit the decrease in β-catenin that occurs upon overexpression of yellow fluorescent protein-eIF6 in SW480 cells (66). Consequently, despite the fact that β-catenin functions as a downstream effector of eIF6, eIF6 expression does not directly regulate the level of β-catenin, indicating that downregulation of β-catenin may only exist in certain vectors transfected with cell lines overexpressing eIF6.

Downstream effector of eIF6: Cell division control protein 42 (Cdc42). In a previous study, several membrane-associated proteins differed in abundance upon eIF6 overexpression in A2780 ovarian cancer cells (11). This effect is particularly notable in Cdc42 (11), a small GTPase belonging to the Ras homolog family (67-69). A number of studies have established that Cdc42 regulates cell differentiation, cell cycle progression, cell polarity, cell fate determination, and cell motility and adhesion (68,70). Aberrant expression of Cdc42 is pivotal in tumorigenesis, including that of breast carcinoma (71). Notably, it was reported that Cdc42 expression is disrupted at the post-transcriptional level by enhanced levels of eIF6 in A2780 ovarian cancer cells (11). In addition, it was observed that downstream of eIF6 activation, Cdc42 levels are increased by a post-transcriptional mechanism (11).

Although the underlying mechanisms of eIF6-mediated Cdc42 expression remain to be elucidated, a possible theory may be that enhanced levels of eIF6 indirectly control the

variation in the abundance of Cdc42. This is supported by the fact that Cdc42 mRNA expression levels exhibit little or no difference following eIF6 overexpression (11), which also demonstrates that eIF6 may target the translation of specific mRNAs. In A2780 cells overexpressing eIF6, ML-141, a selective and potent inhibitor of Cdc42 GTPase, has been demonstrated to significantly decrease migratory activity (11). The tumor-promoting ability of eIF6 is not restricted to the A2780 cell line; the primary melanoma cell line WM793 has also been reported to exhibit upregulated Cdc42 expression, in addition to increased motility and invasiveness (11). Therefore, eIF6 is crucial for Cdc42 upregulation. As eIF6 affects Cdc42 translation in ovarian cancer cells, this indicates that the increased expression of eIF6 is more likely to cause Cdc42 activation in ovarian cancer tissue, which in turn is accountable for increased migration and invasion. Nevertheless, further studies are required to elucidate the mechanisms behind these processes.

7. Conclusions and perspectives

The protein eIF6 possesses a high degree of evolutionary sequence conservation (1-8), and is located subcellularly in the nucleolus and cytoplasm. Phosphorylation of eIF6 regulates nucleocytoplasmic shuttling in mammalian cells and involves the release of eIF6 from the 60S ribosome subunit (3,16,17). In cancer cells, eIF6 is phosphorylated by the RACK1-PKCβII complex, and thus by the Ras cascade (16,72). eIF6 functions as an important component in gene regulatory networks and exerts crucial roles in neoplastic progression (10-15). Nevertheless, the specific molecular mechanisms underlying the role of eIF6 in these processes remain unclear. Oncogenesis typically involves several different signaling pathways. For example, the Ras-extracellular related kinase mitogen-activated protein kinase pathway and phosphoinositide 3-kinase/AKT/mammalian target of rapamycin pathway each take part in the phosphorylation of eIF4E, which is involved in cancer development (73-75). Consequently, it is possible that these signals are involved in the mechanisms of eIF6 overexpression in cancer, since eIF4E is a eukaryotic initiation factor in addition to eIF6.

In conclusion, the following hypothesis is proposed (Fig. 2). Firstly, Notch-1 activated by oncogenic Ras promotes transcription of the eIF6 gene through an RBP-Jκ-dependent mechanism. Ras signaling has a key role in increasing Notch-1 expression in breast carcinoma (75). Secondly, overexpression of eIF6 leads to aberrant activation of the Wnt/β-catenin signaling pathway. Similarly, overexpressed eIF6 controls its downstream effector Cdc42, which in turn affects tumorigenesis. As a consequence, understanding the signaling network in which eIF6 lies may contribute novel insights into the pathogenesis of cancer, and offer a promising target for the development of novel antineoplastic agents.

Acknowledgements

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