



Ubiquitin-Conjugating Enzyme E2S (UBE2S), A Potential Tumor Therapeutic Target

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Abstract

Objective: The ubiquitin-proteasome system plays important roles in the control of numerous processes including cell-cycle, signal transduction, transcriptional regulation, receptor down regulation, and endocytosis. Ubiquitination is a reversible biochemical process mediated or participated by ubiquitin activating enzyme (E1), ubiquitin-conjugating enzyme (E2), ubiquitin ligase (E3), and 26s proteasome. Dysfunction in several ubiquitin-mediated processes has been shown to cause pathological conditions including malignant transformation. This paper reviews Ubiquitin-conjugating enzyme E2S (UBE2S) as a potential tumor therapeutic target.

Methods: In this study, we review the expression of UBE2S in some malignant tumors, its influence on the malignant biological behaviors of cancer and the possible molecular mechanisms in cancer development, according to the related literatures and our recent studies.

Results: A growing body of evidence suggests ubiquitination is associated with the tumorigenesis and development of cancer. Recent studies have shown that UBE2S is overexpressed in some malignant tumors such as breast cancer, esophageal cancer, kidney cancer, cervical cancer and gallbladder cancer; and was found associated with the malignant biological behaviors including proliferation, invasion and metastasis of cancer. The related signaling pathways and the possible molecular mechanisms in cancer development were also shown.

Conclusions: UBE2S is not only overexpressed in some malignant tumors, but also play a crucial role in tumor growth, invasion and metastasis, thus may be a potential tumor therapeutic target.

Keywords: Ubiquitination; Ubiquitin-conjugating enzyme; Ubiquitin-conjugating enzyme E2S; Cancer

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Introduction

A growing body of evidence suggests ubiquitination is associated with the tumorigenesis and development of cancer. Recent studies have shown that ubiquitin-conjugating enzyme E2S (UBE2S) is overexpressed in some malignant tumors such as breast cancer, esophageal cancer, kidney cancer and cervical cancer; and was found associated with the malignant biological behaviors including proliferation, invasion and metastasis of cancer. In this paper, we review the expression of UBE2S in some malignant tumors, its influence on the development of cancer and the possible molecular mechanisms.

Ubiquitin-proteasome system and UBE2S

Ubiquitin is a thermal stability protein composed of 76 amino acid residues, with a kind of molecular weight of 8.5 kD, and the founding member of the ubiquitin protein family of structurally conserved protein that regulate a host of processes in eukaryotic cells, mediates selective proteolysis through its enzymatic conjugation to proteins that contain primary degradation signals, thereby marking such proteins for degradation by the 26S proteasome, an ATP-dependant multi-subunit protease [1,2]. Many studies have showed that the Ubiquitin Proteasome System (UPS) plays important roles in the control of numerous processes, including cell-cycle progression, signal transduction, transcriptional regulation, receptor downregulation, and endocytosis. Ubiquitination is a reversible biochemical process mediated or participated by ubiquitin activating enzyme (E1), ubiquitin-conjugating enzyme (E2), ubiquitin ligase (E3), and 26s or 30s proteasome that attaches ubiquitin to substrate proteins in order to regulate above multiple cellular functions in UPS [3]. The process of ubiquitination usually involves three steps (Figure 1): firstly, the C-terminal Gly residue of ubiquitin is activated in an ATP-requiring step by E1 (Step 1); secondly, activated ubiquitin is next transferred to an active site Cys residue of a ubiquitin-carrier protein, E2 (Step 2); lastly, catalyzed

by a ubiquitin protein ligase, E3, ubiquitin is linked by its C-terminus to the substrate protein's Lys residues (Step 3). Multiple rounds of ubiquitination result in substrate polyubiquitination that can target proteins for proteasome-dependent destruction [3,4]. Dysfunction in several ubiquitin-mediated processes has been shown to cause pathological conditions including malignant transformation [3].

A growing body of evidence has demonstrated that ubiquitination is associated with the progression of cancer [5]. There are more than 600 E3 ligases in humans, some of which regulate the expression of tumor-suppressor or tumor-promoting proteins. For example, p53 is one of the most frequently mutated genes in cancer, and reduced levels of p53 protein can promote cancer initiation. Mdm2 is an E3 ligase for p53 and is one of the major regulators of p53 expression [6,7]. Overexpression of Mdm2 is observed in a variety of cancers, such as breast cancer, oral squamous cell cancer, glioma, lymphoma, and leukemia [8]. COP1 and Pirh1 are also E3 ligases for p53, and are overexpressed in several cancers [9-13]. In addition to E3 ligases, E2-conjugating enzymes are associated with cancer progression [14]. One of the most studied E2s is UBE2C because of its association with cancer. UBE2C is required for the degradation of mitotic regulators in cooperation with anaphase-promoting complex/cyclosome (APC/C) [15,16]. High expression of UBE2C is found in many human cancers of the brain, lung, cervix, colon, liver, thyroid, breast, and nasopharynx [17-20]. Depletion of UBE2C from cancer cells significantly reduced the proliferation of cancer cells and induced cell apoptosis. UBE2C-overexpressed transgenic mice were prone to develop into the carcinogen-induced lung cancers and a variety of spontaneous tumor [21]. These results indicate that UBE2C is involved in cancer development and progression [22].

Ubiquitin-conjugating enzyme E2S (UBE2S) is a 24-kDa protein that is a member of the E2 family of ubiquitin-conjugating enzymes, also known as E2-EPF or E2-EPF Ubiquitin Carrier Protein (UCP), is essential for the elongation of ubiquitin chains to target substrate proteins to the 26S proteasome [22,23]. Once UBE2C attaches ubiquitin onto the target proteins, UBE2S promotes the elongation of ubiquitin chains for the degradation. Recent studies have shown that UBE2S is also overexpressed in cancers.

UBE2S' roles in malignant tumors

UBE2S is highly expressed in a variety of malignant tumors: High expression of UBE2S was observed in cervical, breast, esophagus and kidney cancers in the past decade [22-26]. In 2007, Tedesco et al. identified that E2-EPF mRNA and protein are overexpressed in breast cancers and is regulated by cell cycle in cervical cancer HeLa cells, achieving the highest levels during mitosis; and that ER⁻ breast tumors are more likely to express E2-EPF protein and have detectable E2-EPF expression in a greater percentage of cells than ER⁺ tumors, which is consistent with the presence of E2-EPF in the meta-signature for undifferentiated cancer because ER⁻ tumors are typically poorly differentiated and are of higher mitotic grade than ER⁺ tumors [23]. Ayesha et al. [22] studies also showed that UBE2S is highly expressed in breast cancer, and is localized to both the nucleus and cytoplasm in immunostaining and cell fractionation analysis. Thereafter, Liang et al reported that E2-EPF mRNA was highly expressed in cervical squamous cancer, and its positive expression was correlated with clinical tumor stage, i.e., the relative expression level of E2-EPF in tumors at stage I was significantly lower than the tumors at stage II-IV [26]. Similarly, Chen et al found that UCP mRNA and protein were also more highly expressed in human esophagus cancer tissues

than in nonmalignant esophageal specimens, and the increased UCP was associated with higher expression of HIF-1 α , VEGF, and decreased expression of VHL while the stability of VHL and HIF-1 α are dependent on E2-EPF level [24]. And, Roos et al. [25] reported that E2-EPF mRNA was significantly elevated in Papillary Renal Cell Carcinoma (PRCC), in particular type 2 which patients have poorer prognosis than patients with type 1 PRCC). Recently, our study by immunohistochemical staining for paraffin specimens of gallbladder tissues has showed that UBE2S was highly expressed in gallbladder cancers (n=85) when compared with precancerous lesions (adenoma and severe dysplasia, n=28; 4.08 ± 3.41 vs. 2.68 ± 1.98 score, $P=0.0070$), benign lesions (inflammation and polyps, n=15; 4.08 ± 3.41 vs. 2.07 ± 1.33 score, $P=0.0002$) and normal tissues adjacent to cancer (n=43; 4.08 ± 3.41 vs. 2.42 ± 1.82 score, $P=0.0005$) of the gallbladder; furthermore, western blotting for fresh specimens of gallbladder cancer (n=10) and normal tissues adjacent to cancer (n=10) has showed that expression of UBE2S proteins in gallbladder cancers was higher than that of normal tissues adjacent to cancer (0.7340 ± 0.1806 vs. 0.1972 ± 0.091 , relative grey value; $P=0.0020$). And, the gallbladder cancer patients with high expressed UBE2S had the shortest survival time.

UBE2S associates with proliferation, invasion and metastasis of malignant tumors: As reported in 2006 by Jung et al. [27], forced expression of UCP boosts the proliferation, invasion and metastasis of tumor cells *in vitro* and *in vivo*. But Tedesco et al. [23] reported that reduction of E2-EPF protein levels by >80% using RNAi had no significant effects on the proliferation of cervical cancer HeLa cells or ER⁻ MDA-MB-231 or MDA-MB-453 breast cancer cells; and thought that the possible reason was that the 80% to 90% reduction in E2-EPF levels was not of sufficient magnitude or duration to impact the proliferation of the cancer cells analyzed or that only certain cancers are dependent on E2-EPF for their growth. Subsequently, Liang et al. [26] studies showed that reduction of E2-EPF protein levels by >80% using shRNA decreased the proliferation, invasion and tumorigenicity of another cervical cancer cell line SiHa, so supposed that the role of E2-EPF in cell growth was different depending on the cell type. And, Ayesha et al. [22] found that UBE2S was associated with malignant characteristics of breast cancer cells, UBE2S knockdown suppressed cell spreading, migration, and invasion in three luminal cell lines (MCF7, T47D, and MDA-MB-453) and three basal cell lines (BT20, MDA-MB-231, and Hs578t) of breast cancer. Other studies have also identified that inhibited UCP significantly decrease tumor growth and the capacity for invasion and metastasis in human esophagus and renal cancers [24,25]. Recently, our studies for invasive comparison of high and low aggressive gallbladder cancer cell lines NOZ and SGC996 and microarray analysis for highly aggressive gallbladder cancer NOZ cells by Affymetrix chip gene expression profile scan have found that UBE2S is a target gene related to invasion of gallbladder cancers; and UBE2S knockdown suppressed migration, metastasis and adhesion capabilities of NOZ cells and EMT process. It is thus concluded that UBE2S play a crucial role in tumor growth, invasion and metastasis.

UBE2S affects radiotherapy and chemotherapy

Tedesco et al. [23] reported that E2-EPF knockdown sensitized cervical cancer HeLa cells to the topoisomerase (topo) II inhibitors etoposide and doxorubicin and also increased topo II α protein levels, but had no effect on the sensitivity of HeLa cells to chemotherapeutic agents such as the microtubule stabilizer Taxol or the topoisomerase I inhibitor camptothecin. But Jing et al. [26] studies found that E2-EPF knockdown increases the chemosensitivity to topo I inhibitor

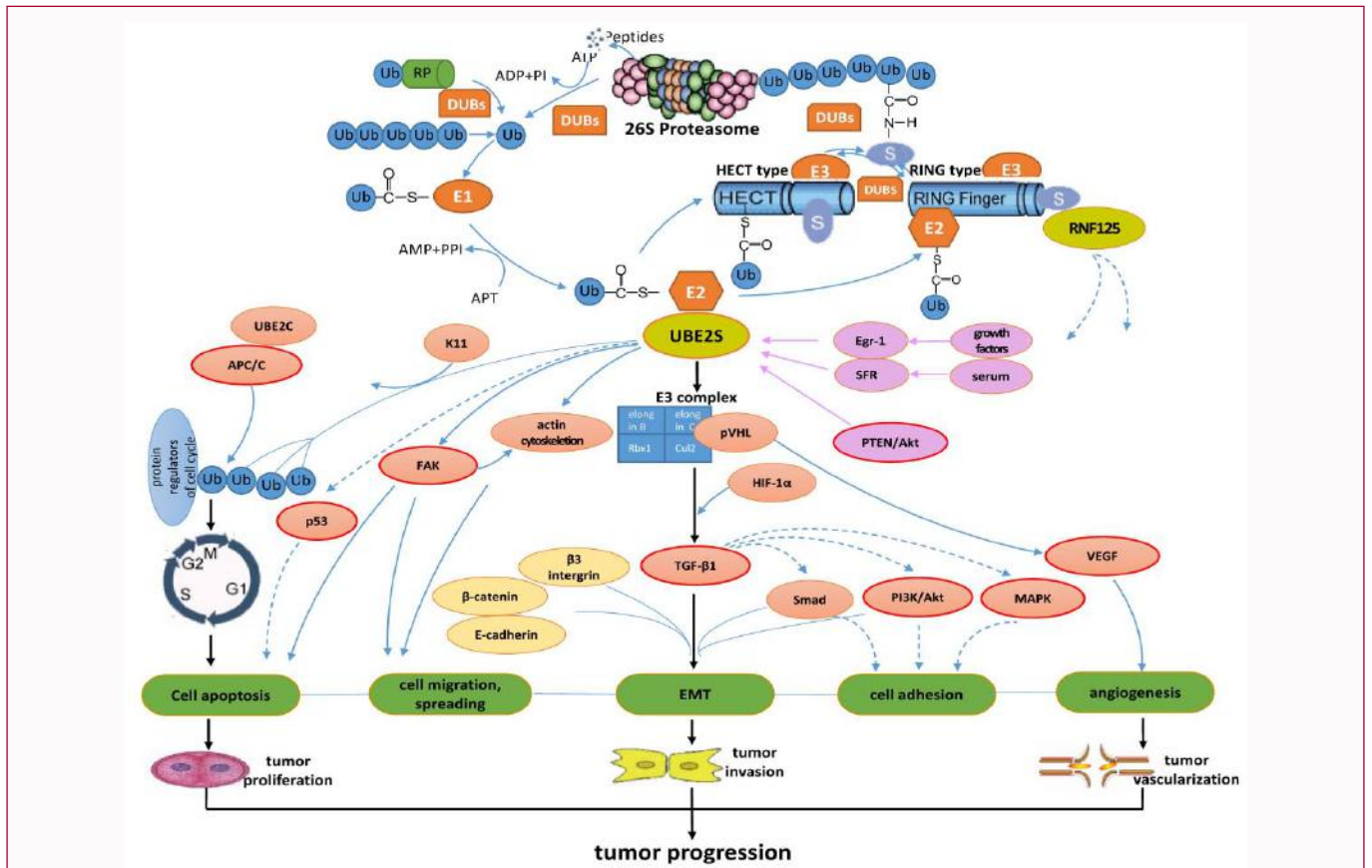


Figure 1: The possible molecular mechanisms and the related signaling pathways of UBE2S in cancer development. Ub: Ubiquitin; E1: Ubiquitin-Activating Enzyme; E2: Ubiquitin-Conjugating Enzyme; E3: Ubiquitin Ligase; DUB: Ubiquitin Decomposition Enzyme; UCP: E2-EPF Ubiquitin Carrier Protein; VHL: von Hippel–Lindau gene; HIF-1α: Hypoxia-Inducible Factor-1α; VEGF: Vascular Endothelial Growth Factor; TGF-β1: Transforming Growth Factor- β 1; APC/C: Anaphase-Promoting Complex/Cyclosome; FAK: Focal Adhesion Kinase; MAPK: Mitogen-Activated Protein Kinase; PI3K: Phosphatidylinositol 3-Kinase; EMT: Epithelial- Mesenchymal Transition.

topotecan though the mechanism is still unknown, these data suggest that combined administration of topo I or topo II drugs and E2-EPF inhibitors may enhance their clinical effectiveness of chemotherapy.

Studies of Liang et al. [26] have demonstrated that overexpression of UCP was significantly associated with poor response to neoadjuvant Computerized Controlled Radiation Therapy (CCRT); and that treatment with UCP silencing vector significantly increase the sensitivity of esophageal cancer to radiation, rather than cisplatin. Recently, Hu et al. [28] reported that UBE2S associated with DNA repair and glioblastoma multiforme resistance to chemotherapy, and that knockdown of UBE2S expression rendered glioblastoma cells more sensitive to chemotherapy. Birle et al. [29] reported that proteasome inhibitor bortezomib decreased tumor Carbonic Anhydrase IX (CAIX) expression in colon cancer patients; and identified that the Hypoxia Inducible Factor-1 (HIF-1) response in cervical cancer xenografts was suppressed by proteasome inhibitor bortezomib. Together with, UBE2S affects radiotherapy and chemotherapy for patients with malignant tumor.

UBE2S associates with the prognosis of patients with malignant tumors: It was showed that negative staining for E2-EPF, response to neo-adjuvant therapy and tumor stage are significant prognostic indicators for overall survival in esophageal cancer by multivariate analysis. And the expression of UCP is associated with clinical outcome in esophageal cancer, i.e., higher UCP was linked to worse Overall Survival (OS) and worse Disease Free Survival (DFS)

[25]. In addition, Roos et al observed that PRCC patients with high E2-EPF expression showed a trend toward increased cancer-related mortality and disease progression *via* immunohistochemistry on tissue microarray and/or Western blot analysis, although a statistical significance could not be reached mainly due to the limited sample size [26]. So, it is suggested that E2-EPF might be a molecular predictor for the prognosis of cancer patients. But, more cases and more clinical specimens need to be collected and analyzed.

Possible molecular mechanisms of UBE2S in cancer development: As shown in Figure 1, Ubiquitin Proteasome System (UPS) and its process of ubiquitination of E1, E2 and E3, and some related signaling pathways of E2 ubiquitin-conjugating enzyme, in particular UBE2S, to cause tumor progression, i.e. possible molecular mechanisms of UBE2S in cancer development are illustrated.

UCP-VHL/HIF-1α-TGF-β1-EMT pathway

Maxwell et al. [30] found in 1999 that the von Hippel-Lindau tumor suppressor (pVHL) targets Hypoxia Inducible Factors (HIFs) for oxygen-dependent proteolysis. pVHL functions as the substrate-recognition module of the E3 ubiquitin ligase complex composed of elongin B, elongin C, Rbx1 and Cul2 which ubiquitinates HIF-1α during normoxia [31-36]; and pVHL is expressed in most tissues and cell types [37], suggesting that loss of pVHL might be generally involved in tumor progression and angiogenesis. The heterodimeric transcription factors HIF-1 and HIF-2, composed of HIF-1α and HIF-2α and HIF-1β, increase the expression of a number of hypoxia-

inducible genes including the gene encoding Vascular Endothelial Growth Factor (VEGF), which promotes tumor growth and vascularization [38,39]. Jung et al. [27] study showed that UCP was correlated inversely with pVHL in most tumor cell lines, and UCP was detected coincidentally with HIF-1 α in human primary liver, colon and breast cancers, and metastatic cholangiocarcinoma and colon cancer cells, and also identified for the first time in 2006 that forced expression of UCP boosts tumor cell proliferation, invasion and metastasis through effects on the pVHL-HIF pathway. However, they cannot exclude the possibility that UCP and its cognate E3 may target other substrates important for cell growth (Figure 1). Thereafter, Chen et al. [24] proposed that the Epithelial Mesenchymal Transition (EMT) induced by the VHL/HIF-1 α TGF- β 1 pathway might be responsible for the effects regulated by UCP. The reasons are as below: firstly, TGF- β 1 is activated by HIF-1 α and plays an important role in the induction of EMT [40-44], TGF- β 1 induces EMT in various epithelial cell types *via* PI3K/Akt and SMADs signaling pathway; secondly, β 3 integrin was reported to facilitate TGF- β -mediated induction of EMT in epithelial cells of breast and colorectal cancers [1,2]; thirdly, downregulation of UCP was associated with TGF- β 1 and β 3 integrin in addition to the decreased HIF-1 α and VEGF in Cuci cells [24]; finally, Rees et al. Reported that TGF- β 1 mediates the EMT associated with loss of β -catenin and E-cadherin, hall marks of EMT, EMT is a key event in the tumor invasion process [45]. Consistent with this, Roos et al. [25] reported for the first time that an inordinately elevated expression of E2-EPF was associated with decreased pVHL and increased HIF-1 α levels in PRCC; and identified that multiple Hypoxia Responsive Elements (HREs) within E2-EPF promoter were capable of recruiting HIF1 and HIF2, E2-EPF transcription is dependent on HIF1.

UBE2S is associated with regulating the actin cytoskeleton and focal adhesions: Ayesha et al. [22] Reported that UBE2S is associated with regulating the actin cytoskeleton and focal adhesions. The depletion of UBE2S induced changes in cellular morphology and significantly disrupted the formation of actin stress fibers and focal adhesions while the actin cytoskeleton plays a critical role in numerous cellular functions such as cell migration and spreading. It was showed that both migration and spreading were delayed in the absence of UBE2S. Focal Adhesion Kinase (FAK), except that it is known to activate survival signals to prevent anoikis, is one of the most studied protein associates with the regulation of actin cytoskeletal organization and cell migration, and its inhibitors are being investigated in clinical trials for cancer treatment [46]. It was found that the phosphorylation of FAK at Tyr397 was reduced in the absence of UBE2S. Although the exact molecular mechanisms of Tyr397 phosphorylation are still not clear, it is believed that integrin binding to extracellular matrix (ECM) promotes dimerization of FAK for the phosphorylation of Tyr397 [47,48]. Src family kinases, which are non-receptor tyrosine kinases, are recruited to the phosphorylated Tyr397, where they phosphorylate other tyrosine residues of FAK for the activation of downstream signals [49,50]. Therefore, a decrease in Tyr397 phosphorylation *via* UBE2S knockdown may suppress numerous signals for cell spreading, migration, and invasion. Further analysis is required to determine whether the functions of FAK or other signal proteins are affected by UBE2S knockdown to suppress cell migration and invasion.

Egr-1/SRF-UCP-VHL pathway

E2-EPF Ubiquitin Carrier Protein (UCP) has been shown to

be highly expressed in common human cancers and target von Hippel-Lindau (VHL) for proteosomal degradation in cells, thereby stabilizing hypoxia-inducible factor HIF-1 α . Lim et al. [51] further investigated cellular factors that regulate the expression of UCP gene, and suggesting that growth factors and serum induced Egr-1 and Serum Response Factor (SRF) can bind the promoter region of E2-EPF, therefore increase the HIF-1 α protein level under non-hypoxic conditions through the Egr-1/SRF-UCP-VHL pathway.

UBE2S involves in the degradation of protein regulators of the cell cycle: Ubiquitination by the Anaphase-Promoting Complex (APC/C) is essential for proliferation in all eukaryotes. Timely degradation of protein regulators of the cell cycle is essential for the completion of cell division. UBE2S is involved in the degradation of proteins by APC/C during mitosis. Once APC/C in combination with UBE2C primes the lysine residues of substrates with ubiquitin, UBE2S promotes elongation of ubiquitin chains via K11-mediated attachment. UBE2S and UBE2C are tightly co-regulated in the cell cycle by APC/C-dependent degradation; they constitute a physiological E2-module for APC/C, the activity of which is required for spindle assembly and cell division. Williamson et al. [52] identified that the conserved Ube2S as a K11-specific chain elongating E2 for human and Drosophila APC/C. While depletion of UBE2S already inhibits APC/C in cells, the loss of the complete UBCH10/UBE2S-module leads to dramatic stabilization of APC/C substrates, severe spindle defects and a strong mitotic delay. Garnett et al. [53] reported that the E2 enzyme UBE2S as an APC/C auxiliary factor that promotes mitotic exit. Thereafter, Wu et al. [54] found that UBE2S drives elongation of K11-linked ubiquitin chains by APC, so they propose that UBE2S is a critical, unique component of the APC ubiquitination pathway. Recently, studies of Ben-Eliezer et al. [55] identified that appropriate expression of UBE2C and UBE2S controls the progression of the first meiotic division. Their depletion reduces the levels of the first meiotic cytokines is by 50%, and their overexpression doubles and accelerates its completion (50% as compared with 4% at 11h). It was also demonstrated that these E2 enzymes take part in ensuring appropriate spindle formation and also control the extrusion of the first polar body.

In studies of Whitfield et al. [56], the genome-wide program of gene expression during the cell division cycle in a human cancer cell line HeLa was characterized using cDNA microarrays. Transcripts of >850 genes showed periodic variation during the cell cycle; E2-EPF was classified as a gene whose expression peaked during the M/G1 phase of the cell cycle, yet its mRNA expression profile was most similar to that of the G2/M phase expressed BUB1, BIRC5, CCNB2, CDC20, PLK1, and other genes involved in mitotic surveillance. Tedesco et al. [23] also noted that E2-EPF was one of 874 cell cycle-regulated gene identified in gene expression analyses of HeLa cells; however, E2-EPF protein was significantly elevated in S phase as well as in G2/M phase compared to serum-deprived cell performed similar cell cycle analyses in HeLa cells. Furthermore, no alteration in cell cycle distribution was observed in HeLa cells with reduced E2-EPF expression. Differentiated with this result, recently studies [26] revealed that E2-EPF knockdown increased the number of cells in the G0/G1 phase and the percentage of cells in G2/M, especially S phase are significantly lower in E2-EPF low expression cells than the normal and control cells.

Other associated pathways

It is newly reported that the stability of UBE2S is regulated by the

PTEN/Akt pathway and that its degradation depends on the ubiquitin-proteasome system. Mechanistically, Akt1 physically interacted with and phosphorylated UBE2S at Thr 152, enhancing its stability by inhibiting proteasomal degradation [28]. UBE2S was recently found to be associated with the components of the Non-Homologous End-Joining (NHEJ) complex and participated in the NHEJ-mediated DNA repair process. Knockdown of UBE2S expression inhibited NHEJ-mediated double-stranded break repair [28].

Conclusions

In summary from literature review, we found that UBE2S is highly expressed in a variety of malignant tumors, and associates with tumor stage, neoadjuvant therapy, and prognosis of malignant tumors. In most cases, downregulation or depletion of UBE2S suppressed malignant characteristics such as proliferation, migration, invasion of cancer cells. In addition, these crucial roles of UBE2S were showed to associate to some related signaling pathways such as VHL/HIF-1 α TGF- β 1 EMT pathway, etc. UBE2S may be a potential tumor therapeutic target. Targeting these signaling pathways may have potential clinical applications for a variety of malignant tumors.

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