Eukaryotic initiation factor 5A2 and human digestive system neoplasms

Qing-Bin Meng, Jing-Jing Peng, Zi-Wei Qu, Xiao-Min Zhu, Zhang Wen, Wei-Ming Kang

Abstract

Eukaryotic initiation factor 5A2 (eIF5A2), as one of the two isoforms in the family, is reported to be a novel oncogenic protein that is involved in multiple aspects of many types of human cancer. Overexpression or gene amplification of EIF5A2 has been demonstrated in many cancers. Accumulated evidence shows that eIF5A2 initiates tumor formation, enhances cancer cell growth, increases cancer cell metastasis, and promotes treatment resistance through multiple means, including inducing epithelial–mesenchymal transition, cytoskeletal rearrangement, angiogenesis, and metabolic reprogramming. Expression of eIF5A2 in cancer correlates with poor survival, advanced disease stage, as well as metastasis, suggesting that eIF5A2 function is crucial for tumor development and maintenance but not for normal tissue homeostasis. All these studies suggest that eIF5A2 is a useful biomarker in the prediction of cancer prognosis and serves as an anticancer molecular target. This review focuses on the expression, subcellular localization, post-translational modifications, and regulatory networks of eIF5A2, as well as its biochemical functions and evolving clinical applications in cancer, especially in human digestive system neoplasms.

Key words: Eukaryotic translation initiation factor 5A2; Hypusine modification;
Acetylation modification; Drug resistance; Cancer therapeutics

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Eukaryotic initiation factor 5A2 (eIF5A2) is one of only two cellular proteins that contain the unusual amino acid hypusine. eIF5A2 initiates tumor formation, enhances cancer cell growth, increases metastasis, and promotes treatment resistance through inducing epithelial–mesenchymal transition, cytoskeletal rearrangement, angiogenesis, and metabolic reprogramming. Isoform eIF5A2 represents a promising target for treatment of human digestive system cancer. Our objective was to consolidate the current literature to better understand the expression, subcellular localization, post-translational modifications, and regulatory networks of eIF5A2, as well as its biochemical functions and evolving clinical applications in human digestive system cancer.

Introduction

In 2000, EIF5A2 was first sequenced and isolated as a novel candidate oncogene from human chromosome 3q26.2[1,2]. Eukaryotic initiation factor 5A2 (eIF5A2) is one of only two eIF5A family members that undergo an unusual post-translational hypusine modification[3]. Unlike isoform eIF5A1, which is ubiquitously expressed, eIF5A2 protein is normally not detected and its mRNA is expressed in a tissue-dependent manner in human tissues[1]. eIF5A2 protein has been shown to be overexpressed in many cancers, including cervical cancer[4-5], ovarian cancer[6-8], colorectal cancer[9,10], gastric cancer[11-12], liver cancer[13-14], lung cancer[15], nasopharyngeal carcinoma[16], bladder cancer[17] and esophageal squamous cell carcinoma (ESCC)[18]. Accumulated evidence shows that eIF5A2 plays important roles as a regulatory molecule in many biological processes, including tumor formation, cancer cell growth, metastasis, maintenance of cancer stem cells (CSCs) and treatment resistance through multiple means including epithelial–mesenchymal transition (EMT), cytoskeletal rearrangement, angiogenesis, and metabolic reprogramming.

In this article, we review eIF5A2-related studies, particularly those about the discovery, subcellular location, functions, upstream and downstream regulation, and modification of eIF5A2, as well as its role as a biomarker and its therapeutic potential for human digestive system cancer.

Literature Search

A literature search was conducted using PubMed Library for “eIF5A2”, “eIF-5A2”, “eIF-5A-2”, “eIF5A-2”, “EIF5A2”, “eukaryotic translation initiation factor 5A2”, “eukaryotic initiation 5A2” or “human eukaryotic initiation factor 5A2”.

Properties and Expression

Human eIF5A2 is a small (approximately 17 kDa) universally conserved acidic protein that contains 153 amino acids and is encoded by EIF5A2 gene, which is located on chromosome 3q26.2; a chromosomal region that is frequently amplified in several human cancers[22]. Multiple forms of EIF5A2 mRNA (5.6, 3.8, 1.6 and 0.7 kb, with one at 3.8 kb being the major form) are the products of one gene with various lengths of 3'-untranslated region (UTR), resulting from the use of different polyadenylation (AAUAAA) signals in various human cancer cell lines[23]. In short, for the structure of eIF5A2, the C-terminal domain consists of a three-turn α-helix α2 and five strands of β7-β11 and the N-terminal domain is dominated by β-strands[23].
Unlike EIF5A1, which is ubiquitously expressed, EIF5A2 is normally not detected and its mRNA is expressed in a tissue-dependent and cell-type-specific manner, and is mainly found in testes, parts of adult brain, human cancer tissues (such as primary ovarian cancers) and some cancer cell lines (such as SW480 and UACC-1598)\(^\text{[1-2]}\). Clement et al\(^\text{[3]}\) described the identification of eIF5A2 protein in human colorectal (SW-480) and ovarian (UACC-1598) cancer cell lines, and were first to report that eIF5A2 has an important role in eukaryotic cell survival similar to that of the ubiquitous eIF5A1. Overexpression of EIF5A2 and/or eIF5A2 protein is observed in several human cancer tissues and/or cell lines such as cervical cancer\(^\text{[3-4]}\), ovarian cancer\(^\text{[5-6]}\), colorectal cancer\(^\text{[7-9]}\), gastric cancer\(^\text{[1]}\), liver cancer\(^\text{[11,12,13]}\), nasopharynx cancer\(^\text{[14]}\), oral squamous cell carcinoma\(^\text{[15,16]}\), pancreatic cancer\(^\text{[17,18]}\), non-small cell lung cancer\(^\text{[19-21]}\), melanoma\(^\text{[22]}\), bladder cancer\(^\text{[23,24]}\), and breast cancer\(^\text{[25-27]}\). In contrast, eIF5A2 is not generally overexpressed in glioblastoma\(^\text{[28]}\) and chronic myeloid leukemia\(^\text{[29]}\). These observations suggest that eIF5A2 overexpression is not an invariable hallmark of cancer. Pällmann et al\(^\text{[30]}\) reported high levels of EIF5A2 mRNA in brain, epididymis, lung, prostate and testis tissues of wild-type mice, as assessed by quantitative real-time polymerase chain reaction.

### POST-TRANSLATIONAL MODIFICATIONS

#### Hypusine modification and activation of eIF5A2

In humans, isoforms eIF5A1 and eIF5A2 are the only two cellular proteins that experience a post-translational hypusination by two essential enzymatic steps involving deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH), which selectively catalyze the polyamine spermidine- to finish eIF5A hypusination\(^\text{[31-33]}\). eIF5A exists mainly as the fully hypusinatin form in mammalian tissues and cells\(^\text{[34]}\). First, the 4- aminobutyl moiety of spermidine are transferred to the amino group of Lys50 to form a deoxyhypusine-containing intermediate by DHS\(^\text{[32,33]}\). Second, DOHH catalyzes the hydroxylation of the deoxyhypusine residue to generate hypusine-containing eIF5A and activates it\(^\text{[22,35]}\). It has been reported that the endogenous activity of DHS and/or DOHH appears to be insufficient for modification of the excess precursors of mature eIF5A2 and eIF5A1\(^\text{[23]}\), and exogenously expressed eIF5A2 and eIF5A1 is largely unhyprusinated, and can be hypusinated only when DHS and DOHH are coexpressed\(^\text{[25,36]}\). Therefore, transfection studies with eIF5A2 expression vectors, such as our previous study\(^\text{[1]}\) and others\(^\text{[11,12,29,30]}\), should be re-assessed by evaluating the real changes in the concentrations of the hypusinated eIF5A2 or its precursor to determine the true cause of the biological effects. Hypusine modification not only activates eIF5A2, but also regulates its subcellular localization. However, in contrast to DHS- and DOHH-mediated hypusination of eIF5A1, which is crucial for embryonic development as well as for viability in adult mice, the cancer-associated isoform eIF5A2 is dispensable for embryonic development and viability in adult organisms\(^\text{[37,38]}\). Future work will be needed to determine the contribution of hypusine biosynthetic enzymes of eIF5A2 in tumorigenesis and metastasis.

#### Acetylation modification

In addition to unique hypusination, eIF5A2 also undergoes reversible acetylation modification at Lys-47, like eIF5A1 does\(^\text{[39,40]}\). Histone deacetylase 6 and sirtuin 2 have been identified as the major deacetylases of eIF5A2\(^\text{[41]}\). However, it has been reported that the carboxyl terminus of Hsc70-interacting protein (CHIP) is not an invariable hallmark of cancer. Pällmann et al\(^\text{[30]}\) reported high levels of EIF5A2 mRNA in brain, epididymis, lung, prostate and testis tissues of wild-type mice, as assessed by quantitative real-time polymerase chain reaction.
oncogenic properties should be elucidated in the future.

**SUBCELLULAR LOCALIZATION OF eIF5A2**

The nuclear membranes force nucleocytoplasmic exchange to proceed through nuclear pore complexes (NPCs). The NPC permeability barrier allows free passage to small molecules, while limiting larger molecules that approach or exceed a limit of > 30kDa in mass or > 5nm in diameter. Most evidence demonstrates that eIF5A2, as a shuttling protein, is responsible for regulating protein translation in the cytoplasm, and only a few studies have shown that it is located and works in the nucleus. More studies are necessary to address its role in the nucleus. eIF5A2 has an invariably small molecular mass of only 17 kDa and can thus cross the NPC permeability barrier rapidly, even without the help of an importin. The nuclear export of eIF5A2 may be mediated by the nuclear exporter exportin (XPO4), which belongs to the importin-β family of nuclear transporters, in a hypusine-dependent manner. In addition, the N-terminal 19 amino acids of eIF5A2 serve as a signal for nuclear localization of eIF5A2. Knockdown of XPO4 in murine hepatoma cells leads to nuclear accumulation of eIF5A2 as well as eIF5A1.

Post-translational modifications including acetylation at Lys-47 and hypusination at Lys-50 of eIF5A2 direct its subcellular localization. Acetylation acts as a molecular switch for eIF5A2, allowing it to exert distinct functions in the cytoplasm and nucleus. The acetylated form of eIF5A2 is primarily enriched in the nucleus, suggesting that acetylation at Lys-47 induces nuclear accumulation. In addition, the study also showed that unhypusinated eIF5A2 is highly acetylated but is significantly deacetylated upon hypusination, implying crosstalk between acetylation and hypusination. Hypusination can reduce acetylation in eIF5A2, leading to its localization in the cytoplasmic compartment where it is required for protein synthesis. Inhibition of the deacetylases or impaired hypusination increases acetylation of eIF5A2, leading to nuclear accumulation. These findings provide strong evidence that cytoplasmic localization of eIF5A2 requires not only hypusination but also hypo-acetylation.

**REGULATION OF EIF5A2 EXPRESSION IN HUMAN DIGESTIVE SYSTEM NEOPLASMS**

Although the mechanisms of EIF5A2 gene upregulation in tumor cells are not clear yet, most researchers believe that the main reason is genomic instability caused by copy number variation. To date, EIF5A2 has been frequently found, but not always, to be amplified in human cancers and cancer cell lines. Although tumors that exhibit gene amplification typically exhibit high EIF5A2 expression, many have high EIF5A2 levels without gene amplification, and thus other mechanisms, such as transcriptional regulation and/or post-transcriptional regulation, must exist in EIF5A2 upregulation. It has been demonstrated that K-ras activation upregulates EIF5A2 expression as well as hypusination via transcriptional regulation during the early stages of pancreatic ductal adenocarcinoma (PDAC) progression. Another study has reported that hypoxia increases EIF5A2 RNA levels, at least in part via hypoxia-inducible factor (HIF)-1α in ESCC cells.

Many studies have demonstrated that miRNAs (miRs) target the 3'-UTR of cytoplasmic mRNA of EIF5A2 to post-transcriptionally regulate mRNA and protein levels. Table 1. EIF5A2 is a putative target for miR-203, miR-30b, miR-9, miR-125b, miR-599 and miR-588, which are predicted by the bioinformatic algorithm TargetScan (www.targetscan.org). miR-203 suppresses growth and invasion of colorectal cancer cells (SW620 and LOVO), at least partly, by binding the 3'-UTR of EIF5A2, and repressing EIF5A2 expression at both the mRNA and protein levels. miR-30b, miR-9, and miR-599 suppress gastric cancer cell metastasis via binding to the 3'-UTR of EIF5A2 and repressing eIF5A2 expression. miR-125b inhibits tumorigenic properties of hepatocellular carcinoma (HCC) cells via suppressing eIF5A2 expression, through binding to the 3'-UTR of EIF5A2. miR-9 enhances sensitivity to cetuximab in epithelial phenotype HCC cells through regulation of eIF5A2.

Zender et al. has reported that eIF5A2 is a key downstream effector of XPO4 in tumor inhibition, and XPO4 is a negative regulator of eIF5A2, which may play a role in inhibiting cell proliferation in the nucleus. In murine hepatoma cells, knockdown of XPO4 leads to accumulation of eIF5A1 and eIF5A2 in the nucleus. The sonic hedgehog-GLI family zinc finger 1 signaling pathway upregulates eIF5A2 in pancreatic cancer cells. Moreover, hypoxia can induce eIF5A2 upregulation and...
Table 1 miRNA action in regulation of EIF5A2 gene expression

| miRs   | Ref.            | Materials                  | Function                                                                 |
|--------|-----------------|----------------------------|---------------------------------------------------------------------------|
| miR-203| Deng et al[26]  | CRC cells (SW620 and LOVO) | Suppressing growth and invasion via miR-203/EIF5A2 axis                  |
| miR-599| Wang et al[71]  | GC cells (BGC823 and MKN-45)| Inhibiting metastasis and EMT via miR-599/EIF5A2 axis                    |
| miR-588| Zhou et al[72]  | GC cells (MGC803)           | Regulating invasion, migration and EMT via miR-588/EIF5A2 axis            |
| miR-30b| Tian et al[29]  | GC cells (AGS and MGC803)   | Downregulation of EIF5A2 by miR-30b inhibits EMT                         |
| miR-9  | Xue et al[74]   | HCC cells (Hep3B and Huh7) | Enhancing sensitivity to cetuximab via miR-9/EIF5A2 axis                 |
| miR-125b| Tsang et al[73] | HCC tissue and cells       | Inhibiting tumorigenic properties via miR-125b/EIF5A2 axis               |

CRC: Colorectal cancer; GC: Gastric cancer.

promote elf5A2 translocation from the cytoplasm to the nucleus in ESCC cell lines (KYSE140, KYSE180, KYSE410, KYSE510 and EC109)[21].

FUNCTIONS OF elf5A2 IN HUMAN DIGESTIVE SYSTEM NEOPLASMS

The cancer-associated isoform elf5A2 is not essential for normal development and viability, which has been confirmed in vivo[50]. Accumulating evidence shows that elf5A2 plays important roles in tumor proliferation[11], metastasis[13], EMT[9,11,13,28-29,35,75,76], cytoskeletal rearrangement[13], angiogenesis[21], metabolic reprogramming[14], maintenance of CSCs[31,77] and drug resistance[33,38,74,75,78-80] via its subsequent signaling pathways. Additionally, elf5A2 is associated with survival of many digestive cancer patients[9,11,12,14,21,32] (Figure 1).

elf5A2 and EMT

Over the past 10 years, many studies have evaluated the role of elf5A2 in activating EMT in human cancer cells. Tang et al[13] first reported that elf5A2 induces EMT, an important event in tumor invasion and metastasis that is chiefly characterized by upregulation of mesenchymal markers (Vimentin, fibronectin, E-cadherin and α-smooth muscle actin) and downregulation of epithelial markers (E-cadherin and β-catenin) in HCC. Shek et al[35] and Lou et al[75] confirmed that elf5A2 enhances the aggressiveness of HCC cells by inducing EMT. Zhu et al[9] found that overexpression of elf5A2 also promotes colorectal carcinoma cell aggressiveness by upregulating Metastasis-associated protein 1 through C-myc to induce EMT[76]. In addition, elf5A2 induces EMT of other human digestive system neoplasms such as gastric cancer[21,28] and pancreatic cancer[9].

elf5A2 and cytoskeletal rearrangement

In HCC, elf5A2 stimulates rearrangement of the cytoskeleton through activation of the RhoA/Rac1 GTPase signaling pathway[21]. That study showed that overexpression of elf5A2 in human liver LO2 cells provokes the formation of stress fibers and lamellipodia, without affecting expression level of Rho/Rac GTPase in the cells[21]. However, the precise mechanism underlying{EIF5A2-mediated Rho-GTPase activation requires further investigation.

elf5A2 and angiogenesis

Increased expression of elf5A2, via hypoxia or gene amplification, contributes to angiogenesis in ESCC via the HIF-1α-mediated signaling pathway[21]. In vitro and in vivo assays have both indicated that elf5A2 increases angiogenesis by enhancing matrix metalloproteinase 2 activity via activation of the p38 mitogen-activated protein kinase pathway, and elf5A2 silencing increases tumor vessel wall continuity, increases blood perfusion, and improves tumor oxygenation in HCC[33].

elf5A2 and metabolic reprogramming

A recent study reported that elf5A2 triggers cellular metabolic reprogramming, including glucose metabolism, by promoting aerobic glycolysis and fatty acid biosynthesis via upregulation of FASN and ACS52 in human liver cancer cells[9].

elf5A2 and maintenance of stemness of cancer cells

CSCs are suggested to be responsible for driving resistance to conventional therapies and for cancer metastasis and/or recurrence. It has been reported that elf5A2
Overexpression of Eukaryotic initiation factor 5A2 (eIF5A2) induces epithelial–mesenchymal transition (EMT) by enhancing RhoA/Rac1-GTPase and ITGB60 GNC5-MTA1 activity in hepatocellular carcinoma (HCC). Overexpression of EIF5A2 also promotes colorectal carcinoma and gastric cancer cell aggressiveness by upregulating the C-myc/MTA axis to induce EMT. Increased expression of eIF5A2 contributes to angiogenesis in esophageal squamous cell carcinoma via the P38 MAPK/MMP2 pathway. eIF5A2 promotes cell proliferation and triggers cellular metabolic reprogramming in HCC cells, including glucose metabolism and fatty acid biosynthesis via upregulation of FASN and ACS5. In HCC, eIF5A2 stimulates rearrangement of the cytoskeleton through activation of the RhoA/Rac1 GTPase signaling pathway. eIF5A2: Eukaryotic initiation factor 5A2; EMT: Epithelial–mesenchymal transition.

Overexpression increases the stemness of ESCC cells (KYSE510)[31]. A recent study showed that eIF5A2 also contributes to the maintenance of HCC CSCs (CD133+ HCC cells) via the c-Myc/miR-29b axis[77].

eIF5A2 and survival of patients

Overexpression of cytoplasmic eIF5A2 detected by immunohistochemistry is correlated with poor survival of patients with digestive system malignancies, including colorectal cancer[31], ESCC[21], gastric cancer[11,12] and liver cancer[14,32]. All these studies suggest that a high level of eIF5A2 expression in the cytoplasm is a potential prognostic indicator in many human cancers. However, a recent study demonstrated that nuclear eIF5A2 expression is also an independent prognostic marker in human melanoma[13]. Therefore, nuclear eIF5A2 may have the potential to serve as a therapeutic marker for some human cancers, and further study is needed to establish the subcellular localization of eIF5A2.

Role of eIF5A2 in treatment resistance of human digestive system neoplasms

Primary or secondary anticancer drug resistance is a clinical problem shared by both chemotherapy and targeted therapy. The development of resistance may be predicted from pre-existing genomic and proteomic profiles in patients[78]. eIF5A2 can be used as a biomarker for predicting drug resistance. N1-guanyl-1,7-diaminoheptane (GC7), an inhibitor of DHS, enhances the therapeutic efficacy of doxorubicin in epithelial HCC cells (Huh7, Hep3B and HepG2)[75,79] by preventing the doxorubicin-induced EMT through inhibition of eIF5A2 activation. GC7 can also enhance the sensitivity of oral cancer cells to cisplatin[37]. eIF5A2 promotes resistance to doxorubicin via regulation of EMT in colon cancer cells[27]. Downregulation of eIF5A2 increases tumor perfusion and reduces tumor hypoxia, thus increasing the chemosensitivity of HCC cells to 5-fluorouracil by remodeling tumor vessels[33]. eIF5A2 is significantly related to gemcitabine sensitivity in PDAC cells[38]. Recently, Xue et al[74] reported that eIF5A2 is associated with cytotoxicity of cetuximab in epithelial HCC cells[80]. A high level of eIF5A2 expression is related to drug resistance in many human digestive system cancers. However, other studies have shown no significant relationship between EIF5A2 expression and effects of preoperative radiotherapy in human rectal cancer[81].

CONCLUSIONS AND PERSPECTIVES

Basic research and clinical evidence show that EIF5A2 is a candidate oncop gene and may be a key biomarker for the prognosis of various human digestive system cancers. There is growing evidence that inhibition of hypusination of eIF5A2 inhibits tumorigenesis. Hypusine modification of eIF5A by DHPS and DOHH forms an attractive platform for therapeutic intervention. Many studies have shown that GC7, as an inhibitor of DHS, enhances the sensitivity of drugs through inhibition of eIF5A2 activation in many kinds of human cancer cells[27,28,36,42,47,75,79,89,82,93]. However, hypusination takes place in all eukaryotic cells and has been shown to be necessary for proliferation of mammalian cell lines[35] and crucial for embryonic development as
well as viability in adult mice\cite{45,84}. So, important questions remain regarding how to selectively target tumors and reduce adverse effects.

In contrast to EIF5A1, the of EIF5A2 is limited to tissue such as testes and a few parts of the adult brain, but it is abundant in many human cancers. The elf5A2 protein is associated with cancer metastasis by influencing the processes of EMT, angiogenesis, cytoskeletal rearrangement, and metabolic reprogramming. Thus, the isoform elf5A2 represents a promising target for the treatment of malignant tumors. Moreover, in contrast to DHS or DOHH, the elf5A2 isoform is not essential for embryonic development or for viability in an adult organism. So, we speculated whether elf5A2, which is only expressed in a few tissues in the normal human body, but abundant in various tumor cells, might represent a better target for therapy. Therefore, we propose that specific inhibitors of elf5A2 will exhibit selective toxicity toward elf5A2-dependent cancer cells. Better understanding of the physiological and pathophysiological functions of elf5A2 may lead to more effective management of many human digestive system cancers with high expression of EIF5A2, via early detection, precise prognostication, and molecular targeted treatment. A recent study demonstrated that Mg(II)-catechin nanocomposite particles (Mg(II)-Cat NPs) delivering siEIF5A2 inhibited bladder cancer cell growth in vitro and in vivo\cite{14,26,28}. These results provide preclinical evidence for use of Mg(II)-Cat/siEIF5A2 combined therapeutic methods in cancer.

However, it is also clear that more researches are needed to clarify the underlying mechanisms that regulate elf5A2 expression, for example, how does noncoding RNA regulate the UTR of EIF5A2 and how is its promoter epigenetically modified. With regard to the downstream pathway, the exact mechanism of elf5A2 in regulating its target and whether it can act as a transcriptional factor have not been elucidated.

**REFERENCES**

1. Jenkins ZA, Hägg PG, Johansson HE. Human elf5A2 on chromosome 3q25-q27 is a phylogenetically conserved vertebrate variant of eukaryotic translation initiation factor 5A with tissue-specific expression. *Genomics* 2001; 71: 101-109 [PMID: 11161802 DOI: 10.1006/geno.2000.6418]

2. Guan XY, Sham JS, Tang TC, Fang Y, Hsu KK, Yang PM. Isolation of a novel candidate oncogene within a frequently amplified region at 3q26 in ovarian cancer. *Cancer Res* 2001; 61: 3806-3809 [PMID: 11325856 DOI: 10.1158/0008-5472.CAN-00-03155.x]

3. Clement PM, Henderson CA, Jenkins ZA, Smit-McBride Z, Wolff EC, Hershey JW, Park MH, Johansson HE. Identification and characterization of eukaryotic initiation factor 5A-2. *Eur J Biochem* 2003; 270: 4254-4263 [PMID: 14622290 DOI: 10.1046/j.1432-1033.2003.03806.x]

4. Yang SS, Gao Y, Wang DY, Xia BR, Liu YD, Qin Y, Ning LX, Yang SS. Overexpression of eukaryotic initiation factor 5A2 (EIF5A2) is associated with cancer progression and poor prognosis in patients with early-stage cervical cancer. *Histopathology* 2016; 69: 276-287 [PMID: 26799223 DOI: 10.1111/his.12933]

5. Liu X, Chen D, Liu J, Chu Z, Liu D. Blocking Modification of Eukaryotic Initiation Factor 5A2 Antagonizes Cervical Carcinoma via Inhibition of RhoA/ROCK Signal Transduction Pathway. *Technol Cancer Res Treat* 2017; 16: 630-638 [PMID: 27609633 DOI: 10.1177/1533034616666722]

6. Quanico J, Franck J, Cardon T, Leblanc E, Wisztorski M, Salzetz M, Fournier I. Nanol-CMS coupling of liquid microjunction microextraction for on-tissue proteomic analysis of a novel Hophip Acta Proteins 2017; 1865: 891-900 [PMID: 27836619 DOI: 10.1111/bjopan.2016.11.002]

7. Guan XY, Fang JM, Ma NF, Lau SH, Tai LS, Xie D, Zhang Y, Hu L, Wu QL, Fang Y, Sham JS. Oncogenic role of elf5A2 in the development of ovarian cancer. *Cancer Res* 2004; 64: 4197-4200 [PMID: 15205331 DOI: 10.1158/0008-5472.CAN-03-3747]

8. Yang GF, Xie D, Liu JH, Luo JH, Li LJ, Hua WF, Wu HM, Kung HF, Zeng YX, Guan XY. Expression and amplification of elf-5A2 in human epithelial ovarian tumors and overexpression of ELF-5A2 is a new independent predictor of outcome in patients with ovarian carcinoma. *Gynecol Oncol* 2009; 112: 314-318 [PMID: 19054548 DOI: 10.1016/j.ygyno.2008.10.024]

9. Zhou W, Cai MY, Tong ZT, Dong SS, Mai SJ, Liao YJ, Bian XW, Lin MC, Kung HF, Zeng YX, Guan XY, Xie D. Overexpression of ELF5A2 promotes colorectal carcinoma cell aggressiveness by upregulating MT1A through C-myc to induce epithelial-mesenchymal transition. *Gut* 2012; 61: 562-575 [PMID: 21813470 DOI: 10.1136/gut.2011-300207]

10. Xie D, Ma NF, Pan ZZ, Wu HX, Liu YD, Wu QQ, Kung HF, Guan XY. Overexpression of ELF5A2 is associated with metastasis of human colorectal cancer. *Hum Pathol* 2008; 39: 80-86 [PMID: 17949776 DOI: 10.1016/j.humpath.2007.05.011]

11. Meng QB, Kang WM, Yu JC, Liu YQ, Ma ZQ, Zhou L, Cui QC, Zhou WX. Overexpression of eukaryotic translation initiation factor 5A2 (EIF5A2) correlates with cell aggressiveness and poor survival in gastric cancer. *PloS One* 2013; 10: e0119229 [PMID: 24073713 DOI: 10.1371/journal.pone.0119229]

12. Yang Q, Ye Z, Zhang Q, Zhao Z, Yuan H. Expression of eukaryotic translation initiation factor 5A-2 (elf5A2) associated with poor survival in gastric cancer. *Tumour Biol* 2016; 37: 1189-1195 [PMID: 26282002 DOI: 10.1007/s13277-015-3894-0]

13. Tang DJ, Dong SS, Ma NF, Xie D, Chen L, Fu L, Lau SH, Li Y, Li Y, Guan XY. Overexpression of eukaryotic initiation factor 5A2 enhances cell motility and promotes tumor metastasis in hepatocellular carcinoma. *Hepatology* 2010; 51: 1255-1263 [PMID: 20112245 DOI: 10.1002/hep.23451]

14. Cao TT, Lin SH, Fu L, Tang Z, Che CM, Zhang LY, Ming XY, Liu TF, Tang XM, Tan BB, Xiang D, Li F, Chan OY, Xie D, Cai Z, Guan XY. Eukaryotic translation initiation factor 5A2 promotes metabolic reprogramming in hepatocellular carcinoma cells. *Carcinogenesis* 2017; 38: 94-104 [PMID: 27879277 DOI: 10.1093/carcin/bgw119]
Meng QB et al. EIF5A2 in human digestive system neoplasms
pancreatic ductal adenocarcinoma cells to gemcitabine via the inhibition of eukaryotic translation initiation factor 5A2. Exp Ther Med 2017; 14: 2101-2107 [PMID: 28962130 DOI: 10.3892/etm.2017.4740]

40 Cao D, Hustins SR, Sui G, Bala P, Sato N, Martin S, Maitra A, Murphy KM, Cameron JL, Yeo CJ, Kern SE, Goggins M, Pandey A, Heuman RH. Identification of novel highly expressed genes in pancreatic ductal adenocarcinomas through a bioinformatics analysis of expressed sequence tags. Cancer Biol Ther 2004; 3: 1081-1089, discussion 1090-1091 [PMID: 15467436 DOI: 10.4161/cbt.3.11.11757]

41 Xu G, Shao G, Pan Q, Sun L, Zheng D, Li M, Li N, Shi H, Ni Y. MicroRNA-9 regulates non-small cell lung cancer cell invasion and migration by targeting eukaryotic translation initiation factor 5A2. Am J Transl Res 2017; 9: 478-488 [PMID: 28337276]

42 Wang X, Jiang R, Cui EH, Feng WM, Guo HH, Gu DH, Tang CW, Xue T, Bao Y. N1 guanylyl-1,7-diaminohexane enhances the chemosensitivities of NSCLC cells to cetuximab through inhibition of eukaryotic translation initiation factor 5A2 activation. Eur Rev Med Pharmacol Sci 2016; 20: 1244-1250 [PMID: 27097842]

43 Chen C, Zhang B, Wu S, Song Y, Li J. Knockdown of EIF5A2 inhibits the malignant potential of non-small cell lung cancer cells. Oncol Lett 2018; 15: 4541-4549 [PMID: 29541224 DOI: 10.3892/ol.2018.7832]

44 Wei JH, Cao JZ, Zhang D, Liao R, Zhong WM, Lu J, Zhao HW, Zhang JX, Tong ZT, Fan S, Liang CZ, Liao YB, Pang J, Wu RH, Fang Y, Chen ZH, Li B, Xie D, Chen W, Luo JH. EIF5A2 predicts outcome in localised invasive bladder cancer and promotes bladder cancer cell aggressiveness in vitro and in vivo. Br J Cancer 2014; 110: 1767-1777 [PMID: 24504366 DOI: 10.1038/bjc.2014.52]

45 Chen Z, Yu T, Zhou H, Wei J, Fang Y, Lu J, Guo L, Chen W, Liu ZP, Luo J. Mg(II)-Catechin nanoparticles delivering siRNA targeting EIF5A2 inhibit bladder cancer cell growth in vitro and in vivo. Biomaterials 2016; 81: 125-134 [PMID: 26715756 DOI: 10.1016/j.biomaterials.2015.11.022]

46 Liu Y, Du F, Chen W, Yao M, Lv K, Fu P. EIF5A2 is a novel chemoresistance gene in breast cancer. Breast Cancer 2015; 22: 602-607 [PMID: 24638963 DOI: 10.1016/j.bjca.2014.01.030]

47 Liu Y, Liu R, Fu D, Du F, Hong Y, Yao M, Zhang X, Zheng S. N1-Guanyl-1,7-Diaminohexane Sensitizes Estrogen Receptor Negative Breast Cancer Cells to Doxorubicin by Preventing Epithelial-Mesenchymal Transition through Inhibition of Eukaryotic Translation Initiation Factor 5A2 Activation. Cell Physiol Biochem 2015; 36: 2494-2503 [PMID: 26279450 DOI: 10.1007/s00972-015-2455-2]

48 Preukuschas M, Hagel C, Schulte A, Weber K, Lamskus Z, Sievert H, Pällmann N, Bokemeyer C, Hauber J, Braig M, Balabanov S. Expression of eukaryotic initiation factor 5A and hypusine forming enzymes in glioblastoma patient samples: implications for new targeted therapies. PLoS One 2012; 7: e43468 [PMID: 22927971 DOI: 10.1371/journal.pone.0043468]

49 Ziegler P, Chahoud T, Wilhelm T, Pällmann N, Braig M, Wiehle V, Ziegler V, Schröder M, Meier C, Kolodzik A, Rarey M, Pasje J, Hauber J, Balabanov S, Brümmendorf TH. Evaluation of deoxypseudusynhe synthase inhibitors targeting BCR-ABL positive leukemias. Invest New Drugs 2012; 30: 2274-2283 [PMID: 22415796 DOI: 10.1007/s10637-010-9810-0]

50 Pällmann N, Braig M, Sievert H, Preukuschas M, Herrmanns-Borgmeyer I, Schweizer M, Nagel CH, Neumann M, Wild P, Haralambieva E, Hagel C, Bokemeyer C, Hauber J, Balabanov S. Biological Relevance and Therapeutic Potential of the Hypusine Modification System. J Biol Chem 2015; 290: 18343-18360 [PMID: 26027925 DOI: 10.1074/jbc.M115.664490]

51 Park MH, Nishimura K, Zanelli CF, Valentini SR. Functional significance of eIF5A and its hypusine modification in eukaryotes. Amino Acids 2010; 38: 491-500 [PMID: 19997760 DOI: 10.1007/s00726-009-0408-7]

52 Park MH. The post-translational synthesis of a polyamine-derived amino acid, hypusine, in the eukaryotic translation initiation factor 5A (eIF5A). J Biochem 2006; 139: 161-169 [PMID: 16452363 DOI: 10.1093/jb/mvl034]

53 Park MH, Lee YB, Joe YA. Hypusine is essential for eukaryotic cell proliferation. Bio Signals 1997; 6: 115-123 [PMID: 9285094 DOI: 10.1159/000109117]

54 Klier H, Csonga R, João HC, Eckerskorn C, Auer M, Lottspeich F, Eder J. Isolation and structural characterization of different isoforms of the hypusine-containing protein eIF-5A from HeLa cells. J Biol Chem 1997; 272: 26803-26810 [PMID: 9273082 DOI: 10.1074/jbc.272.42.26803]

55 Pang J, Wu SI, Song Y, Li J. Knockdown of EIF5A2 inhibits the malignant potential of non-small cell lung cancer cells. Invest New Drugs 2012; 30: 1244-1250 [PMID: 22771473 DOI: 10.1016/j.ijid.2012.06.042]

56 Lee SB, Park JH, Folk JE, Deck JA, Pegg AE, Sokabe M, Fraser CS, Park MH. Inactivation of eukaryotic initiation factor 5A (eIF5A) by specific acetylation of its hypusine residue by spermidine/spermine acetyltransferase 1 (SSAT1). J Biochem 2011; 433: 205-213 [PMID: 20492800 DOI: 10.1042/BJ20101322]

57 Kang HA, Schwalberger HG, Hershey JW. Translation initiation factor eIF-5A, the hypusine-containing protein, is phosphorylated on serine in Saccharomyces cerevisiae. J Biol Chem 1993; 268: 14750-14756 [PMID: 8325852 DOI: 10.1111/j.1365-2142.1993.tb18061.x]

58 Chung J, Roela AA, Tonelli RR, Castillo BA, Schenkman S. Eukaryotic initiation factor 5A dephosphorylation is required for translational arrest in stationary phase cells. Biochem J 2013; 451: 257-267 [PMID: 23368777 DOI: 10.1042/BJ20121553]

59 Shang Y, Zhao X, Tian B, Wang Y, Ren F, Jia B, Zhai Y, Chen W, He D, Chang Z. CHIP/Stub1 interacts with eIF5A and mediates its degradation. Cell Signal 2014; 26: 1098-1104 [PMID: 24509416 DOI: 10.1016/j.cellsig.2014.01.030]

60 Beninati S, Nicolini L, Jakus J, Passaggio A, Abbruzzese A. Identification of a substrate site for transglutaminases on the human protein synthesis initiation factor 5A. Biochem J 1995; 305: 725-728 [PMID: 7848820 DOI: 10.1042/30507275]

61 Kabachinski G, Schwartz TU. The nuclear pore complex--structure and function at a glance. J Cell Sci 2015; 128: 423-429 [PMID: 26046137 DOI: 10.1242/jcs.195246]

62 Schmidt HB, Görlitch D. Transport Selectivity of Nuclear Pores, Phase Separation, and Membraneless Organelles. Trends Biochem Sci 2016; 41: 46-61 [PMID: 26705895 DOI: 10.1016/j.tibs.2015.11.001]
Meng QB et al. EIF5A2 in human digestive system neoplasms

Zender L, Xue W, Zuber J, Semignini CP, Krasnitz A, Ma B, Zender P, Kubicka S, Luk JM, Schirmacher P, McCombie WR, Wigler M, Hicks J, Hamon GJ, Powers S, Lowe SW. An oncogenics-based in vivo RNAi screen identifies tumor suppressors in liver cancer. Cell 2008; 135: 852-864 [PMID: 19012953 DOI: 10.1016/j.cell.2008.09.061]

Aksu M, Trakhanov S, Görlich D. Structure of the exportin Xpo4 in complex with RanGTP and the hypusine-containing translation factor eIF5A. Nat Commun 2016; 7: 11952 [PMID: 27306458 DOI: 10.1038/ncomms11952]

Lipowsky G, Bischoff FR, Schwarzmaier P, Knafl B, Kostka S, Hartmann E, Kutay U, Görlach D. Exportin-4: a mediator of a novel nuclear export pathway in human eryocytes. EMBO J 2000; 19: 4362-4371 [PMID: 10944119 DOI: 10.1093/emboj/19.16.4362]

Parreira-E-Silva LT, Gomes MD, Oliveira EB, Costa-Neto CM. The N-terminal region of eukaryotic translation initiation factor 5A signals to nuclear localization of the protein. Biochem Biophys Res Commun 2007; 362: 393-398 [PMID: 17707773 DOI: 10.1016/j.bbrc.2007.07.185]

Wang FW, Guan XY, Xie D. Roles of eukaryotic initiation factor 5A in human cancer. Int J Biol Sci 2013; 9: 1013-1020 [PMID: 24250246 DOI: 10.7150/ijbs.7191]

Rasko JE, Wong JJ. Nuclear microRNAs in normal hemopoiesis and cancer. J Hematol Oncol 2017; 10: 8 [PMID: 28057040 DOI: 10.1186/s13045-016-0375-x]

Wang X, Jin Y, Zhang H, Huang X, Zhang Y, Zhu J. MicroRNA-599 inhibits metastasis and epithelial-mesenchymal transition via targeting EIF5A2 in gastric cancer. Biomed Pharmacother 2018; 97: 473-480 [PMID: 29091897 DOI: 10.1016/j.biopha.2017.10.069]

Zhou X, Xu M, Guo Y, Ye L, Long L, Wang H, Tan P, Xu M. MicroRNA-588 regulates invasion, migration and epithelial-mesenchymal transition via targeting EIF5A2 pathway in gastric cancer. Cancer Manag Res 2018; 10: 5187-5197 [PMID: 30469616 DOI: 10.2174/CMR.S176954]

Tsang FH, Au V, Lu WJ, Shek FH, Liu AM, Luk JM, Fan ST, Poon RT, Lee NP. Prognostic marker microRNA-125b inhibits tumorigenic properties of hepatocellular carcinoma cells via suppressing tumorigenic molecule eIF5A2. Dig Dis Sci 2014; 59: 2477-2487 [PMID: 24811246 DOI: 10.1007/s00412-013-3184-5]

Xue F, Liang Y, Li Z, Liu Y, Zhang H, Wen Y, Yan L, Tang Q, Xiao E, Zhang D. MicroRNA-9 enhances sensitivity to cetuximab in epithelial phenotype hepatocellular carcinoma cells through regulation of the eukaryotic translation initiation factor 5A-2. Onco Lett 2018; 15: 813-820 [PMID: 29399149 DOI: 10.3892/ol.2017.7399]

Lou B, Fan J, Wang K, Chen W, Zhou X, Zhang J, Lin S, Lv F, Chen Y. Ni-guanyl-1,7-diaminoheptane (GC7) enhances the therapeutic efficacy of doxorubicin by inhibiting activation of eukaryotic translation initiation factor 5A2 (eIF5A2) and preventing the epithelial-mesenchymal transition in hepatocellular carcinoma cells. Exp Cell Res 2013; 319: 2708-2717 [PMID: 23958463 DOI: 10.1016/j.yexcr.2013.08.010]

Kolligs FT. An alternative way for epithelial-to-mesenchymal transition in colorectal cancer via EIF5A2? Gut 2012; 61: 473-474 [PMID: 22180609 DOI: 10.1136/gutjnl-2011-301091]

Bai HY, Liao YJ, Cai MY, Ma NF, Zhang Q, Chen JW, Wang CY, Chen WH, Jin XH, Xu RH, Guan XY, Xie D. Eukaryotic Initiation Factor 5A2 Contributes to the Maintenance of CD133(+) Hepatocellular Carcinoma Cells via the c-Myc/microRNA-29b Axis. Stem Cells 2016; 34: 180-189 [PMID: 27197081 DOI: 10.1002/stem.2734]

Cree IA, Clayton P. Molecular chess? Hallmarks of anti-cancer drug resistance. BMC Cancer 2017; 17: 10 [PMID: 28056859 DOI: 10.1186/s12885-016-2999-1]

Zhou QY, Tu CY, Shao CX, Wang WK, Zha JD, Cai Y, Mao JY, Chen WC. GC7 blocks epithelial-mesenchymal transition and reverses hypoxia-induced chemotherapy resistance in hepatocellular carcinoma cells. Am J Transl Res 2017; 9: 2688-2697 [PMID: 28560008]

Xue F, Liu Y, Chu H, Wen Y, Yan L, Tang Q, Xiao E, Zhang D. EIF5A2 is an alternative pathway for cell proliferation in cetuximab-treated epithelial hepatocellular carcinoma. Am J Transl Res 2018; 10: 4670-4681 [PMID: 27940670]

Ojima E, Inoue Y, Miki C, Mori M, Kusunoki M. Effectiveness of gene expression profiling for response prediction of rectal cancer to preoperative radiotherapy. J Gastroenterol 2007; 42: 730-736 [PMID: 17876542 DOI: 10.1007/s00535-007-0209-x]

Liu Y, Xue F, Zhang Y, Lei P, Wang Z, Zhu Z, Sun K. Ni-guanyl-1,7-diaminoheptane enhances the chemosensitivity of acute lymphoblastic leukemia cells to vincristine through inhibition of eIF5A-2 activation. Anticancer Drugs 2017; 28: 1097-1105 [PMID: 28883268 DOI: 10.1097/CAD.0000000000000550]

Xu G, Yu H, Shi X, Sun L, Zhou Q, Zheng D, Shi H, Li N, Zhang X, Shao G. Cisplatin sensitivity is enhanced in non-small cell lung cancer cells by regulating epithelial-mesenchymal transition through inhibition of eukaryotic translation initiation factor 5A2. BMC Med 2014; 14: 174 [PMID: 25380840 DOI: 10.1186/1471-2466-14-174]

Aata A. Re: Mg(II)-Catechin Nanoparticles Delivering siRNA Targeting EIF5A2 Inhibit Bladder Cancer Cell Growth In Vitro and In Vivo. J Urol 2017; 198: 258-259 [PMID: 29370654 DOI: 10.1016/j.juro.2017.05.010]