DEPDC1/ EEF1A1 Complex Promotes the Progression of Human Osteosarcoma via Downregulation of FOXO3a

Lin Shen
    Shandong First Medical University

Han Lin
    Shandong University

Aijun Zhang
    Shandong University

Ronghan Liu
    Shandong First Medical University

Chendan Zhou
    Shandong University

Ying Zhang
    Shandong First Medical University

Kai Zhao
    Shandong First Medical University

Laitong Lu
    Shandong First Medical University

Bin Ning (✉ bning@sdfmu.edu.cn)
    Shandong First Medical University

Research

Abstract

Background

Osteosarcoma is a common primary malignant bone tumor with poor prognosis. Currently there is no effective therapeutic strategies primarily due to the insufficient understanding its underlying mechanisms. Here we aimed to decipher the molecular mechanisms underlying the osteosarcoma progression.

Methods

GEO data analysis, immunohistochemistry, qRT-PCR and western blotting were performed to evaluate the expression of differentially genes in human osteosarcoma tissues. Stably transfected human osteosarcoma cells were injected in mouse model to assess the effect of DEPDC1 in vivo. The function of DEPDC1−EEF1A1−FOXO3a axis was detected by mass spectrometry analysis, co-immunoprecipitation (co-IP) experiments and RNA sequencing in vitro.

Results

By exploring differentially expressed genes, we found *DEPDC1* is highly expressed in human osteosarcoma cells and tissues. Mechanistically, we found the protein expressed by DEPDC1 can directly bind to EEF1A1 through three binding regions, thus forming a complex. Importantly, DEPDC1/EEF1A1 complex can directly inhibit the transcription and expression of FOXO3a in vitro and in vivo, thus promoting the metastasis and proliferation of osteosarcoma. The clinical relevance study showed that overexpression of DEPDC1/EEF1A1 complex is correlated with reduced survival rate of osteosarcoma patients.

Conclusions

Collectively, this study demonstrated the DEPDC1/EEF1A1−FOXO3a axis as a critical pathway that promotes the progression of osteosarcoma and leads to poor prognosis. Genetically targeting or pharmacologically inhibiting DEPDC1/EEF1A1−FOXO3a axis may serve a promising strategy for targeting human osteosarcoma.

Background

Osteosarcoma is a common primary malignant bone tumor in children and adolescents. Surgical resection and drug chemotherapy are the main treatment for osteosarcoma. However, the side effects of chemotherapy drugs and the drug resistance of osteosarcoma often lead to poor prognosis [1-3]. Thus deciphering the molecular mechanisms underlying the osteosarcoma progression is of urgent.

Recent studies mainly focused on exploring the critical oncogenes that promote the occurrence and development of osteosarcoma. Because the occurrence and development of malignant tumor is a

continuous and complex process, involving a variety of changes in gene expression. A large number of studies have found that *XIAP*, *COX-2*, *Livin* and other genes are highly expressed in osteosarcoma, while *Caspase-3* and *tp53* genes are low expressed in osteosarcoma [4-8]. XIAP and Livin are the main members of apoptosis inhibitory protein family, which can directly affect Caspase-3, Caspase-7i and Caspase-9, thus inhibiting the apoptosis of tumor cells [5, 7-9]. High expression of *COX-2* is mainly involved in promoting tumor angiogenesis and inhibiting tumor apoptosis [6]. As a tumor suppressor gene, *tp53* gene induces apoptosis of tumor cells through Bax/Bcl2, Fas/Apol and IGF-BP3 [9]. According to the change of spatial conformation, mutant p53 losts the regulatory effect on cell growth, apoptosis and DNA repair, and will transform from tumor suppressor gene into oncogene [10]. Therefore, it has been confirmed that the gene regulation network of osteosarcoma mainly suppresses the apoptosis of tumor cells, but the underlying mechanism in proliferation and metastasis of osteosarcoma is still unclear. Our aim is to screen the differentially expressed genes of osteosarcoma, and explore the mechanism of promoting tumor proliferation and metastasis in vivo and in vitro, which is conducive to the development of new molecular targeted drugs, improve the therapeutic effect, reduce the risk of adverse reactions, and improve the survival rate of osteosarcoma patients [11].

EEF1A1, a subtype of eukaryotic translation extension factor 1A (EEF1A), is involved in the process of protein translation. Recently, it has been found that EEF1A1 mediates the epithelial-mesenchymal transition (EMT) of breast cancer cells to promote the occurrence and metastasis through the formation of TGF-β-activated-translational (BAT) mRNP complex [12]. Slega E et al. found that EEF1A1 was overexpressed among the pancreatic cancer, leukemia and osteosarcoma cell lines, and siRNA treatment against EEF1A1 produced a chemosensitization toward MTX. In addition, biological association networks identified DKK1, UGT1As and EEF1A1 as important gene nodes in MTX-resistance [13]. However, it is still unclear which oncogenes regulate EEF1A1 and how it participates in the proliferation and metastasis of osteosarcoma.

FOXO3a belongs to Forkhead transcription factor (FOXO) family. Its role in tumor inhibition has been widely studied. Phosphorylation of FOXO3a by AKT promotes its translocation from the nucleus to the cytoplasm, and then enhancing the expression of cyclin D1 and tumor cell proliferation and metastasis [14-17]. However, it is unclear whether FOXO3a plays a critical role in osteosarcoma is elusive.

DEP domain-containing 1 (*DEPDC1*) is a newly identified tumor-related gene. Recent studies have shown that *DEPDC1* is overexpressed in bladder cancer, breast cancer, lung adenocarcinoma and other malignant tumor types [18-21]. *DEPDC1* inhibits cell apoptosis by activating the NF−κB pathway to promote the progression of bladder cancer [19]. However, It is not clear whether *DEPDC1* can promote the development of cancer in human osteosarcoma, and whether *DEPDC1* can promote the proliferation and invasion of osteosarcoma cells. From the online database, we screened differentially expressed genes in osteosarcoma patients and found that *DEPDC1* was highly expressed in human osteosarcoma tissues and cells. Therefore, *DEPDC1* might be a crucial oncogene for the progression of osteosarcoma.

In this study, we revealed that DEPDC1 combined with EEF1A1 to inhibit the expression of FOXO3a and promote its phosphorylation, ultimately inducing the proliferation and migration of osteosarcoma cells and significantly reducing the survival rate of osteosarcoma patients. Our data suggest that the DEPDC1−EEF1A1−FOXO3a axis may be a new effective target for treating human osteosarcoma.

Methods

Clinical samples and survival time analysis

Osteosarcoma tissue samples, its adjacent tissues, and the corresponding clinical data were obtained from Jinan Central Hospital, Qilu Hospital of Shandong University, and Xi'an Best Biotechnology Co., Ltd (Xian, China). Details are provided in Additional file 1: Materials and Methods.

Gene Expression Omnibus data analysis of differential gene expression in human osteosarcoma

Eight datasets (species: *Homo sapiens*) were downloaded from the NCBI Gene Expression Omnibus (GEO) database including GSE11414, GSE12865, GSE14359, GSE16088, GSE19276, GSE28424, GSE36001, and GSE9508. Details are provided in Additional file 1: Materials and Methods.

Lentiviral constructs and cell infection

Overexpression or downregulation of DEPDC1 was induced with a GV-based lentiviral vector system. Details are provided in Additional file 1: Materials and Methods. The core sequences of target gene fragments in each group are shown in Additional file 3: Supplementary Table. S1.

Plasmids

The construction of *DEPDC1* plasmid and four segments of its full-length sequence (15−106, 107−180, 181−406, 407−527) are listed in Additional file 3: Supplementary Table S1. The construction of plasmids containing EEF1A1/siEEF1A1/siFOXO3a is also listed in Additional file 3: Supplementary Table S1 and Additional file 4: Supplementary Fig. S3b.

Immunofluorescentstaining

For a detailed description of cell and tissue immunofluorescence staining, please refer to Additional file 1: Materials and Methods and Additional file 2: Supplementary Method 1, respectively.

Construction of FOXO3-GFP reporter plasmid

The sequence of the FOXO3-GFP reporter plasmid is listed in Additional file 4: Supplementary Fig. S2l. Details are provided in Additional file 1: Materials and Methods. and Additional file 2: Supplementary Method 2.

Statistical analysis

Data were processed in GraphPad Prism 6.0 (GraphPad Software, CA, USA) and SPSS v. 20.0 (IBM Corp., NY, USA). Details are provided in Additional file 1: Materials and Methods.

Further applied methods

Additional cell culture, clinical samples, survival time analysis, Gene Expression Omnibus data analysis of differential gene expression in human osteosarcoma, lentiviral constructs and cell infection, quantitative real-time PCR, western blotting, immunohistochemical staining and scoring, cell proliferation assay, clonogenic survival assay, apoptosis analysis, wound healing assay, plasmids, immunoprecipitation, immunofluorescent staining, animal xenograft tumorigenesis assay, RNA sequencing, construction of FOXO3-GFP reporter plasmid, H&E staining and statistical analysis are further described in the Additional file 1: Materials and Methods.

Results

DEPDC1 is highly expressed in human osteosarcoma tissues and cells

To explore the crucial oncogenes related to osteosarcoma progression, we analysed the differential gene expression between an osteosarcoma group and a normal group by using the Gene Expression Omnibus (GEO) database including 25,035 annotated genes to (**Additional file 3: Supplementary Table. S2**). Genes with |logFC|>1 and P<0.05 were selected as considered as differentially expressed genes. A total of 1,355 differentially expressed genes were screened out (523 upregulated genes and 832 downregulated genes). A heatmap was constructed for these interested genes (**Fig. 1A**). For the combined adjusted P- and logFC values, the logFC value of *DEPDC1* was the largest (logFC=1.204), with a corresponding adjusted P-value of<0.05, thus reaching significance, and the relative expression level of *DEPDC1* in the osteosarcoma group was 2.3 times higher than that in the control group (**Fig. 1B**). We next examined the protein and RNA expression of DEPDC1 in the osteosarcoma of clinical patients. We found that DEPDC1 was widely expressed in osteosarcoma tissues and osteosarcoma cell lines (HOS, MG-63, and Saos-2) compared with the para-carcinoma tissues and the human osteoblastic cell line Hfob1.19 (**Fig. 1C–E**).

Suppression of DEPDC1 expression could significantly inhibit the proliferation and metastasis, but induce apoptosis of osteosarcoma cells

To further explore the functional role of DEPDC1 in osteosarcoma, compared with the control group (shCtrl), we obviously silenced the expression of *DEPDC1* in osteosarcoma cells using siDEPDC1 lentivirus infection (**Additional file 4: Supplementary Fig. S1C, D**). We found that the DEPDC1 knockdown led to cell growth arrest (**Fig. 1F, G**), increased apoptosis induction (**Fig. 1H**), and repressed clone formation (**Fig. 1I**) and cell migration (**Fig. 1J**). Taken together, all of these findings demonstrate that DEPDC1 is an oncogene to promote the osteosarcoma cancer cell growth and cell migration while inhibit apoptosis.

DEPDC1 promotes the proliferation and migration of osteosarcoma cells by binding to and upregulating EEF1A1

To determine the specific signalling pathway of DEPDC1 involved in the proliferation and metastasis of osteosarcoma cells, we performed the immunoprecipitation (IP) and mass spectrometry to probe into the candidate proteins interacting with DEPDC1. We found that EEF1A1 was one of the most likely proteins to bind with DEPDC1 (**Fig. 2A, B and Table. 1**). Then we performed co-immunoprecipitation (co-IP) experiments, which showed that they formed a complex in osteosarcoma cells (**Fig. 2C**). Overexpression of DEPDC1 in osteosarcoma cells by DEPDC1 lentivirus infection (**Additional file 4: Supplementary Fig. S3B**) increased the expression of EEF1A1, while downregulation of *DEPDC1* suppressed the expression of EEF1A1 (**Fig. 2d**). To identify the binding site of DEPDC1 to EEF1A1, we constructed four plasmids concluding different *DEPDC1* fragments (15−106, 107−180, 181−406, and 407−527) (**Additional file 3: Supplementary Table S1**). We co-transfected FLAG-tagged-*DEPDC1* and His-tagged-*EEF1A1* into 293T cells and HOS cells. DEPDC1 and its four fragments were successfully expressed after transfection, as detected with a FLAG antibody (**Fig. 2E top**). 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 (**Fig. 2E bottom**). These results suggested that DEPDC1 binds to EEF1A1 at three domains, 15−106, 107−180, and 407−527. Immunofluorescence staining showed that when DEPDC1 was over expressed, DEPDC1 and EEF1A1 were co-localized in the nucleus, further supporting the interaction between DEPDC1 and EEF1A1 (**Fig. 2F**). Subsequently, we silenced EEF1A1 expression with siRNAs targeting the open reading frame of *EEF1A1* (siEEF1A1; **Additional file 3: Supplementary Table S1**). Silencing of *EEF1A1* decreased DEPDC1-induced proliferation (**Fig. 2F**) and migration (**Fig. 2G**) of both HOS and MG-63 cells. Collectively, these results demonstrated that DEPDC1 interacts with EEF1A1, which is crucial for DEPDC1 promotion on the proliferation and migration of osteosarcoma cells .

DEPDC1 inhibits FOXO3a expression by regulating the binding of EEF1A1 to DEPDC1

To further explore the signalling pathway involved in the promotion of DEPDC1−EEF1A1 complex on human osteosarcoma, we performed RNA-seq to investigate the differentially expressed genes in *DEPDC1*-knockdown cells (**Fig. 3A**). Transcription factor enrichment analysis with TFactS, showed FOXO3 as a transcription factor significantly regulated by DEPDC1 (**Fig. 3B**). Moreover, overexpression of DEPDC1 decreased the mRNA and protein expression of FOXO3a, while upregulated cyclin D1 expression. Furthermore, the low expression of DEPDC1 increased the mRNA and protein expression of FOXO3a, decreasing cyclin D1 expression (**Fig. 3C, D**). Meanwhile, after downregulating DEPDC1, the lowexpression of FOXO3a inhibited the apoptosis of osteosarcoma cells and promoted their migration (**Fig. 3E, F**). DEPDC1 and EEF1A1 were co-localized in the nucleus, further supporting the interaction between DEPDC1 and EEF1A1 (**Fig. 3G left**). FOXO3a and p-FOXO3a were mainly colocalized in the cytoplasm. FOXO3a was mainly located in the nuclei of osteosarcoma cells after knockdown of DEPDC1 expression (**Fig. 3G right**). Compared with the control group, FOXO3a was up-regulated in the cytoplasm after DEPDC1 expression was inhibited, while EEF1A1 and p-FOXO3a were down-regulated in the nucleus and cytoplasm, respectively (**Fig. 3G middle**). To further explore the regulatory effect of DEPDC1 and

EEF1A1 on FOXO3a, we successfully constructed a FOXO3-GFP reporter plasmid (**Additional file 4:Supplementary Fig. S2I**). Compared with the control group, after overexpression of DEPDC1 in HOS and MG-63 osteosarcoma cell lines, the GFP fluorescence intensity of FOXO3 was significantly inhibited. Then the GFP fluorescence intensity of FOXO3 was gradually enhanced while decreasing EEF1A1 expression (**Fig. 3H**). Conversely, when DEPDC1 was downregulated, the GFP fluorescence intensity of FOXO3 was significantly enhanced, and the GFP fluorescence intensity of FOXO3 was decreased while increasing EEF1A1 expression (**Fig. 3H**). These results suggest that the combination of DEPDC1 and EEF1A1 promotes FOXO3a to translocate from the nucleus for phosphorylation, thus promoting the migration of osteosarcoma cells and inhibiting their apoptosis.

Perturbed expression of DEPDC1 inhibits the proliferation of osteosarcoma cells in vivo

To investigate the role of DEPDC1 in the progression of osteosarcoma cells in vivo, we successfully constructed a nude mouse xenograft model (**Fig. 4A**). In this model, downregulation of DEPDC1 in human osteosarcoma cells significantly suppressed tumor growth compared with control cells (**Fig. 4B, D, E**). Hematoxylin and eosin (H&E) staining showed that the *DEPDC1*-knockdown tumor cells presented with nuclear concentration and cytoplasmic fibrosis (**Fig. 4C**). Moreover, EEF1A1 expression was decreased, but FOXO3a was increased, in the *DEPDC1*-knockdown xenograft model of nude mice, as detected by immunohistological staining (**Fig.4F, H**) and immunofluorescence (**Fig. 4G, I**), respectively. This implied that the DEPDC1/EEF1A1–FOXO3a axis also promotes the proliferation of human osteosarcoma cells in vivo.

DEPDC1 expression was correlated with human osteosarcoma progression, high level of EEF1A1 and low level of FOXO3a

To explore the relationship between the DEPDC1/EEF1A1/FOXO3a axis and the clinicopathological characteristics of osteosarcoma patients, we analysed the expression of DEPDC1, EEF1A1, and FOXO3a in human osteosarcoma tissue samples. We used immunohistochemical staining to investigate the relationship between the expression of DEPDC1, EEF1A1, and FOXO3a and the different clinicopathological features of patients with osteosarcoma (**Fig. 5A**). At the same time, the expression of DEPDC1 protein was positively correlated with EEF1A1 but negatively correlated with FOXO3a, which indicated that the development of human osteosarcoma may be related to the DEPDC1/EEF1A1–FOXO3a pathway (**Fig. 5B**). The protein expression levels of either DEPDC1 or EEF1A1 were higher in the advanced TNM stage group and the lymphatic metastasis-positive group. However, FOXO3a was expressed at lower levels in either the advanced TNM stage or lymphatic metastasis-positive groups (**Fig. 5C and Additional file 3 Supplementary Table. S3**). Subsequently, ROC curves demonstrated that the area under the curves (AUC) of the DEPDC1-, EEF1A1-, and FOXO3a-based predictions were 0.7908, 0.79, and 0.7528, respectively, suggesting that they all could be used to predict the survival rate of osteosarcoma patients (**Fig. 5D**). Importantly, the higher expression levels of either DEPDC1 or EEF1A1 and lower expression of FOXO3a were highly associated with the decreased survival time of osteosarcoma patients ($P<0.001$, **Fig. 5E**). Interestingly, the survival time was shortest in the high

expression groups of DEPDC1 and EEF1A1 (**Fig. 5F**) and the groups with high DEPDC1 but low FOXO3a expression (**Fig. 5G**). **Fig. 5H** showed that the group with high expression of both DEPDC1 and EEF1A1, but low expression of FOXO3a, had the shortest survival time. Furthermore, the χ^2 value of log-rank (Mantel-Cox) test in the three-index group is 74.05, demonstrating that it is better to predict the patients' survival time by combining three indices. Therefore, we conclude that the DEPDC1/EEF1A1−FOXO3a axis is closely related to the clinicopathological characteristics of osteosarcoma patients, and can accurately predict the survival time of osteosarcoma patients, which indicates that this axis can be used as a molecular marker of human osteosarcoma.

Discussion

In this study, we explored a new mechanism of DEPDC1-induced proliferation and migration of osteosarcoma cells. The expression of DEPDC1 in osteosarcoma was similar to that of EEF1A1 but was negatively correlated with FOXO3a. Therefore, we speculated that the complex formed by DEPDC1 and EEF1A1 enhanced the expression of EEF1A1 and ultimately led to the downregulation of FOXO3a, thus promoting the proliferation and migration of osteosarcoma cells in vitro and in vivo (**Fig. 6**).

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 [22]. Recent studies have shown that DEPDC1 is overexpressed in bladder cancer, breast cancer, lung adenocarcinoma, and other malignant tumor types [19-21]. In addition, Harada et al. found that DEPDC1 mainly inhibited cell apoptosis through the NF−κB signalling pathway, and then promoted the progression of bladder cancer [19]. Furthermore, DEPDC1 can also be used in the diagnosis and treatment of various tumors. Kretschmer et al. found that DEPDC1 and FOXM1 are significantly upregulated in ductal carcinoma in situ (DCIS), and thus can be used to identify early molecular markers of breast cancer [23]. S-288310, a cancer peptide vaccine containing oncoantigens against DEPDC1, is well tolerated and can effectively prolong the survival time of patients with urothelial carcinoma of the bladder [18]. However, it remains unclear whether *DEPDC1* is the main mechanism of promoting the proliferation and migration of malignant tumors. In this study, we found that *DEPDC1* was highly expressed in human osteosarcoma through the GEO database analysis and confirmed this result in osteosarcoma tissues and cells (**Fig. 1**). At the same time, through co-IP and RNA-seq experiments, we found that *DEPDC1* inhibits the expression of FOXO3a in combination with EEF1A1, thereby promoting the proliferation and migration of osteosarcoma in vitro and in vivo (**Fig. 2−5**).

Eukaryotic translation elongation factor 1A (EEF1A) is an important molecule involved in the translation function in protein synthesis. EEF1A can be divided into two subtypes, EEF1A1 and EEF1A2. In humans, EEF1A is encoded by two genes on chromosomes 6 and 20, which are mainly involved in apoptosis, cell cycle regulation, protein degradation, and post-translation modifications [24-26]. EEF1 complex members are necessary in the eukaryotic elongation process. Recently, it has been found that EEF1A1 is related to cancer occurrence [13, 27]. Genetic changes in *EEF1A1* were detected by The Cancer Genome Atlas

(TCGA) to explore its potential impact on selected epigenetic modulators. However, the specific upstream and downstream regulatory molecules that bind to EEF1A1 were not accurately investigated, nor the clinical correlation between the expression of EEF1A1 and the prognosis of tumor patients [28]. Furthermore, the role of EEF1A1 in the proliferation and metastasis of osteosarcoma has not been studied. In our study, we found that DEPDC1 directly binds to and promotes the expression of EEF1A1 in the nuclei of osteosarcoma cells, thus promoting the proliferation and migration of osteosarcoma cells in vitro and in vivo (**Fig. 2 and 4**).The 15–106, 107–180, and 407–527 fragments of *DEPDC1* showed strong binding with EEF1A1 (**Fig. 2E bottom**). Furthermore the 15–106 domain of DEPDC1 is the DEP domain, named after Dishevelled, Egl-10, and Pleckstrin, in which this domain was first discovered. The function of this domain remains unclear, but is believed to be important for the membrane association of the signalling proteins in which it is present [29, 30]. In addition, this report for the first time revealed the relationship between the expression of DPEDC1/EEF1A1 and the clinical prognosis of osteosarcoma patients (**Fig. 5**). The expression of EEF1A1 in osteosarcoma is positively correlated with DEPDC1, and the high expression of both can reduce the survival time of osteosarcoma patients, which indicates that DEPDC1 and EEF1A1 are potential prognostic markers and therapeutic molecular targets of osteosarcoma.

The Forkhead transcription factors (FOXO), also named Forkhead-like protein (FKHR), is a family transcription factors that was identified in 2000. There are four types in mammals, FOXO1, FOXO3a, FOXO4, and FOXO6, which are distributed on different chromosomes [31]. The common feature of this family is the conserved DNA domain, namely, the Forkhead box. This protein family regulates apoptosis, cell cycle, cell proliferation, DNA damage repair, and cancer development, and inhibits tumor cell proliferation [28]. FOXO3a is among the most widely studied members of the Forkhead family. It is located on human chromosome 6q21 and is expressed in gastrointestinal, liver, ovary, prostate, and breast tissue as well as others [32, 33]. FOXO3a dysfunction leads to uncontrolled cell proliferation and DNA damage accumulation, resulting in tumorigenic effects [34, 35]. The main mechanism regulating FOXO3a activity and its target genes is the control of the nuclear–cytoplasmic shuttling of FOXO3a. The phosphorylation of FOXO3a leads to its translocation from the nucleus to cytoplasm, followed by binding to 14-3-3 protein in the cytoplasm, and then FOXO3a is degraded in a ubiquitin-/proteasome-dependent manner [36, 37]. The balance of nuclear–cytoplasmic shuttling is crucial for maintaining the function of FOXO3a. The loss of this balance leads to the occurrence and development of various diseases including cancer [38, 39]. Studies have shown that the Wnt–β-catenin and PI3K–AKT–FOXO3a pathways have a central role in cancer. AKT phosphorylates FOXO3a, promoting its translocation from the cell nucleus to the cytoplasm. When this effect was reversed by PI3K and AKT inhibitors, the accumulation of FOXO3a in the nucleus increased, which promoted the apoptosis of colon cancer cells and inhibited their metastasis [40]. Additionally, Hu et al. found that nuclear exclusion of FOXO3a by AKT contributed to cell survival. They also observed that Iκβ kinase (IKK) can promote FOXO3a phosphorylation, inhibiting the expression of FOXO3a, and finally causing FOXO3a protein hydrolysis through the ubiquitin-dependent proteasome pathway. The expression and accumulation of FOXO3a in the nucleus is reduced by IKKβ, which promotes the proliferation of breast cancer cells and is related to the low survival rate of breast

cancer [41]. However, there have been no further studies on whether oncogenes are involved in the transcription and expression of FOXO3a, affecting the balance of nuclear−cytoplasmic shuttling and promoting the occurrence and development of tumors. Lei et al. found that *DEPDC1* overexpression facilitated cell proliferation and tumor growth through increasing the expression of FOXM1 in TNBC cells [23]. FOXM1 is negatively regulated by FOXO3a, supports cell survival, drug resistance, colony formation and proliferation in vitro, and promotes tumor development in vivo [42]. At the same time, the FOXO3-FOXM1 axis is a key cancer drug target and a modulator of cancer drug resistance [43]. But so far, there is no report to explore the relationship between *DEPDC1* and FOXO3a and their interaction mechanism. In this study, we found that *FOXO3* had the best correlation with *DEPDC1* expression in human osteosarcoma cells by RNA-seq (**Fig. 3A, B and Additional file 4: Supplementary Fig. S2**). Later, we found that DEPDC1 forms a complex with EEF1A1. Upregulation of DEPDC1 promoted the accumulation of EEF1A1 in the nuclei of osteosarcoma cells and then inhibited the expression of FOXO3a, restricting its distribution to the cytoplasm, while the expression of phosphorylated FOXO3a increased (**Fig. 2−4**). When DEPDC1 was downregulated, the opposite result was obtained (**Fig. 2−4**). Furthermore, the expression of FOXO3a was negatively correlated with DEPDC1 in human osteosarcoma and lowexpression of FOXO3a shortened the survival time of patients with osteosarcoma (**Fig. 5**). Therefore, we observed that inhibition of FOXO3a expression could significantly promote tumor proliferation and migration while reducing the survival time of tumor patients, which is consistent with many previous reports. Wasim et al. found that EEF1A1 can activate AKT dependent cell migration and tumor proliferation [44, 45]. In addition, AKT phosphorylates FOXO3a, promoting tumor proliferation and metastasis [46].

Cyclin D1 is an important regulator of the cell cycle and has a vital role in tumor development. In the cell cycle, cyclin D1 levels are regulated by many factors and change periodically. Cyclin D1 regulates cell proliferation from G1 phase to S phase. Overexpression of cyclin D1 shortens the time from G1 to S phase and accelerates the transformation of the cell cycle, leading to uncontrolled cell proliferation and migration and finally tumor occurrence [17]. In addition, cyclin D1 is the critical downstream molecule of the FOXO3a signalling pathway, and is negatively correlated with FOXO3a expression (**Fig. 3C, D**). Tudzarova et al. found that FOXO3a activates the ARF−Hdm2−p53−p21 pathway, and p53 in turn activates expression of the Wnt/β-catenin signalling antagonist DKK3, leading to cyclin D1 downregulation [17]. Zheng et al. also found that FOXO transcription factors repress cyclinD1 transcription. Failure to hydroxylate FOXO3a promotes its accumulation in cells, which in turn suppresses cyclin D1expression [47]. Lin et al. found that *FLOT1* knockout inhibited the proliferation and tumorigenicity of breast cancer cells by upregulating FOXO3a and then downregulating cyclin D1 [48]. Meanwhile, the overexpression of EEF1A1 regulates G1-phase progression to promote HCC proliferation through the STAT1-cyclin D1 pathway [49]. STAT1 has been proved to have the same effect as AKT, which can phosphorylate FOXO3a [50]. However, it has not been reported whether the complex formed by DEPDC1 and EEF1A1 in human osteosarcoma weakens the inhibitory effects of FOXO3a on cyclin D1 to ultimately affect tumor progression.

Combined with this study and many previous studies, we first found that the proliferation and migration of osteosarcoma cells are affected through the DEPDC1−EEF1A1−FOXO3a−cyclin D1 signalling

pathway in vitro and in vivo. Therefore, we speculated that in human osteosarcoma cells, high expression of DEPDC1 permits it to form a complex with EEF1A1 and promote the expression of EEF1A1, thus inhibiting the synthesis of FOXO3a. At the same time, FOXO3a translocates from the nucleus and is phosphorylated, which eventually decreases the inhibitory effect of FOXO3a on cyclin D1 and promotes the growth of human osteosarcoma. However, there are many limitations in this study, such as how FOXO3a is degraded after leaving the nucleus and how it affects the expression of downstream cyclin D1 in osteosarcoma, thus inhibiting the progression of osteosarcoma. Further studies are required to elucidate this. In conclusion, our study suggests that the DEPDC1−EEF1A1−FOXO3a−cyclin D1 pathway may be a promising target for the prevention and treatment of human osteosarcoma.

Conclusion

In this study, we deciphered the mechanism of DEPDC1 promoting the development of osteosarcoma, which will provide new therapeutic targets for further development of new anticancer drugs. At the same time, DEPDC1−EEF1A1−FOXO3a axis can accurately predict the clinical characteristics and prognosis of patients, thus providing new methods and strategies for tumor diagnosis and treatment.

Abbreviations

DEPDC1: DEP domain-containing 1; EEF1A1: Eukaryotic translation extension factor 1A1; EMT: Epithelial-mesenchymal transition; FOXO: Forkhead transcription factor; GEO: Gene Expression Omnibus; TCGA: The Cancer Genome Atlas

Declarations

Author contributions

L Shen and H Li performed the experiments, analyzed the data and wrote the manuscript. C Zhou, Y Zhang, K Zhao and L Lu participated in the experiments and manuscript preparation. H Li contributed to the animal model. A Zhang, R Liu, and B Ning conceived the study and participated in manuscript preparation.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

Ethics approval and consent to participate

All the clinical studies were approved by the Institutional Ethical Review Boards of Jinan Central Hospital, and written informed consent was obtained from all patients. All animal experiments were carried out in accordance with the guidelines approved by the Institutional Animal Care and Use Ethics Committee of Shandong University (SYXK20150015).

Consent for publication

All authors have agreed to publish this manuscript.

Competing interests

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. No writing assistance was utilized in the production of this manuscript.

Author details

[1]Department of Orthopaedics, Jinan Central Hospital, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan, Shandong, 250013, China. [2]Department of Endocrine, Jinan Central Hospital, Shandong University, Jinan, Shandong, 250013, China. [3]Department of Pediatrics, Qilu Hospital, Shandong University, Jinan, China.

References

1. J Ritter, SS Bielack: **Osteosarcoma.***Annals of oncology : official journal of the European Society for Medical Oncology* 2010**:**vii320-325.
2. DJ Harrison, DS Geller, JD Gill, VO Lewis, R Gorlick: **Current and future therapeutic approaches for osteosarcoma.***Expert review of anticancer therapy* 2018, **18:**39-50.
3. L Shen, K Zhao, H Li, B Ning, W Wang, R Liu, Y Zhang, A Zhang: **UBE2TDownregulation of can enhance the radiosensitivity of osteosarcoma and.***Epigenomics* 2019, **11:**1283-1305.
4. M Notarbartolo, P Poma, D Perri, L Dusonchet, M Cervello, N D'Alessandro: **Antitumor effects of curcumin, alone or in combination with cisplatin or doxorubicin, on human hepatic cancer cells. Analysis of their possible relationship to changes in NF-kB activation levels and in IAP gene expression.***Cancer letters* 2005, **224:**53-65.

5. B Spee, MD Jonkers, B Arends, GR Rutteman, J Rothuizen, LC Penning: **Specific down-regulation of XIAP with RNA interference enhances the sensitivity of canine tumor cell-lines to TRAIL and doxorubicin.***Molecular cancer* 2006, **5:**34.

6. FM Huang, MY Chou, YC Chang: **Induction of cyclooxygenase-2 mRNA and protein expression by epoxy resin and zinc oxide-eugenol based root canal sealers in human osteoblastic cells.***Biomaterials* 2003, **24:**1869-1875.

7. X Li, S Fan, L Li, L Wang, G Fan, Q Zhao, Y Li: **RNA interference-mediated knockdown of Livin suppresses cell proliferation and invasion and enhances the chemosensitivity to cisplatin in human osteosarcoma cells.***International journal of oncology* 2013, **43:**159-168.

8. L Wang, F Jin, A Qin, Y Hao, Y Dong, S Ge, K Dai: **Targeting Notch1 signaling pathway positively affects the sensitivity of osteosarcoma to cisplatin by regulating the expression and/or activity of Caspase family.***Molecular cancer* 2014, **13:**139.

9. JS Wunder, N Gokgoz, R Parkes, SB Bull, S Eskandarian, AM Davis, CP Beauchamp, EU Conrad, RJ Grimer, JH Healey, et al: **TP53 mutations and outcome in osteosarcoma: a prospective, multicenter study.***Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2005, **23:**1483-1490.

10. DF Lee, J Su, HS Kim, B Chang, D Papatsenko, R Zhao, Y Yuan, J Gingold, W Xia, H Darr, et al: **Modeling familial cancer with induced pluripotent stem cells.***Cell* 2015, **161:**240-254.

11. MF Heymann, HK Brown, D Heymann: **Drugs in early clinical development for the treatment of osteosarcoma.***Expert opinion on investigational drugs* 2016, **25:**1265-1280.

12. GS Hussey, A Chaudhury, AE Dawson, DJ Lindner, CR Knudsen, MC Wilce, WC Merrick, PH Howe: **Identification of an mRNP complex regulating tumorigenesis at the translational elongation step.***Molecular cell* 2011, **41:**419-431.

13. E Selga, C Oleaga, S Ramírez, MC de Almagro, V Noé, CJ Ciudad: **Networking of differentially expressed genes in human cancer cells resistant to methotrexate.***Genome medicine* 2009, **1:**83.

14. SP Tenbaum, P Ordóñez-Morán, I Puig, I Chicote, O Arqués, S Landolfi, Y Fernández, JR Herance, JD Gispert, L Mendizabal, et al: **β-catenin confers resistance to PI3K and AKT inhibitors and subverts FOXO3a to promote metastasis in colon cancer.***Nature medicine* 2012, **18:**892-901.

15. R Kumarswamy, AR Lyon, I Volkmann, AM Mills, J Bretthauer, A Pahuja, C Geers-Knörr, T Kraft, RJ Hajjar, KT Macleod, et al: **SERCA2a gene therapy restores microRNA-1 expression in heart failure via an Akt/FoxO3A-dependent pathway.***European heart journal* 2012, **33:**1067-1075.

16. K Naka, T Hoshii, T Muraguchi, Y Tadokoro, T Ooshio, Y Kondo, S Nakao, N Motoyama, A Hirao: **TGF-beta-FOXO signalling maintains leukaemia-initiating cells in chronic myeloid leukaemia.***Nature* 2010, **463:**676-680.

17. S Tudzarova, MW Trotter, A Wollenschlaeger, C Mulvey, J Godovac-Zimmermann, GH Williams, K Stoeber: **Molecular architecture of the DNA replication origin activation checkpoint.***The EMBO journal* 2010, **29:**3381-3394.

18. W Obara, M Eto, H Mimata, K Kohri, N Mitsuhata, I Miura, T Shuin, T Miki, T Koie, H Fujimoto, et al: **A phase I/II study of cancer peptide vaccine S-288310 in patients with advanced urothelial carcinoma of the bladder.***Annals of oncology : official journal of the European Society for Medical Oncology* 2017, **28:**798-803.

19. Y Harada, M Kanehira, Y Fujisawa, R Takata, T Shuin, T Miki, T Fujioka, Y Nakamura, T Katagiri: **Cell-permeable peptide DEPDC1-ZNF224 interferes with transcriptional repression and oncogenicity in bladder cancer cells.***Cancer research* 2010, **70:**5829-5839.

20. C Kretschmer, A Sterner-Kock, F Siedentopf, W Schoenegg, PM Schlag, W Kemmner: **Identification of early molecular markers for breast cancer.***Molecular cancer* 2011, **10:**15.

21. H Okayama, T Kohno, Y Ishii, Y Shimada, K Shiraishi, R Iwakawa, K Furuta, K Tsuta, T Shibata, S Yamamoto, et al: **Identification of genes upregulated in ALK-positive and EGFR/KRAS/ALK-negative lung adenocarcinomas.***Cancer research* 2012, **72:**100-111.

22. K Spring, C Chabot, S Langlois, L Lapointe, NT Trinh, C Caron, JK Hebda, J Gavard, M Elchebly, I Royal: **Tyrosine phosphorylation of DEP-1/CD148 as a mechanism controlling Src kinase activation, endothelial cell permeability, invasion, and capillary formation.***Blood* 2012, **120:**2745-2756.

23. L Zhang, Y Du, S Xu, Y Jiang, C Yuan, L Zhou, X Ma, Y Bai, J Lu, J Ma: **DEPDC1, negatively regulated by miR-26b, facilitates cell proliferation via the up-regulation of FOXM1 expression in TNBC.***Cancer letters* 2019, **442:**242-251.

24. LD Kapp, JR Lorsch: **The molecular mechanics of eukaryotic translation.***Annual review of biochemistry* 2004, **73:**657-704.

25. E Behrmann, J Loerke, TV Budkevich, K Yamamoto, A Schmidt, PA Penczek, MR Vos, J Bürger, T Mielke, P Scheerer, CM Spahn: **Structural snapshots of actively translating human ribosomes.***Cell* 2015, **161:**845-857.

26. CB Andersen, T Becker, M Blau, M Anand, M Halic, B Balar, T Mielke, T Boesen, JS Pedersen, CM Spahn, et al: **Structure of eEF3 and the mechanism of transfer RNA release from the E-site.***Nature* 2006, **443:**663-668.

27. S Liu, S Hausmann, SM Carlson, ME Fuentes, JW Francis, R Pillai, SM Lofgren, L Hulea, K Tandoc, J Lu, et al: **METTL13 Methylation of eEF1A Increases Translational Output to Promote Tumorigenesis.***Cell* 2019, **176:**491-504.e421.

28. J Tayou, Q Wang, GF Jang, AN Pronin, C Orlandi, KA Martemyanov, JW Crabb, VZ Slepak: **Regulator of G Protein Signaling 7 (RGS7) Can Exist in a Homo-oligomeric Form That Is Regulated by Gαo and R7-binding Protein.***The Journal of biological chemistry* 2016, **291:**9133-9147.

29. PV Lishko, KA Martemyanov, JA Hopp, VY Arshavsky: **Specific binding of RGS9-Gbeta 5L to protein anchor in photoreceptor membranes greatly enhances its catalytic activity.***The Journal of biological chemistry* 2002, **277:**24376-24381.

30. H Jonsson, SL Peng: **Forkhead transcription factors in immunology.***Cellular and molecular life sciences : CMLS* 2005, **62:**397-409.

31. Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma.*Cell* 2017, **169:**1327-1341.e1323.

32. Z Mao, L Liu, R Zhang, X Li: **Lithium reduces FoxO3a transcriptional activity by decreasing its intracellular content.***Biological psychiatry* 2007, **62:**1423-1430.

33. H Tran, A Brunet, JM Grenier, SR Datta, AJ Fornace, PS DiStefano, LW Chiang, ME Greenberg: **DNA repair pathway stimulated by the forkhead transcription factor FOXO3a through the Gadd45 protein.***Science (New York, NY)* 2002, **296:**530-534.

34. Y Liu, X Ao, W Ding, M Ponnusamy, W Wu, X Hao, W Yu, Y Wang, P Li, J Wang: **Critical role of FOXO3a in carcinogenesis.***Molecular cancer* 2018, **17:**104.

35. C Hu, Z Ni, BS Li, X Yong, X Yang, JW Zhang, D Zhang, Y Qin, MM Jie, H Dong, et al: **hTERT promotes the invasion of gastric cancer cells by enhancing FOXO3a ubiquitination and subsequent ITGB1 upregulation.***Gut* 2017, **66:**31-42.

36. KL Tsai, YJ Sun, CY Huang, JY Yang, MC Hung, CD Hsiao: **Crystal structure of the human FOXO3a-DBD/DNA complex suggests the effects of post-translational modification.***Nucleic acids research* 2007, **35:**6984-6994.

37. I Kapoor, Y Li, A Sharma, H Zhu, J Bodo, W Xu, ED Hsi, BT Hill, A Almasan: **Resistance to BTK inhibition by ibrutinib can be overcome by preventing FOXO3a nuclear export and PI3K/AKT activation in B-cell lymphoid malignancies.***Cell death & disease* 2019, **10:**924.

38. A Fluckiger, A Dumont, V Derangère, C Rébé, C de Rosny, S Causse, C Thomas, L Apetoh, A Hichami, F Ghiringhelli, M Rialland: **Inhibition of colon cancer growth by docosahexaenoic acid involves autocrine production of TNFα.***Oncogene* 2016, **35:**4611-4622.

39. K Levanon, S Sapoznik, K Bahar-Shany, H Brand, R Shapira-Frommer, J Korach, MS Hirsch, MH Roh, A Miron, JF Liu, et al: **FOXO3a loss is a frequent early event in high-grade pelvic serous carcinogenesis.***Oncogene* 2014, **33:**4424-4432.

40. MC Hu, DF Lee, W Xia, LS Golfman, F Ou-Yang, JY Yang, Y Zou, S Bao, N Hanada, H Saso, et al: **IkappaB kinase promotes tumorigenesis through inhibition of forkhead FOXO3a.***Cell* 2004, **117:**225-237.

41. J George, JS Lim, SJ Jang, Y Cun, L Ozretić, G Kong, F Leenders, X Lu, L Fernández-Cuesta, G Bosco, et al: **Comprehensive genomic profiles of small cell lung cancer.***Nature* 2015, **524:**47-53.

42. M Buchner, E Park, H Geng, L Klemm, J Flach, E Passegué, H Schjerven, A Melnick, E Paietta, D Kopanja, et al: **Identification of FOXM1 as a therapeutic target in B-cell lineage acute lymphoblastic leukaemia.***Nature communications* 2015, **6:**6471.

43. S Yao, LY Fan, EW Lam: **The FOXO3-FOXM1 axis: A key cancer drug target and a modulator of cancer drug resistance.***Seminars in cancer biology* 2018, **50:**77-89.

44. W Abbas, A Kumar, G Herbein: **The eEF1A Proteins: At the Crossroads of Oncogenesis, Apoptosis, and Viral Infections.***Frontiers in oncology* 2015, **5:**75.

45. KW Lin, I Yakymovych, M Jia, M Yakymovych, S Souchelnytskyi: **Phosphorylation of eEF1A1 at Ser300 by TβR-I results in inhibition of mRNA translation.***Current biology : CB* 2010, **20:**1615-1625.

46. G Giamas, A Filipović, J Jacob, W Messier, H Zhang, D Yang, W Zhang, BA Shifa, A Photiou, C Tralau-Stewart, et al: **Kinome screening for regulators of the estrogen receptor identifies LMTK3 as a new therapeutic target in breast cancer.***Nature medicine* 2011, **17:**715-719.

47. X Zheng, B Zhai, P Koivunen, SJ Shin, G Lu, J Liu, C Geisen, AA Chakraborty, JJ Moslehi, DM Smalley, et al: **Prolyl hydroxylation by EglN2 destabilizes FOXO3a by blocking its interaction with the USP9x deubiquitinase.***Genes & development* 2014, **28:**1429-1444.

48. C Lin, Z Wu, X Lin, C Yu, T Shi, Y Zeng, X Wang, J Li, L Song: **Knockdown of FLOT1 impairs cell proliferation and tumorigenicity in breast cancer through upregulation of FOXO3a.***Clinical cancer research : an official journal of the American Association for Cancer Research* 2011, **17:**3089-3099.

49. J Huang, C Zheng, J Shao, L Chen, X Liu, J Shao: **Overexpression of eEF1A1 regulates G1-phase progression to promote HCC proliferation through the STAT1-cyclin D1 pathway.***Biochemical and biophysical research communications* 2017, **494:**542-549.

50. de Mel Sanjay, Hue Susan Swee-Shan, Jeyasekharan Anand D., Chng Wee-Joo, Ng Siok-Bian: **Molecular pathogenic pathways in extranodal NK/T cell lymphoma.***Journal of hematology & oncology* 2019, **12:**33.

Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.
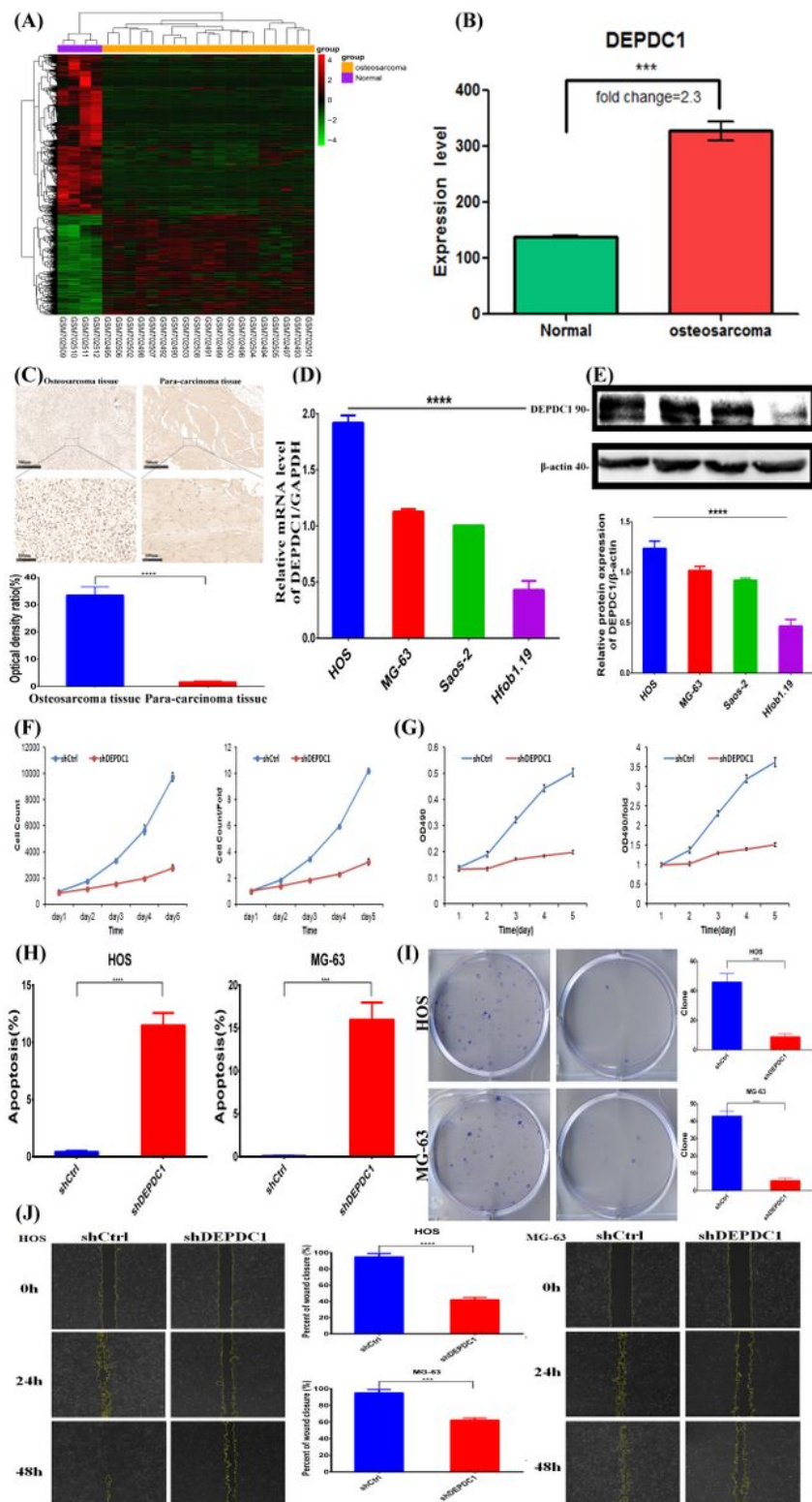
Figures

Figure 1

DEPDC1 is highly expressed in GEO database and human osteosarcoma tissues and cells, meanwhile, down-regulation of DEPDC1 significantly inhibits the growth, metastasis and promoted the apoptosis of osteosarcoma cells. A, B Heat map and bar chart showing the expression levels of DEPDC1 across the human osteosarcoma among GEO database. C Representative immunohistochemical (IHC) staining of DEPDC1 protein expression classified in osteosarcoma patient tissues. Scale bar, 100 μm. D, E RT-PCR

and Western blot analysis of DEPDC1 expression in osteosarcoma cells and normal human osteoblasts. F, G Celigo cytometer detection and MTT assays showed that knockdown of DEPDC1 expression in HOS cells significantly suppressed the cell proliferation. H Flow cytometry was used to determine the apoptosis percentages of each group. I Cell clone formation of HOS and MG-63 cell lines after knockdown of DEPDC1. J The cell scratch assay was used to examine the metastatic abilities of HOS and MG-63 osteosarcoma cells after inhibiting the expression of DEPDC1. ***p<0.001,****p<0.001.
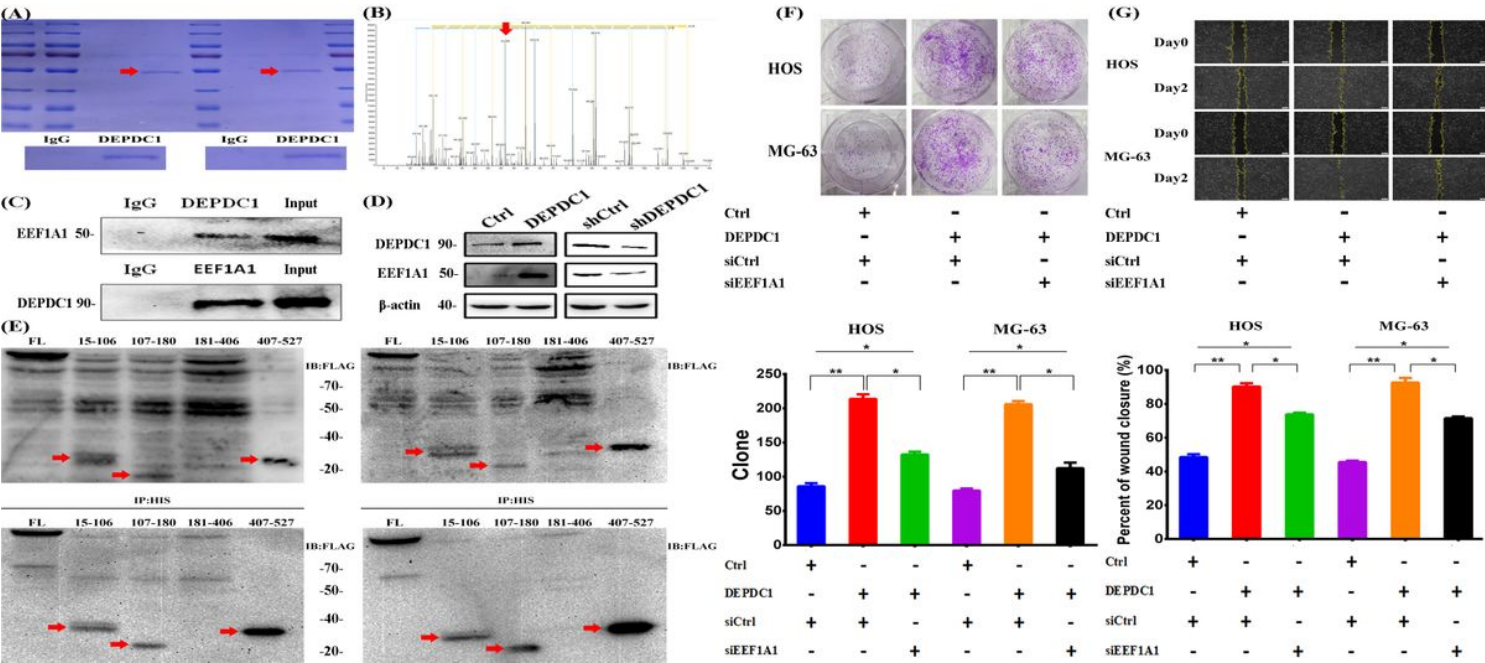
Figure 2

DEPDC1 enhances the growth and metastasis of osteosarcoma cells by binding to the extension factor 1-alpha 1 (EEF1A1) and promoting the expression of EEF1A1. A Coomassie blue staining of IgG and DEPDC1 in HOS and MG63 cells. The differential protein expression molecular weight is 55kda. B Adapted image of a mass spectra for EEF1A1. C Co-immunoprecipitation (Co-IP) of DEPDC1 and EEF1A1 in osteosarcoma cells. D Western blot analysis of the correlation between DEPDC1 and EEF1A1 after up-regulation and down-regulation of the DEPDC1 gene in osteosarcoma cells. E Co-IP assay to detect the binding site of DEPDC1 to EEF1A1 in 293T cells and HOS cells. Plasmids of FLAG-tagged DEPDC1 and its N-terminal deletion mutants or C-terminal deletion mutants and His-tagged EEF1A1 were co-transfected. Cell lysates were immunoprecipitated with His antibody and probed with FLAG antibody. Expression of FLAG-tagged N-terminal deletion or C-terminal deletion mutants of DEPDC1 probed by FLAG antibody after transfection for 48h (top). Co-IP assay immunoprecipitated (bottom) with His antibody and probed with FLAG antibody. The binding regions of DEPDC1 and EEF1A1 were 15-106,107-180 and 407-527. F,G Cell clone formation and cell scratch assay were used to examine the proliferation and metastatic abilities of HOS and MG-63 osteosarcoma cells, while knockdown of EEF1A1 after DEPDC1 overexpression.*p<0.05, **p<0.01.
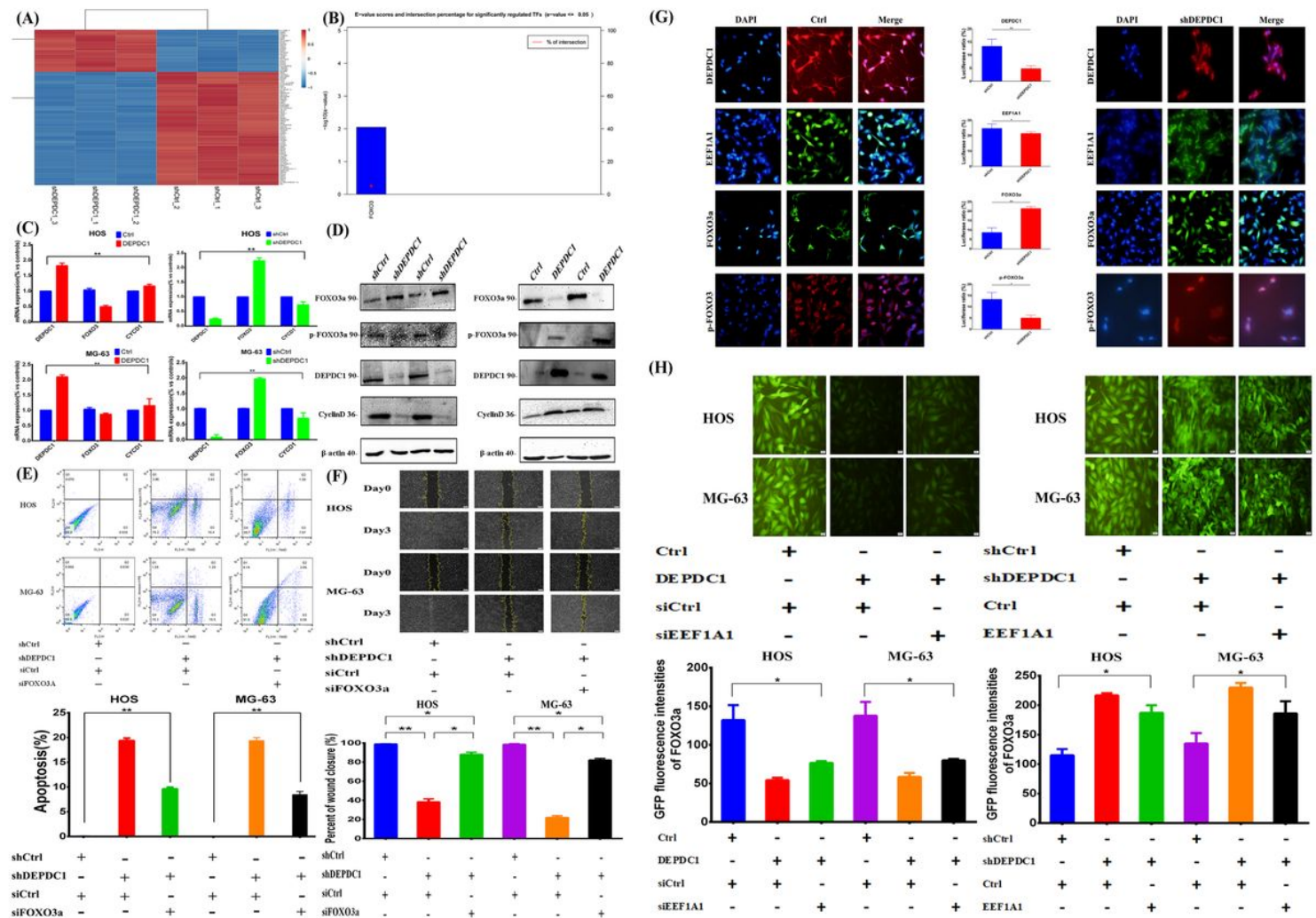
Figure 3

Down-regulation of DEPDC1 inhibits the metastasis and promotes the apoptosis of osteosarcoma cells by up-regulating forkhead box O3 activation (FOXO3a). A Heat map of differential mRNA clustering microarray analysis in osteosarcoma cells after down-regulation of DEPDC1. B E−value scores and intersection percentage for significantly regulated TFs showed that FOXO3a was the best candidate molecule for the downstream regulation of DEPDC1. C, D Western blot analysis and Real-time PCR were used to analyze the correlation between DEPDC1 and FOXO3a pathway after up-regulation and down-regulation of DEPDC1 gene in HOS and MG-63 cells. After down-regulating DEPDC1, FOXO3a was down-regulated in HOS and MG-63 osteosarcoma cells for flow cytometry of apoptosis (E) and cell scratch assay (F). G Immunofluorescence staining of DEPDC1, EEF1A1, FOXO3a and p-FOXO3 in Ctrl group and shDEPDC1 group. H The GFP fluorescence intensity of FOXO GFP Reporter Plasmid regulated by DEPDC1 and EEF1A1. *p<0.05, **p<0.01.
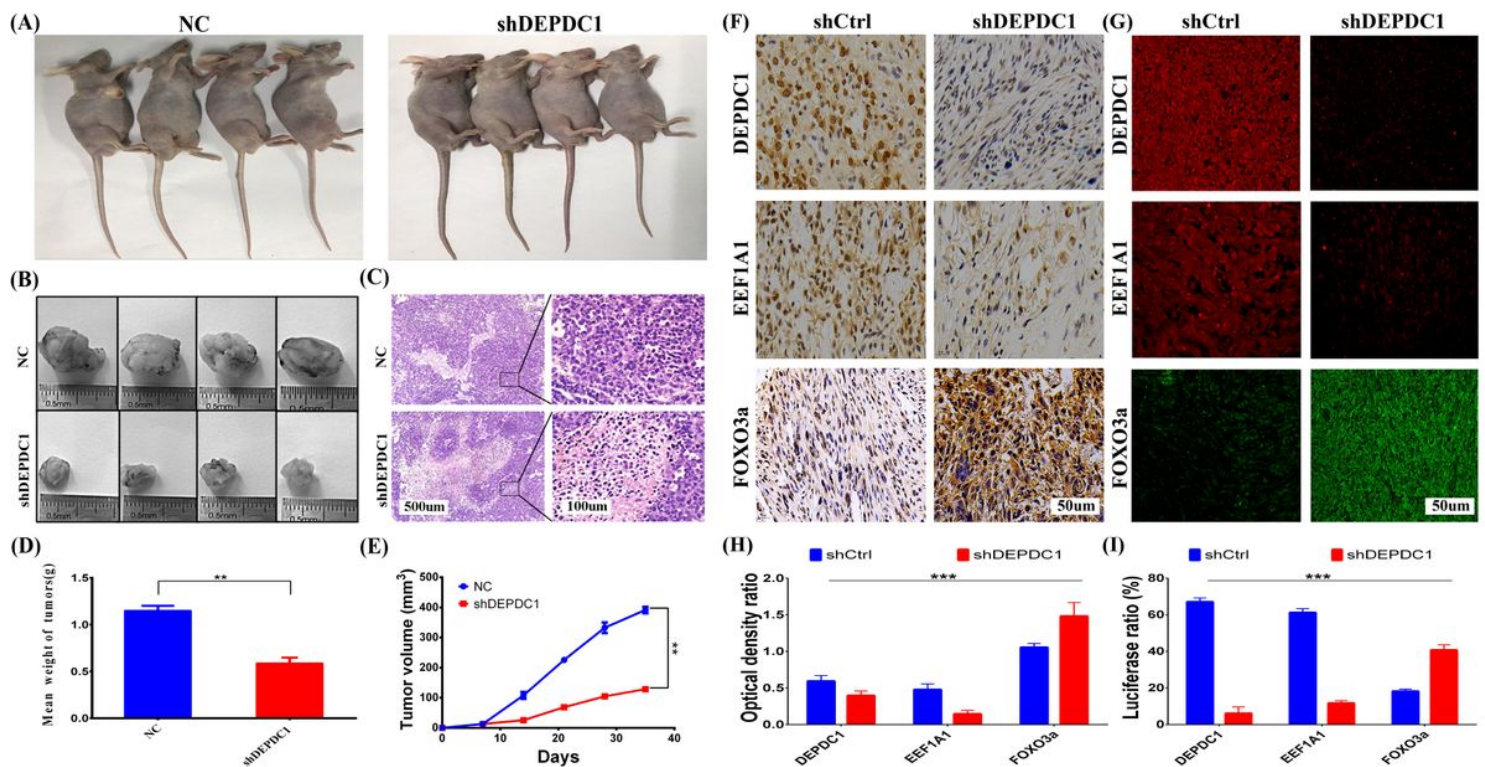
Figure 4

Down-regulation of DEPDC1 inhibits the growth of osteosarcoma cells in vivo. A The sizes of xenograft tumors in NC group and shDEPDC1group. B Representative primary tumor volumes and tumor images are shown. C Representative H&E staining images of the two groups. Scale bar, 100 μm. Mean tumor weight (D) and tumor volume (E) of NC group and shDEPDC1group. F Representative IHC image of xenograft tumors for DEPDC1, EEF1A1 and FOXO3a in shCtrl group and shDEPDC1group. G The immunofluorescent staining of DEPDC1, EEF1A1 and FOXO3a in the two groups. H Quantitative results were derived from (F), using Image J software. I Quantitative results were derived from (G) using Image J software. **p<0.01, ***p<0.001.
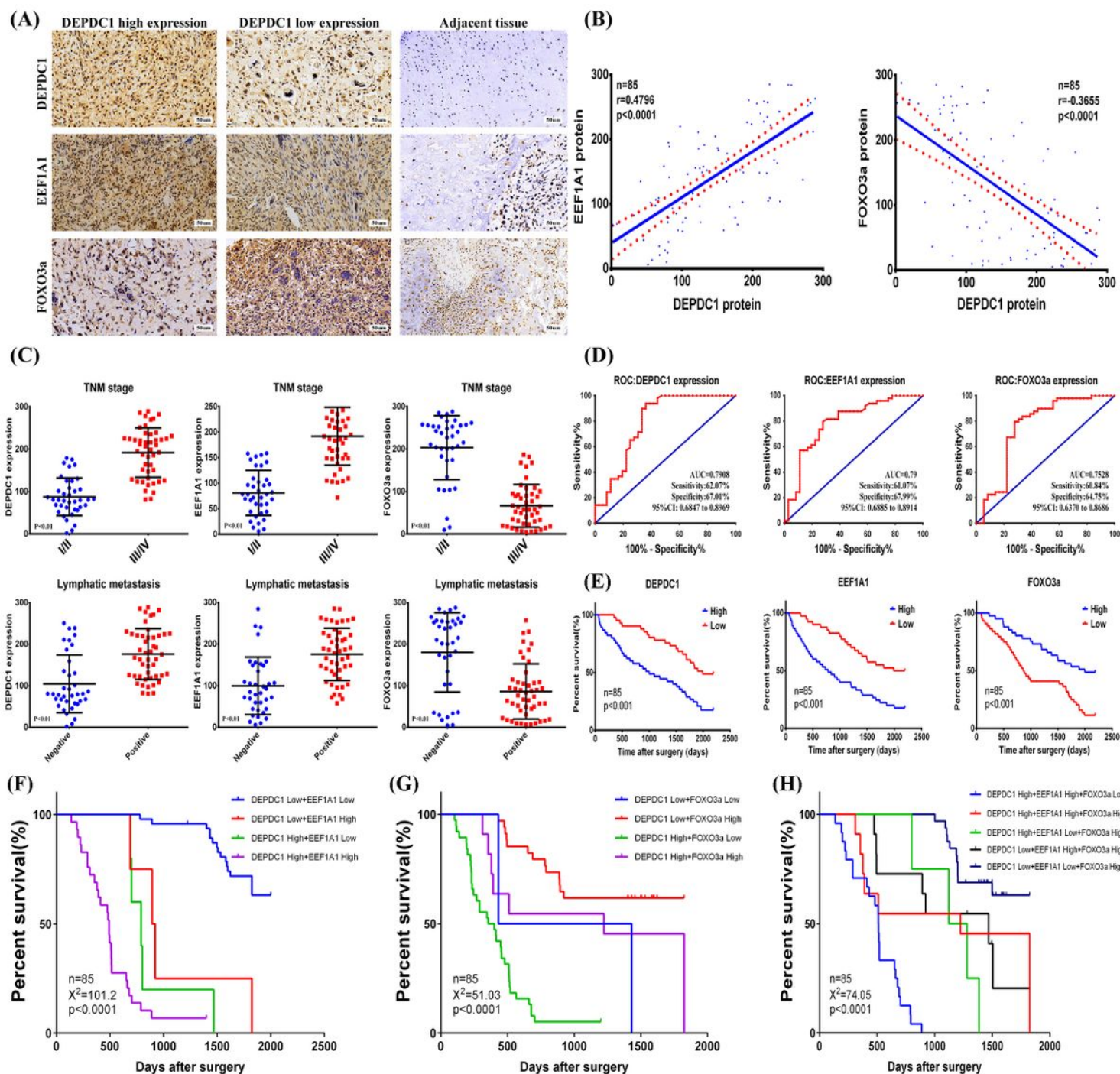
Figure 5

The expression of DEPDC1 in human osteosarcoma is consistent with EEF1A1, but negatively correlated with FOXO3a. A Representative immunohistochemical staining image of DEPDC1, EEF1A1, FOXO3a in osteosarcoma and related adjacent non-cancerous tissues. B The correlation between the protein levels of DEPDC1 and EEF1A1 (left). The correlations between the protein levels of DEPDC1 and FOXO3a (right) (p<0.0001). C The association between DEPDC1/ EEF1A1/ FOXO3a expression and TNM stage/ lymphatic metastasis in patients with osteosarcoma (p<0.01). D The receiver operating characteristic (ROC) curves for predicting patients' survival time using DEPDC1/ EEF1A1/ FOXO3a expression. E Kaplan−Meier analyses of overall survival according to DEPDC1/ EEF1A1/ FOXO3a expression levels

(p<0.01). AUC, area under the curve; TNM, tumor node metastases. F, G Kaplan-Meier analyses of overall survival time combining two indices (p<0.0001). H Kaplan-Meier analyses of overall survival time combining three indices (p<0.0001).
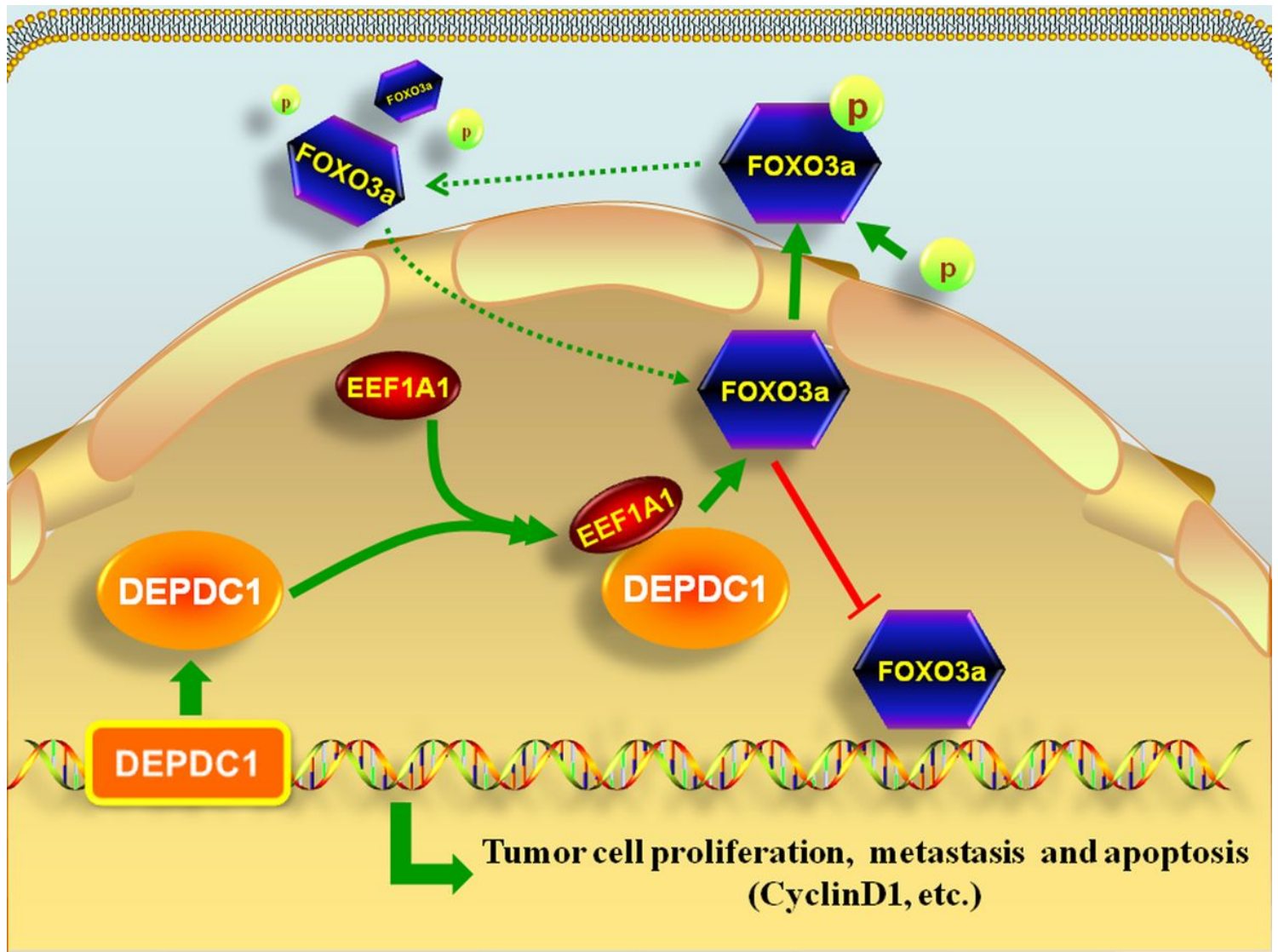

Figure 6

A proposed model of the signaling pathway for DEPDC1 regulating the human osteosarcoma proliferation, metastasis and apoptosis. In this model, DEPDC1 improves the expression of EEF1A1 and enhances the binding to EEF1A1, thus form a complex to inhibit the FOXO3a which could block tumor progression. This process ultimately promotes the proliferation, migration and inhibits the apoptosis of osteosarcoma.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table.S3Osteosarcomapatientinformation.xlsx

- Table.S2GeneexpressionofosteosarcomainGEOdatabase..xls
- Table.S1Constructionofplasmidsorlentivirus..xlsx
- SupplementaryMethod.2FOXO3aGFPReporterPlasmid.docx
- SupplementaryMethod.1Tissueimmunofluorescentstaining.doc
- Additionalfile1MaterialsandMethods.docx
- Table.1Proteinidentificationlistofsamples.pdf