



EIF5A1 promotes epithelial ovarian cancer proliferation and progression

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ABSTRACT

Epithelial ovarian cancer (EOC) is one of the most common gynecological cancers and has the highest mortality rate thereof. We found abundant eukaryotic translation initiation factor 5A1 (EIF5A1) in 54 EOC tissues, and high EIF5A1 levels predicted poor survival. EIF5A1 ectopic expression enhanced EOC cell proliferative, migration, and invasive capabilities, while EIF5A1 knockdown suppressed them. Most importantly, GC7 (N1-guanyl-1,7-diaminoheptane, an EIF5A1 hypusination inhibitor) could reverse the effect of EIF5A1 upregulation on EOC cell proliferation, migration, and invasion and mutant type EIF5A1_{K50A} plasmid [bearing a single point mutation (K50 → A50) that prevents hypusination] had no effects on these malignant behaviors. Our findings imply that EIF5A1 is a vital regulator of EOC proliferation and progression and is a potential prognostic marker and therapeutic target in EOC.

1. Introduction

Epithelial ovarian cancer (EOC) accounts for the highest proportion of ovarian cancer, the leading cause of cancer-related death among female reproductive tumors [1,2]. Despite improved treatment strategies, more than 14,080 women die from ovarian cancer annually and the overall survival rate is still as low as about 46%, which mainly results from the intricate, obscure pathogenesis of EOC, rendering the discovery of an effective treatment regimen and the improvement of prognosis difficult [3–5]. Accordingly, we focused on the mechanisms of EOC proliferation and metastasis and hope to find a key target for improving the survival rate.

Eukaryotic translation initiation factor 5A (EIF5A) is a highly conserved protein evolutionarily; its lysine at position 50 (Lys50) can be hypusinated (polyamine-derived amino acid hypusine, Nε-[4-amino-2-hydroxybutyl]-lysine) after translation, catalyzed by deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH) [6]. EIF5A is the only protein known to contain hypusine, which is necessary for EIF5A function [7]. N1-guanyl-1,7-diaminoheptane (GC7) is the most potent and the most specific DHS inhibitor and is used in many studies [8,9].

Although EIF5A was originally identified as a eukaryotic translation initiation factor, no evidence supports its role in initiating translation [10]. To date, the identified EIF5A1 functions in eukaryotic cells include RNA and ribosome binding, shuttling between the nucleus and cytoplasm, exporting mRNA from the nucleus to the cytoplasm, and

translation elongation and termination [7,11,12]. In humans, EIF5A has two isoforms: EIF5A1 (17p13.1) and EIF5A2 (3q26.2). Both contain the hypusine residue. EIF5A1 is abundant in most cells and tissues; EIF5A2 shows tissue specificity [13,14]. EIF5A can promote cancer cell proliferation, metastasis, and chemoresistance [15]. In pancreatic cancer, EIF5A1–PEAK1 (pseudopodium-enriched atypical kinase 1) signaling regulates YAP1 (Yes-associated protein 1)/TAZ (tafazzin) protein expression and cancer cell growth [16]. EIF5A2 overexpression enhances hepatocellular carcinoma cell motility and promotes tumor metastasis [17]. EIF5A1 can also promote leukemia cell proliferation and may be a novel therapeutic target in BCR-ABL–positive leukemia [18]. EIF5A2 regulates chemoresistance in colorectal cancer through epithelial–mesenchymal transition (EMT) [19]. EIF5A1 also plays a role in gynecological cancers. For example, blocking EIF5A1 modification in cervical cancer cells alters the expression of cancer-related genes and suppresses cell proliferation [20], while EIF5A2 plays an oncogenic role in ovarian cancer development [21,22]. However, whether EIF5A1 is critical to ovarian cancer progression is unknown.

Here, we report that EIF5A1 is upregulated in EOC tissues and that high EIF5A1 levels predict both poor progression-free survival (PFS) and overall survival (OS). EIF5A1 ectopic expression promoted EOC cell proliferation, migration, and invasion, while EIF5A1 knockdown inhibited it; GC7 suppression of EIF5A1 hypusination inhibited EOC cell proliferative, migration, and invasive abilities. Most importantly, GC7 reversed the effects of wild type EIF5A1 overexpression on EOC cell proliferation, migration, as well as invasion; mutant type EIF5A1_{K50A}

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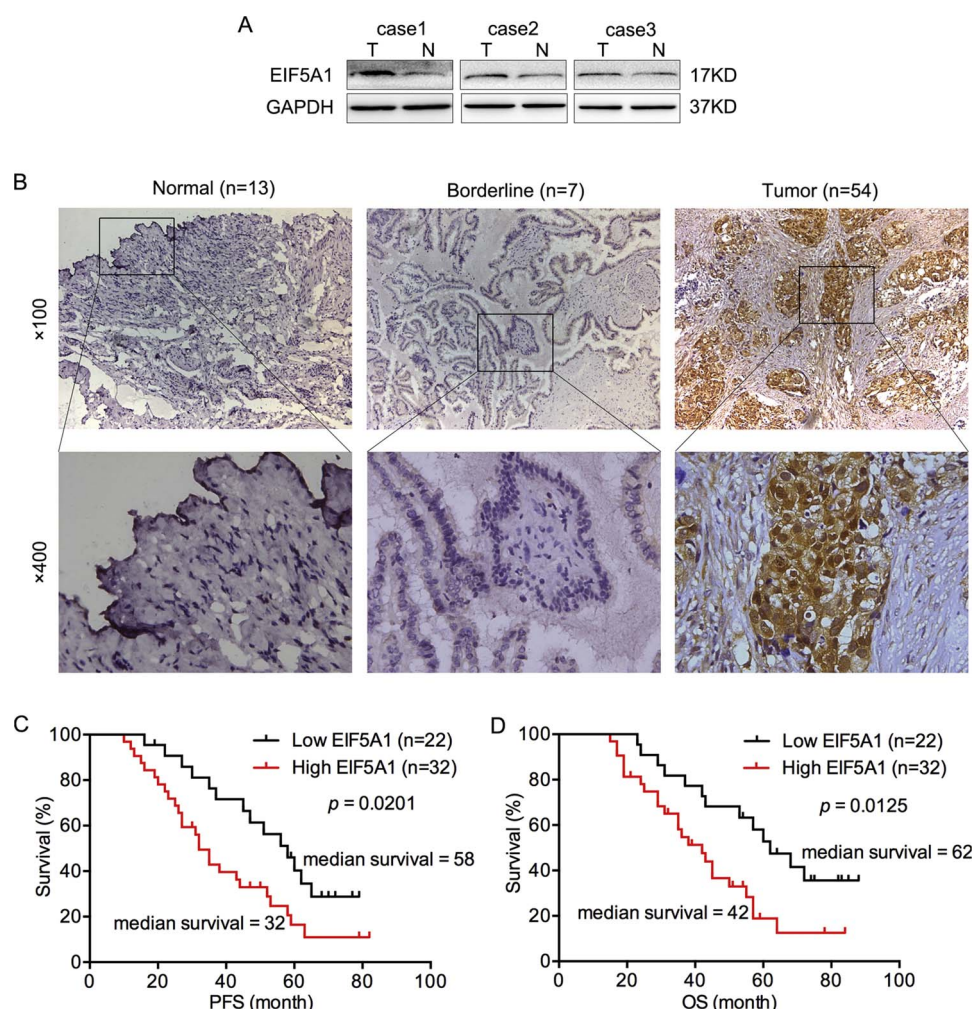


Fig. 1. EIF5A1 is enriched in EOC tissues and predicts poor survival. (A) Western blotting measurement of EIF5A1 levels in three pairs of tumor (T) and normal (N) tissues. (B) Representative images of IHC staining of EIF5A1 in 13 normal, 7 borderline, and 54 malignant epithelial ovarian tissues. Top: $\times 100$ magnification; bottom, $\times 400$ magnification. (C, D). PFS and OS curves of 54 cases of EOC with high and low levels of EIF5A1 expression.

plasmid [bearing a single point mutation (K50 $\rightarrow$ A50) that prevents hypusination] had no effects on these malignant behaviors. We suggest that EIF5A1 is a vital positive regulator of EOC progression and a potential prognostic marker and therapeutic target in EOC.

2. Materials and methods

2.1. Patient samples and immunohistochemical (IHC) staining

We obtained 13 normal ovarian tissue samples, 7 borderline ovarian tissue samples, and 54 EOC samples between June 2008 and June 2009 from the Department of Gynecology and Obstetrics, Shanghai General Hospital, and diagnosed them according to FIGO (International Federation of Gynecology and Obstetrics) stage clinically and histopathologically. All 54 patients with EOC had not undergone preoperative chemotherapy. Supplementary Table S1 shows the patients' basic clinicopathological data. The Shanghai General Hospital Institutional Research Ethics Committee approved the use of patient samples in this study.

EIF5A1 expression in ovarian tissues was tested with IHC staining. We have described the IHC staining protocol and scoring method previously [23].

2.2. Cell culture and transfection

The human EOC cell lines Hey, HO-8910, and SKOV3 were from the Cell Bank of Chinese Academy of Sciences and were cultured in RPMI 1640 medium with 10% fetal bovine serum (Gibco, USA) and 1% penicillin/streptomycin. A normal ovarian epithelial cell line (Moody) and a serous cystadenoma cell line (MCV152) was kindly provided by the Shanghai General Hospital laboratory and cultured in minimum essential medium containing 15% FBS [24]. All cell lines were tested with short tandem repeat analysis and then used within 6 months. The last instance of authentication was June 2017. The cells were maintained in a humidified atmosphere with 5% CO₂ at 37 °C.

EIF5A1 has two isoforms: isoform1 (identifier: P63241-1, 16,832 Da mass) and isoform2 (identifier: P63241-2, 20,170 Da mass), encoding 154-amino acid and 184-amino acid protein respectively (<http://www.uniprot.org/uniprot/P63241>). Wild type EIF5A1 overexpression plasmid (P63241-1) and mutant type EIF5A1_{K50A} plasmid [bearing a single point mutation (K50 $\rightarrow$ A50) that prevents hypusination] were constructed by Genechem (China); vector plasmid was used as the negative control. The sequences of the PCR primers for cDNA amplification were: EIF5A1, forward, 5'-CGGGATCCATGGCAGATGACTTGGAC TTCG-3'; reverse, 5'-ACCTCGAGTTATTTTGGCATGGCCTTGAT-3'; EIF5A1_{K50A}, forward, GATGTCTACTTCGAAGACT-GGCGCGCACGGCC ACGCAA; reverse, TTGGCGTGGCCGTGCGCGCCAGTCTTCGAAGTAG ACATC. Small interference RNA (siRNA; RiboBio, China) was used to

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