# DEPDC1/ EEF1A1 complex promotes the progression of human osteosarcoma

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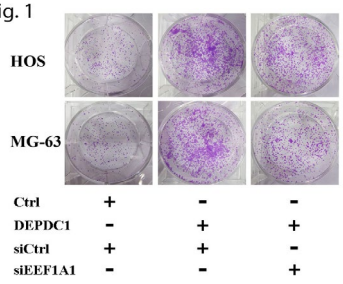
INTRODUCTION: Osteosarcoma remains a high mortality malignancy in youth. Effort has been placed on deciphering the molecular mechanisms underlying the osteosarcoma progression in our research laboratories, due to the fact that development of malignant tumor is a continuous and complex process involving a variety of changes in gene expression. Previous studies in our labs have suggested that a newly identified tumor-related gene DEPDC1 (DEP domain-containing 1) is highly expressed in human osteosarcoma patients and experimental osteosarcoma models, while down-regulation of DEPDC1 resulted in ceasing of osteosarcoma cell proliferation and decelerating of experimental mouse osteosarcoma tumor growth [1]. The extended study here has identified a potentially critical interplay of the DEPDC1 and EEF1A1 (eukaryotic translation elongation factor 1A) axis during the osteosarcoma progression.

METHODS: *Lentiviral constructs and cell infection.* Overexpression or downregulation of DEPDC1 was induced with a GV-based lentiviral vector system, referred to as lenti-DEPDC1/shDEPDC1 and lenti-Ctrl/shCtrl that were used to infect osteosarcoma cells at 1.0 multiplicity of transfection (MOI). In addition, four segments of DEPDC1 full-length sequence (15–106, 107–180, 181–406, 407–527) were incorporated into plasmids for EEF1A1 binding assays. They were constructed in FLAG-tagged plasmids, while EEF1A1 plasmids were HIS-tagged. The plasmids containing siEEF1A1+ GFP-reporter were also constructed.
*Cell Culture*. Human osteosarcoma cell lines HOS, MG-63, and Saos-2 were cultured in Dulbecco's modified Eagle's medium supplemented with 10% FBS, streptomycin (100 mg/mL), and penicillin (100 U/mL) with medium changed every 3rd day prior to transgene modification.
*Cell proliferation and clonogenic survival assays.* Cells infected with lenti-DEPDC1, shDEPDC1, siEEF1A1, or shCtrl were cultured at $5x10^4$/well in 96-well plates at 37°C and 5% CO2 for 5 days before testing with a MTT cell proliferation assay. For the clonogenic assay, $1\times10^3$ HOS and MG-63 cells infected with DEPDC1, Ctrl, and DEPDC1+siEEF1A1 were cultured into each well of six-well culture plates for 14 days. The cell colonies were fixed with 4% paraformaldehyde and stained with Giemsa. Colonies consisting of >50 cells were counted.
*Immunoprecipitation (IP).* 293T and HOS cells were co-transfected with FLAG-tagged-DEPDC1, its four segments (15–106, 107–180, 181–406, 407–527), and HIS-tagged-EEF1A1 plasmids for 24 h. The cells were lysed in Radio-Immunoprecipitation Assay (RIPA) Lysis Buffer. 1 mg of extracted protein was incubated with anti-FLAG-antibody or anti-HIS-antibody for immunoprecipitation to reveal binding complex of EEF1A1 and segment of DEPDC1.
*Immunofluorescent (IF) and immunohistochemical (IHC) staining.* Osteosarcoma cells were cultured to 60%–80% confluence on glass coverslips in culture dishes. After fixation with 4% paraformaldehyde, cells were incubated with anti-DEPDC1-antibody (1:200) or anti-EEF1A1-antibody (1:1000) at 8°C overnight. The cells were next incubated with a fluorescent dye-conjugated secondary antibody (1:300) and phalloidin prior to examination using a Zeiss LSM710 confocal microscope.

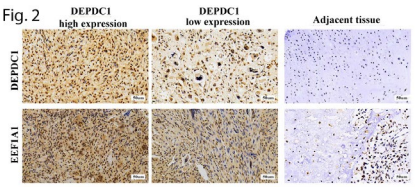
RESULTS: *DEPDC1 promotes the proliferation and migration of osteosarcoma cells by binding to and upregulating EEF1A1.* To determine the specific signaling pathway of DEPDC1 in the proliferation and metastasis of osteosarcoma cells, immunoprecipitation (IP) and mass spectrometry confirmed that EEF1A1 appears the most likely protein to bind DEPDC1. Co-immunoprecipitation experiments showed that they formed a complex in osteosarcoma cells. To identify the binding site of DEPDC1 to EEF1A1, the constructed plasmids containing the 4 different DEPDC1 fragments co-transfected with FLAG-tagged-DEPDC1 or His-tagged-EEF1A1 into HOS and MG-63 cells. DEPDC1 and its four fragments were successfully expressed after transfection, as detected with a FLAG antibody. The fragments of 15–106, 107–180, and 407–527 showed strong binding with His-tagged-EEF1A1, while the binding was lost in 181–406. These results suggested that DEPDC1 binds to EEF1A1 at three domains, 15–106, 107–180, and 407–527. Subsequently, we silenced EEF1A1 expression with siRNAs targeting the open reading frame of EEF1A1 (siEEF1A1), and the silencing of EEF1A1 effectively reduced the proliferation (Fig. 1) and migration induced by overexpression of DEPDC1 in both HOS and MG-63 cells.

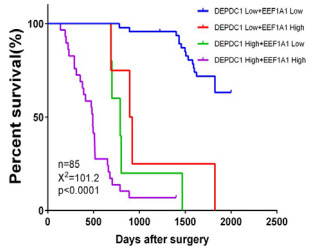
Fig. 1




*DEPDC1 expression was correlated with human osteosarcoma progression and high level of EEF1A1.* To explore the relationship between the DEPDC1/EEF1A1 interplay and the clinicopathological characteristics of osteosarcoma patients, we analyzed the positive staining patterns of DEPDC1 and EEF1A1 in the human osteosarcoma tissue samples by immunohistochemistry. The data clearly shown that the expression of DEPDC1 protein was positively correlated with the expression of EEF1A1 (Fig. 2), indicating that the development of human osteosarcoma may be related to the DEPDC1/EEF1A1 pathway. Further, the IHC data correlated with the clinical disease progressions of the patients. The protein expression levels of either DEPDC1 or EEF1A1 were higher in the advanced TNM stage group and the lymphatic metastasis-positive group. Subsequently, receiver operating characteristic (ROC) curves demonstrated that the area under the curves (AUC) of the DEPDC1- and EEF1A1-based predictions were 0.7908, and 0.79, respectively, suggesting that they could be used to predict the survival rate of osteosarcoma patients. Importantly, the higher expression levels of either DEPDC1 or EEF1A1 were highly associated with the decreased survival time of osteosarcoma patients (P<0.001). indeed, the survival time was shortest in the high expression groups of DEPDC1 and EEF1A1 (Fig. 3).

Fig. 2




DISCUSSION: DEPDC1 is a newly discovered tumor-related gene that has a highly conserved domain. Many studies have found that proteins with DEP domains can regulate many cellular functions, such as cell membrane anchoring, signal transduction, cell polarity establishment, and regulation of small molecule GTP enzyme activity. Eukaryotic translation elongation factor 1A (EEF1A) is an important molecule involved in the translation function in protein synthesis. In vitro and in vivo experiments in this investigation suggested that DEPDC1 directly binds to and promotes the expression of EEF1A1 in the nuclei of osteosarcoma cells and accelerates the proliferation and migration of osteosarcoma cells. More importantly, this report for the first time revealed the relationship between the expression of DPEDC1/EEF1A1 and the clinical prognosis of osteosarcoma patients.

SIGNIFICANCE/CLINICAL RELEVANCE: This study deciphered the mechanism of DEPDC1 promoting the development of osteosarcoma, which will provide new therapeutic targets for further development of new anticancer drugs. Also, DEPDC1–EEF1A1 interaction can accurately predict the clinical characteristics and prognosis of patients, thus providing new indication and strategies for tumor diagnosis and treatment.

REFERENCE: [1] L Shen, *et al*. DEPDC1 as a crucial factor in the progression of human osteosarcoma. CANCER MEDICINE, 2023, 12(5):5798-5808