Clinical significance and potential mechanisms of the RNA methyltransferase KIAA1429 in osteosarcoma

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Abstract

Background: KIAA1429, a member of the RNA methyltransferase complex, is involved in cancer progression. However, the clinical significance of KIAA1429 in osteosarcoma (OS) and the underlying mechanisms by which it contributes to disease progression remain unclear.

Methods: The clinical significance of KIAA1429 in OS was evaluated based on the RT-qPCR, microarray, RNA sequencing, and published data. Two lentivirus-mediated KIAA1429-targeting siRNA constructs were transfected into SW1353 cells, and CCK-8 and flow cytometry assays were applied to investigate the biological function of KIAA1429 in OS cells. KIAA1429-related genes in OS were identified from lists of co-expressed genes (CEGs) and differentially expressed genes (DEGs). Functional enrichment analysis was employed to explore the potential mechanisms by which KIAA1429 contributes to the progression of OS.

Results: The mRNA expression of KIAA1429 was notably overexpressed in 250 OS samples than 71 non-cancer samples (SMD=0.74). SROC curve analysis showed that KIAA1429 exhibits diagnostic capacity between OS samples and non-cancer samples (AUC=0.83). Survival analysis showed that overexpression of KIAA1429 was associated with shorter overall survival time. Knocking down KIAA1429 reduced the level of m6A methylation, inhibited proliferation, and accelerated apoptosis in OS cells. A total of 395 KIAA1429-related genes were identified, and were enriched in the cell cycle pathway. Protein–protein interaction (PPI) network analysis showed that CDK1, CCNA2, and CCNB1 were KIAA1429-related genes that act as major network hubs in OS.

Conclusion: KIAA1429 plays an oncogenic role in OS and may facilitate the progression of OS through a mechanism involving the regulation of CDK1, CCNA2, and CCNB1.

Background

Osteosarcoma (OS) is the most common primary malignant bone tumor derived from mesenchymal tissue, and accounts for approximately 35% of primary malignant bone tumors [1]. OS occurs predominantly in the metaphysis of long bones, and the average annual incidence of OS is about 4 cases per million [2]. While OS occurs in patients of all ages, it is more likely to occur in adolescents and is the third deadliest tumor among adolescents [3]; it is characterized by high metastatic potential, rapid progression, and poor prognosis. Despite advances in neoadjuvant chemotherapy and surgical techniques, OS generally remains insensitive to common adjuvant treatment methods, such as radiotherapy and chemotherapy [4, 5]. In the past 30 years, the survival rate of patients with OS has not improved [6]. Therefore, there is an urgent need to better understand the molecular mechanisms of OS development and to identify novel therapeutic targets.

In eukaryotes, N6-methyladenosine (m6A) is the commonest post-transcriptional modification of RNA [7]. M6A modification is mainly impacted by three types of proteins, including methyltransferase, demethylase, and m6A binding protein [8]. Proteins that modify or regulate m6A can accelerate the proliferation, migration, and metastasis of malignant tumor cells. For example, METTL3 was shown to

post-transcriptionally silence SOCS2 expression through a YTHDF2-dependent pathway to promote liver cancer progression [9]. Ma *et al.* found that METT14 affected the metastasis of liver cancer by mediating the maturation of miRNA-126 [10]. Zhou *et al.* reported that the FTO expression levels were notably increased in cervical squamous cell carcinoma, and elevated FTO was associated with poorer clinical prognosis [11]. Li *et al.* found that FTO promoted progression of leukemia by reducing ASB2 and RARA m6A levels [12]. ALKBH5 was found to maintain the tumorigenicity of glioma stem cells by regulating FOXM1 expression [13]. ALKBH5 promotes OS progression by upregulating PVT1. METTL3 promotes OS growth by upregulating ATAD2 through m6A methylation modification. Additionally, METTL3 functions as an oncogene in OS by upregulating DRG1 and promoting progression of OS.

KIAA1429 is a subunit component of the m6A methyltransferase complex, and participates in the modification of m6A residues in RNA [14]. In human A549 cells, deletion of KIAA1429 significantly reduced the maximum levels of m6A in RNA, indicating that KIAA1429 plays an important role in mediating m6A methyltransferase activity [15]. KIAA1429 has also been shown to participate in tumor progression through both m6A-dependent and m6A-independent mechanisms [16-19]. In OS, only one study reported that KIAA1429 is regulated by miR-143-3p and promotes OS progression [20]. However, the clinical significance of the KIAA1429 RNA methyltransferase in OS and the potential mechanisms by which it contributes to OS progression remain incompletely understood.

In present study, we comprehensively assessed the clinical significance of RNA methyltransferase KIAA1429 and investigated its biological role in OS. Our research shows that KIAA1429 is significantly increased in OS and plays an oncogenic role in the progression of OS. Furthermore, functional enrichment analysis identified a potential biological mechanism through which KIAA1429 contributes to OS, and indicated that KIAA1429 may contribute to OS disease pathogenesis by regulating CDK1, CCNA2, and CCNB2.

Materials And Methods

Clinical samples

OS tissue and adjacent non-cancer tissue samples were collected from patients (n = 30) who underwent surgical resection at the First Affiliated Hospital of Guangxi Medical University. No patient received chemo- or radiotherapy before surgery.

Ethical approval

Informed consent was obtained from all patients. This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University.

Date collection

OS gene expression data were retrieved from the following databases: Gene Expression Omnibus (GEO), ArrayExpress, and Sequence Read Archive (SRA). The search terms used were: (bone OR bones) OR

(osteosarcoma OR osteosarcomas). The data inclusion criteria were: (1) human samples; (2) the study included an OS group and a non-cancer group; (3) the study included KIAA1429 expression data. We obtained KIAA1429 expression data for OS samples and corresponding clinical data from the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) database. In addition, microarray, RNA sequencing, and published studies including KIAA1429 expression and prognostic clinical data were used to assess the prognostic value of KIAA1429 in OS.

Cell culture and lentivirus infection

Human OS cell lines MG-63, Saos2, and SW1353, and osteoblast cell line hFOB1.19 were obtained from the Cell Bank of the Chinese Academy of Science (Shanghai, China). Cells were cultured in Roswell Park Memorial Institute-1640 medium containing 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin (Solarbio) in humidified air (containing 5% CO_2) at 37°C. Two lentivirus-mediated si-KIAA1429 siRNA constructs (si-KIAA1429-1, CAGUGAUGUUCAAAUGCUAGA; si-KIAA1429-2, GGAAGAACCAAGACUACUAAA) and a negative control siRNA construct (si-NC) were synthesized by Shanghai Genechem Company Ltd. SW1353 cells were infected with lentiviral particles, and the infected cells were selected with 3 μg/ml puromycin.

RT-qPCR

Total RNA was extracted using TRIzol (Invitrogen, USA). Synthesis of cDNA was performed using a One-Step RT-PCR Kit (Thermo Fisher, USA). Real-time PCR assay was performed using an ABI Vii7 system (Applied Biosystems, USA). GAPDH mRNA expression was used as a reference gene. The primers synthesized were: KIAA1429-hF GTTGTGCCACCACCAAGAGG and KIAA1429-hR AACCCACCACGGGAAGAAAT; GAPDH-hF TGACAACTTTGGTATCGTGGAAGG and GAPDH-hR AGGCAGGGATGATGTTCTGGAGAG. Relative expression data were calculated using the $2^{-\Delta\Delta Ct}$ method.

Western blot protein analysis

Western blotting was performed as described [21] with antibodies against KIAA1429 and GAPDH (Santa Cruz Biotechnology, USA); the latter was used as an endogenous control to normalize expression values of KIAA1429.

M6A RNA methylation quantification

EpiQuik m6A RNA Methylation Quantification Kit (Colorimetric) was used to assess m6A methylation levels, as previously described [22].

Cell proliferation assay

Cell counting kit-8 assay (CCK-8, Beyotime, Shanghai, China) was used to measure the cell proliferation. Briefly, SW1353 cells (5×10^3 cells/well) were seeded into 96-well plates, incubated overnight, and treated

with control, si-NC, si-KIAA1429-1, or si-KIAA1429-2 for 0, 24, 48, 72, and 92 h. Then, cells were incubated with CCK-8 reagent (10 μL) for 2 h at 37°C and the absorbance was measured at 450 nm.

Apoptosis assay

An Annexin V-FITC Apoptosis Detection Kit (Becton Dickinson San Jose, CA) was used. After treatment, 5×10^5cells were pelleted by centrifugation, resuspended in binding buffer (200 μl), and incubated with 5 μl fluorescein isothiocyanate-Annexin V and 1 μl propidium iodide (PI) solution for 30 min at room temperature. Cells were detected using a Calibur Flow Cytometer (BD); apoptotic cells stained positive for Annexin V and negative for PI.

Identification of KIAA1429-related genes

KIAA1429-related genes were identified from co-expressed genes (CEGs) and differentially expressed genes (DEGs) in OS. To define genes as CEGs, we estimated the relationships between expression of genes and KIAA1429 expression in the microarray and RNA sequencing datasets; we considered genes with |Pearson's r| $\geq$ 0.3 and $P < 0.05$ as KIAA1429 CEGs. DEGs were defined using the Limma-Voom package, based on microarray and RNA sequencing datasets; $P < 0.05$ and |log2FC| > 1 were established as screening criteria. Genes fitting within the criteria in both screens from the CEGs and the DEGs were considered to be KIAA1429-related genes.

Bioinformatics analysis

The Database for Annotation, Visualization, and Integrated Discovery (DAVID) database was used to perform enrichment analysis for Gene Ontology (GO) functions and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched in the KIAA1429-related genes. The Search Tool for the Retrieval of Interacting Genes (STRING) database and Cytoscape software were used to construct the protein–protein interaction (PPI) network of KIAA1429-related genes. Hub related genes were screened based on the degree of connectivity among KIAA1429-related genes.

Validation of hub related genes

Firstly, the expression of hub related genes in OS was evaluated by analyzing microarray and mRNA sequencing data. Secondly, the diagnostic potential of hub related genes was evaluated by a summarized receiver operating characteristic (SROC) curve. Finally, the prognostic value of hub related genes was analyzed based on the microarray, RNA sequencing, and published data.

Statistical analysis

Independent t-tests were employed to assess the expression differences of KIAA1429 between two independent groups using SPSS22.0. Receiver operating characteristic (ROC) curves were applied to determine the sensitivity and specificity of KIAA1429 in each study. Stata12.0 was applied to merge standardized mean difference (SMD), SROC, hazard radio (HR) and its 95% confidence interval (95% CI).

Cochran's Q test and I^2 index were employed to evaluate the heterogeneity between the included studies. If $P < 0.05$ or $I^2 > 50\%$, there was significant heterogeneity between the included studies, and a random-effects model was applied; otherwise, a fixed-effects model was used. Sensitivity analysis was employed to assess the robustness of each study included in the meta-analysis. Begg's test and Egger's test were used to evaluate whether included studies exist publication bias. A p-value of $P < 0.05$ was considered to be statistically significant. A flow chart outlining this study is shown in Fig. 1.

Results

Expression of KIAA1429 in OS

KIAA1429 expression was significantly higher in 30 OS clinical samples vs. 30 non-cancer clinical samples, as indicated by RT-PCR (Fig. 2a). ROC curve analysis demonstrated that KIAA1429 has the capability to distinguish between OS samples and non-cancer samples (Fig. 3a). Five studies met the inclusion criteria for the ROC analysis. In three studies (GSE99671, GSE126209, and GSE42352), KIAA1429 was significantly overexpressed in OS samples; in one study (GSE19276), KIAA1429 was significantly lower in OS samples; in the last study (GS87624), there was no notable difference in KIAA1429 expression between OS and non-cancer samples (Fig. 2b-f). The potential of KIAA1429 to distinguish OS samples from non-cancer samples in each study is shown in Fig. 3b-f.

Clinical significance of KIAA1429 in OS

RT-qPCR, microarray, and RNA sequencing data were acquired for a total of 250 OS samples and 71 non-cancer samples (Table 1). KIAA1429 expression was notably increased in OS samples than non-cancer samples, as indicated by the SMD = 0.67 (0.07-0.68) obtained from the random effects model (Fig. 4a). Sensitivity analysis showed that no studies had a significant impact on the entire study cohort (Fig. 4b); Begg's test and Egger's test results suggested that no publication bias was observed ($P > 0.05$) (Fig. 4c). The area under the curve (AUC) value of the SROC curve was 0.83, showing that KIAA1429 demonstrated good diagnostic capacity to distinguish between OS samples and non-cancer samples (Fig. 4d), and no publication bias was observed (Fig. 4e). The relationship between KIAA1429 expression and clinical features in patients with OS was analyzed using data from the TARGET database. Higher expression of KIAA1429 was significantly associated with disease recurrence, but not with gender, age, primary location, disease metastasis, or survival status (Table 2). Survival analysis results showed that patients with high KIAA1429 expression had a shorter overall survival time [HR, 1.94 (1.24, 3.04)] (Fig. 4f).

Silencing KIAA1429 reduces m6A methylation levels, inhibits cell proliferation, and promotes apoptosis in OS cells

The expression of KIAA1429 in OS cells was notably increased than in osteoblast cells (Fig. 5a). To explore the function of KIAA1429 in OS, we knocked down the expression of KIAA1429 in the SW1353 cell line, which had the highest expression of KIAA1429 among three OS cell lines we assessed. RT-qPCR and western blot analysis demonstrated that both siRNA constructs, si-KIAA1429-1 and si-KIAA1429-2,

exhibited good KIAA1429 knockdown efficiency (Fig. 5b-c). Silencing KIAA1429 reduced m6A methylation levels in SW1353 cells (Fig. 5d). Knocking down KIAA1429 significantly inhibited the proliferation SW1353 cells, as determined by CCK-8 assays (Fig. 5e). Apoptotic cells in the both si-KIAA1429-1 and si-KIAA1429-2 treated groups were significantly increased compared with the si-NC group (Fig. 5f).

Bioinformatics analysis of genes related to KIAA1429

A total of 5924 CEGs and 2920 DEGs related to KIAA1429 were identified in OS; 395 genes represented the intersection between both gene lists, and were collectively termed the KIAA1429-related genes (Fig. 6a). GO enrichment analysis of the 395 KIAA1429-related genes demonstrated that these genes were enriched most significantly in cellular response to DNA damage stimulus, nucleoplasm, and RNA binding (Table 3) (Fig. 7a-c). KEGG pathway analysis results suggested that the cell cycle pathway was most significantly enriched pathway among the KIAA1429-related genes (Table 4) (Fig. 7d). Three genes, CDK1, CCNA2, and CCNB1, were screened as hub related genes in OS using PPI network analysis (Fig. 6b).

Validation of hub related genes

By integrating microarray and RNA sequencing datasets (Table 5), we found that CDK1, CCNA2, and CCNB1 were significantly upregulated in OS samples than non-cancer samples (Fig. 8a, 8c, and 8e). The SROC curve analysis shows that CDK1, CCNA2, and CCNB1 have a good capability to distinguish between OS samples and non-OS samples (Fig. 8b, 8d, and 8f). Survival analysis showed that overexpression of CCNA2 was associated with shorter overall survival time, and that expression of CDK1 and CCNB1 was not associated with overall survival outcomes in OS (Fig. 9).

Discussion

KIAA1429, a member of the RNA methyltransferase complex, is involved in the tumorigenesis and development of a variety of cancers. Lan *et al.* reported that KIAA1429 promotes the progression of liver cancer by reducing GATA3 expression through an m6A-dependent pathway [16]. Cheng *et al.* found that KIAA1429 upregulates the m6A modification levels of ID2 mRNA, leading to inhibition of ID2 mRNA expression and promoting the metastasis of liver cancer [17]. In breast cancer, KIAA1429 was shown to upregulate the expression of CDK1, thereby promoting the proliferation of breast cancer cells [18]. KIAA1429 is also involved in the progression of gastric cancer, where it upregulates the expression of c-Jun in an m6A-independent manner [19]. The only notable study of KIAA1429 in OS reported that KIAA1429 expression was higher in OS lesions compared to matched non-cancerous tissue [20]. However, that study involved a small sample size (n = 30), and the observations required confirmation in a larger study including many more OS samples. In the present study, we analyzed 321 samples from six studies, and we found that KIAA1429 expression is significantly elevated in OS. Moreover, we observed that overexpression of KIAA1429 is related to poorer disease prognosis for patients with OS. Furthermore, we demonstrated that silencing KIAA1429 reduced m6A methylation levels, inhibited proliferation, and promoted apoptosis in OS cells. These results suggested that KIAA1429 plays a role as an oncogene in OS, and is a potential prognostic biomarker of poorer clinical outcomes.

To further explore the potential biological mechanisms by which KIAA1429 facilitates OS, we performed enrichment analysis of KIAA1429-related genes. We found that KIAA1429-related genes were significantly enriched in the cell cycle pathway. Multiple studies have shown that differentially expressed genes are involved in the progression of cancer by regulating the cell cycle pathway. For example, the lncRNA PITPNA-AS1/miR-876-5p/c-MET axis regulates the cell cycle, and promotes the progression of cervical cancer [23]. Additionally, HE4 overexpression reduces the sensitivity of pancreatic cancer to paclitaxel by deregulating the cell cycle pathway [24]. Glycyrrhizic acid represses the proliferation of gastric cancer cells by inhibiting the cell cycle [25]. High expression of FOXF1 inhibits the progression of lung cancer cells by inducing G1 cell cycle arrest [26]. Silencing of REG γ mediates apoptosis of OS cells by inhibiting the cell cycle [27]. Furthermore, miRNA-98-5p regulates the cell cycle in OS by downregulating the expression of CDC25A, which in turn inhibits the progression of OS [28]. We hypothesize that KIAA1429 is involved in OS progression through regulation of the cell cycle pathway. However, further experiments are needed to verify the relationship between KIAA1429 and regulation of the cell cycle.

In the present study, three hub related genes, CDK1, CCNA2, and CCNB1, were identified by PPI network analysis, and were shown to play important roles in the development of OS. Yang *et al*. found that CDK1 expression might be relate to methotrexate resistance in OS [29]. FGFR1 was shown to upregulate the expression of CDK1 to promote the proliferation of OS cells [30]. Piperine inhibited the progression of OS by reducing the expression of CDK1 [31]. Ginsenosides induced apoptosis and cell cycle arrest of OS cells by reducing CDK1 expression [32]. PDCD5 downregulated the expression of CDK1, which induced apoptosis in MG-63 cells [33]. In this study, by merging microarray and RNA sequencing data, we found that CDK1 was significantly overexpressed in OS, and had the capability to distinguish between OS and non-OS, as demonstrated by SROC analysis.

By analyzing 419 samples collected from microarray and sequencing datasets, we found that the expression of CCNA2 is upregulated in OS, and that CCNA2 is a good candidate for a diagnostic biomarker in OS. Liu *et al.* showed that knockdown of CCNA2 remarkably inhibited proliferation of OS cells [34]. The regulation of CCNA2 by miR-449a and miR-424 has been reported to be involved in the progression of OS [35]. Additionally, Wu *et al.* found that CCNA2 expression is significantly upregulated in OS cell lines, and reported that patients with high expression of CCNA2 had worse prognosis [36]; this is consistent with our finding that elevated CCNA2 was associated with poorer clinical outcome.

CCNB1, a cell cycle correlated gene, may play a key role in the tumorigenesis and progression of OS [37]. FKBP14 increased the number of OS cells in G0/G1 phase by regulating the expression of CCNB1 [38]. In a study that evaluated 5 OS samples and 22 normal tissue samples, Bekim *et al*. showed that there was no difference of CCNB1 expression between OS tissue and normal tissue [39]. Wang *et al*. reported that CCNB1 is significantly downregulated in OS tissue compared with normal tissue, but their study evaluated just 14 OS samples and 3 normal tissue samples [40]. In the present study, we found that CCNB1 was upregulated in 321 OS samples than 66 non-cancer samples; the larger sample size that we used in our analysis gives our study stronger statistical power compared to the small sample sizes in prior studies.

Of course, this study has certain limitations. First, we only tested the mRNA level of KIAA1429 in OS, and a further immunohistochemistry experiment is needed to evaluate the expression of KIAA1429 at the protein level. Second, the heterogeneity of this study was 68.8%, which weakened the credibility of our results to a certain extent. Due to insufficient sample information, we cannot perform subgroup analysis or meta-regression to find the source of heterogeneity, and a random effects model was applied. Third, our results showed that KIAA1429 plays an oncogenic role in the progression of OS, but its underlying molecular mechanism needs to be further investigated through *in vivo* and *in vitro* experiments.

In conclusion, KIAA1429 may contribute to the progression of OS by targeting CDK1, CCNA2, and CCNB2. KIAA1429 could serve as a potential biomarker and therapeutic target in OS.

Abbreviations

OS: osteosarcoma; CEGs: co-expressed genes; DEGs: differentially expressed genes; PPI: Protein–protein interaction; m6A: N6-methyladenosine; SRA: Sequence Read Archive; TARGET: Therapeutically Applicable Research to Generate Effective Treatments; CCK-8: Cell counting kit-8; PI: propidium iodide; DAVID: Database for Annotation, Visualization, and Integrated Discovery; GO: Gene Ontology; KEGG: functions and Kyoto Encyclopedia of Genes and Genomes; STRING: Search Tool for the Retrieval of Interacting Genes; SROC: summarized receiver operating characteristic; ROC: Receiver operating characteristic; SMD: standardized mean difference; HR: hazard radio; 95%CI: 95% confidence interval; AUC: area under the curve.

Declarations

Ethics approval and consent to participate

The studies involving human participants were reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (Nanning, China), and were performed according to the relevant guidelines and regulations. The patients/participants provided their written informed consent to participate in this study.

Consent for publication

Not applicable.

Availability of data and materials

All datasets generated for this study are included in the article. The original data are available upon request to the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Author' contributions

Mao-lin He, Gang Chen and Yun Liu conceived and designed the study; Shu-Fan Ji and Hao Wu performed data analysis; Sheng-Lian Wen and Ming-Hui Wei collected the data; Yi-wu Lei and Yu Sun wrote the manuscript. Mao-lin He, Yun Liu, Shu-Fan Ji, Hao Wu, Sheng-Lian Wen and Ming-Hui Wei revised the manuscript.

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Tables

1. The KIAA1429 expression in OS samples and non-OS samples based on RT-qPCR, microarray NA sequencing data.

	Country	Year	Platform	Non-OS			OS		
	M	SD	N	M	SD				
)671	Estonia	2017	GPL20148	18	9.389	0.223	18	9.642	0.411
26209	China	2019	GPL20301	5	3.039	0.081	6	3.475	0.266
7624	USA	2016	GPL11154	3	3.513	0.276	44	3.847	0.498
)276	Australia	2009	GPL6848	5	0.588	0.000	44	0.023	0.720
2352	Norway	2012	GPL10295	10	9.288	0.162	108	9.575	0.381
CR	China	2019	NA	30	0.521	0.405	30	1.447	0.999

an; N: number; OS: osteosarcoma; SD: standard deviation.

Table 2. Association between KIAA1429 expression and clinicopathological parameters in OS samples based on TARGET database.

Clinicopathological parameters	KIAA1429 expression			T-test	
	N	M	SD	T-value	P-value
Gender					
Female	37	11.511	0.898	-1.447	0.152
Male	48	11.790	0.869		
Age					
<18	67	11.598	0.825	-1.413	0.161
≥18	18	11.929	1.074		
Primary location					
Femur/Tibia	61	11.619	0.904	-0.824	0.412
Others	24	11.795	0.849		
Recurrence					
No	45	11.353	1.017	-2.884	0.005
Yes	40	11.873	0.617		
Metastasis					
No	63	11.760	0.892	1.62	0.109
Yes	22	11.407	0.840		
Survival state					
Alive	58	11.713	0.925	0.673	0.503
Dead	27	11.573	0.809		

TARGET: Therapeutically Applicable Research To Generate Effective Treatments; M: mean; N: number; OS: osteosarcoma; SD: standard deviation.

. The 10 most significant items of the GO analyses based on 395 KIAA1429-related genes.

y	Term	Count	P value
M_BP_DIRECT	cellular response to DNA damage stimulus	13	0.000547
M_BP_DIRECT	transcription from RNA polymerase II promoter	22	0.000658
M_BP_DIRECT	DNA duplex unwinding	6	0.001305
M_BP_DIRECT	double-strand break repair	7	0.00142
M_BP_DIRECT	DNA repair	13	0.001576
M_BP_DIRECT	cell division	16	0.002471
M_BP_DIRECT	cilium morphogenesis	9	0.003998
M_BP_DIRECT	viral process	14	0.004159
M_BP_DIRECT	telomere maintenance	5	0.00436
M_BP_DIRECT	peptidyl-threonine phosphorylation	5	0.005307
M_CC_DIRECT	nucleoplasm	131	1.21E-27
M_CC_DIRECT	nucleus	167	5.34E-16
M_CC_DIRECT	nucleolus	51	1.28E-13
M_CC_DIRECT	cytoplasm	131	7.41E-06
M_CC_DIRECT	centrosome	21	9.04E-05
M_CC_DIRECT	transcription factor TFIID complex	6	0.000425
M_CC_DIRECT	nuclear membrane	13	0.000896
M_CC_DIRECT	transcription factor complex	11	0.002562
M_CC_DIRECT	NURF complex	3	0.006313
M_CC_DIRECT	nuclear matrix	7	0.007928
M_MF_DIRECT	RNA binding	58	4.28E-12
M_MF_DIRECT	transcription factor activity	43	2.4E-07
M_MF_DIRECT	helicase activity	12	5.01E-07
M_MF_DIRECT	ATP binding	53	8.28E-06
M_MF_DIRECT	DNA helicase activity	5	0.000941
M_MF_DIRECT	transcription coactivator activity	13	0.002492
M_MF_DIRECT	cyclin-dependent protein serine/threonine kinase activity	5	0.003548
M_MF_DIRECT	TBP-class protein binding	4	0.006672
M_MF_DIRECT	core promoter binding	6	0.006804
M_MF_DIRECT	transcription factor binding	12	0.018026

ıe ontology; BP: biological process; CC: cellular component; MF: molecular function.

ł. The 10 most significant items of the KEGG analyses based on 395 KIAA1429-related genes.

Category	Term	Count	P value
KEGG_PATHWAY	Cell cycle	10	5.65E-05
KEGG_PATHWAY	Basal transcription factors	6	0.000393
KEGG_PATHWAY	RNA transport	10	0.00067
KEGG_PATHWAY	Ribosome biogenesis in eukaryotes	7	0.001355
KEGG_PATHWAY	Progesterone-mediated oocyte maturation	6	0.007468
KEGG_PATHWAY	RNA degradation	5	0.022971
KEGG_PATHWAY	Viral carcinogenesis	8	0.025259
KEGG_PATHWAY	Ubiquitin mediated proteolysis	6	0.033748
KEGG_PATHWAY	Homologous recombination	3	0.042462
KEGG_PATHWAY	RNA polymerase	3	0.044282

KEGG: Kyoto Encyclopedia of Genes and Genomes.

. The descriptions of selected microarray and RNA sequencing datasets.

Study	Year	Country	Platform	0S samples	Non-OS samples
E-MEXP-3628	2012	USA	NA	4	14
GSE12865	2009	Canada	GPL6244	12	2
GSE126209	2019	China	GPL20301	6	5
GSE99671	2017	Estonia	GPL20148	18	18
GSE87624	2016	USA	GPL11154	44	3
GSE68591	2015	USA	GPL11028	10	2
GSE39262	2012	UK	GPL96	10	3
GSE14359	2010	Germany	GPL96	18	2
GSE11414	2009	Canada	GPL6244	4	2
GSE42352	2012	USA	GPL10295	108	10
GSE36001	2012	Norway	GPL6102	19	6
GSE33383	2011	Norway	GPL10295	84	15

OS: osteosarcoma.

Figures

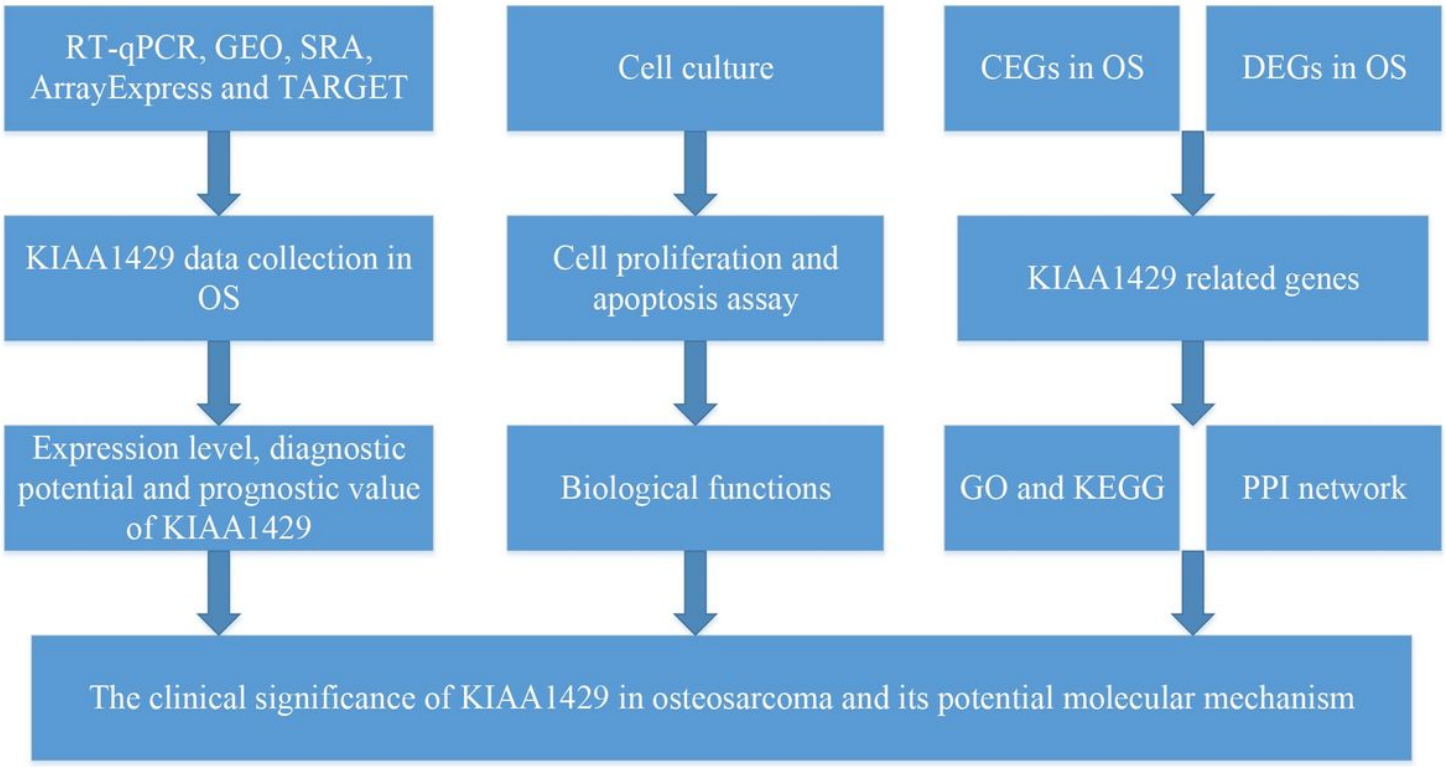

Figure 1

Flow diagram of the study procedure. GEO: Gene Expression Omnibus; SRA: Sequence Read Archive; TARGET: Therapeutically Applicable Research To Generate Effective Treatments; CEGs: co-expressed

genes; DEGs: differentially expressed genes; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: protein–protein interaction.

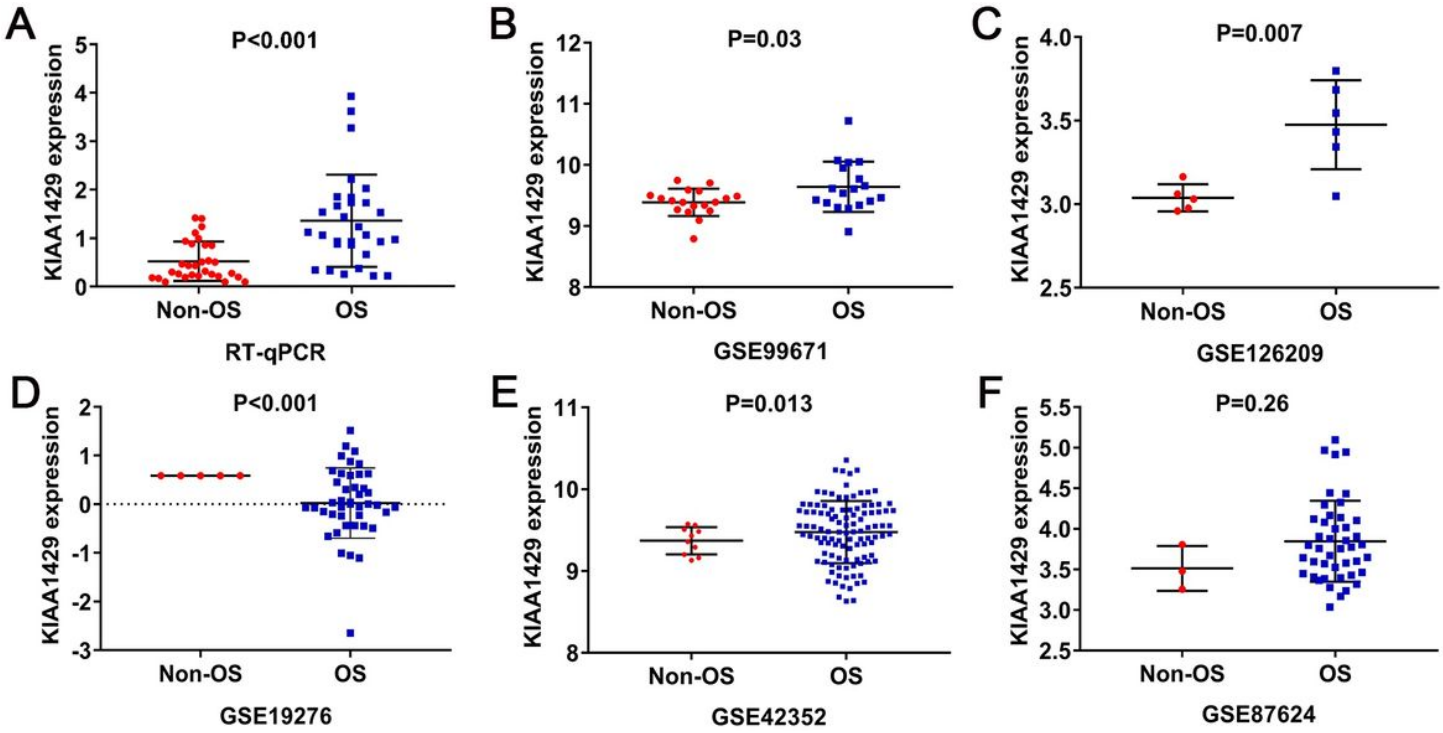

Figure 2

The expression level of KIAA1429 in OS based on RT-qPCR, microarray and RNA sequencing data. (a) RT-qPCR. (b) GSE99671. (c) GSE126209. (d) GSE19276. (e) GSE42352. (f) GSE87624. OS：osteosarcoma.

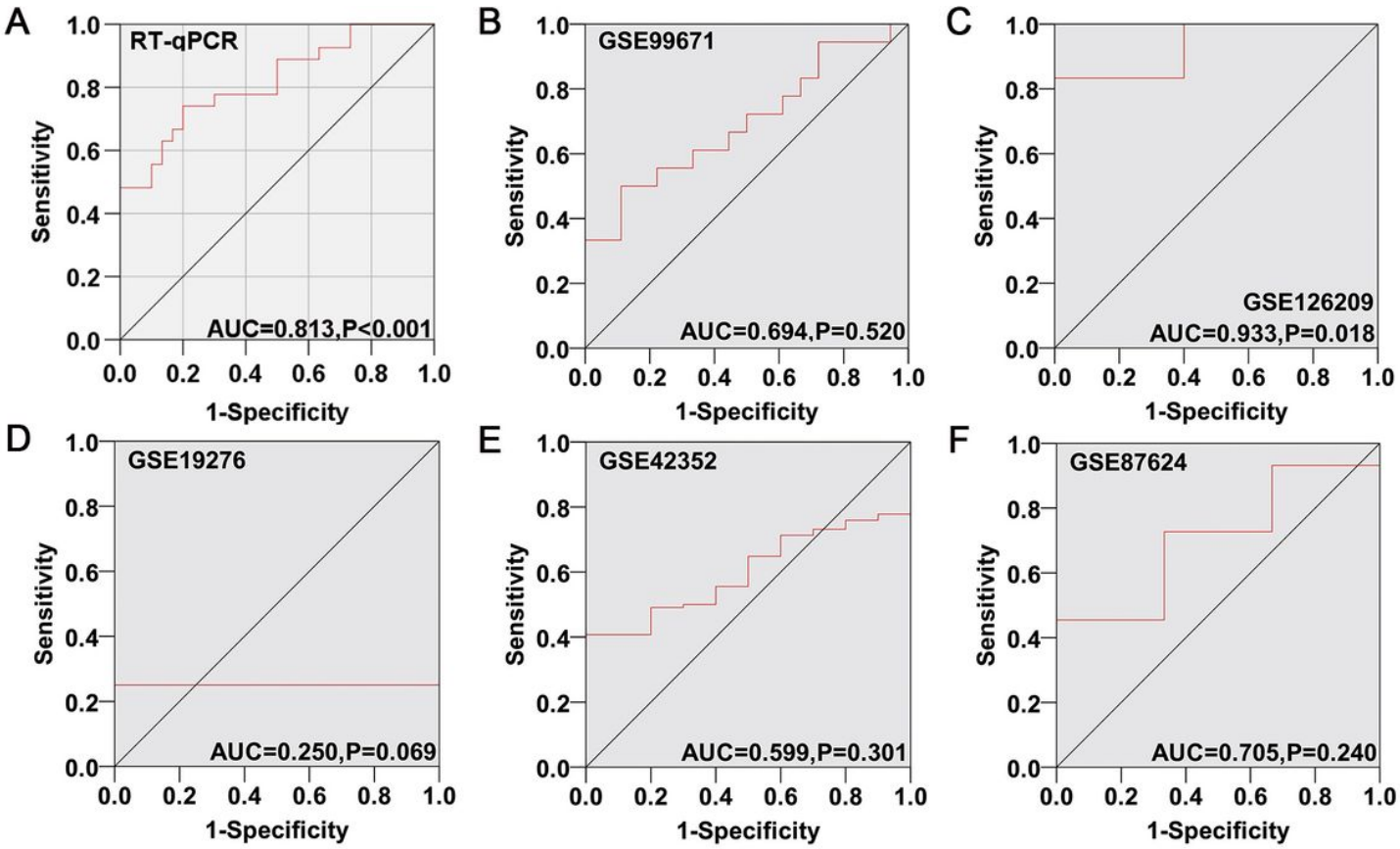

Figure 3

Diagnostic capability of KIAA1429 in OS based on RT-qPCR, microarray and RNA sequencing data. (a) RT-qPCR. (b) GSE99671. (c) GSE126209. (d) GSE19276. (e) GSE42352. (f) GSE87624. OS□osteosarcoma.

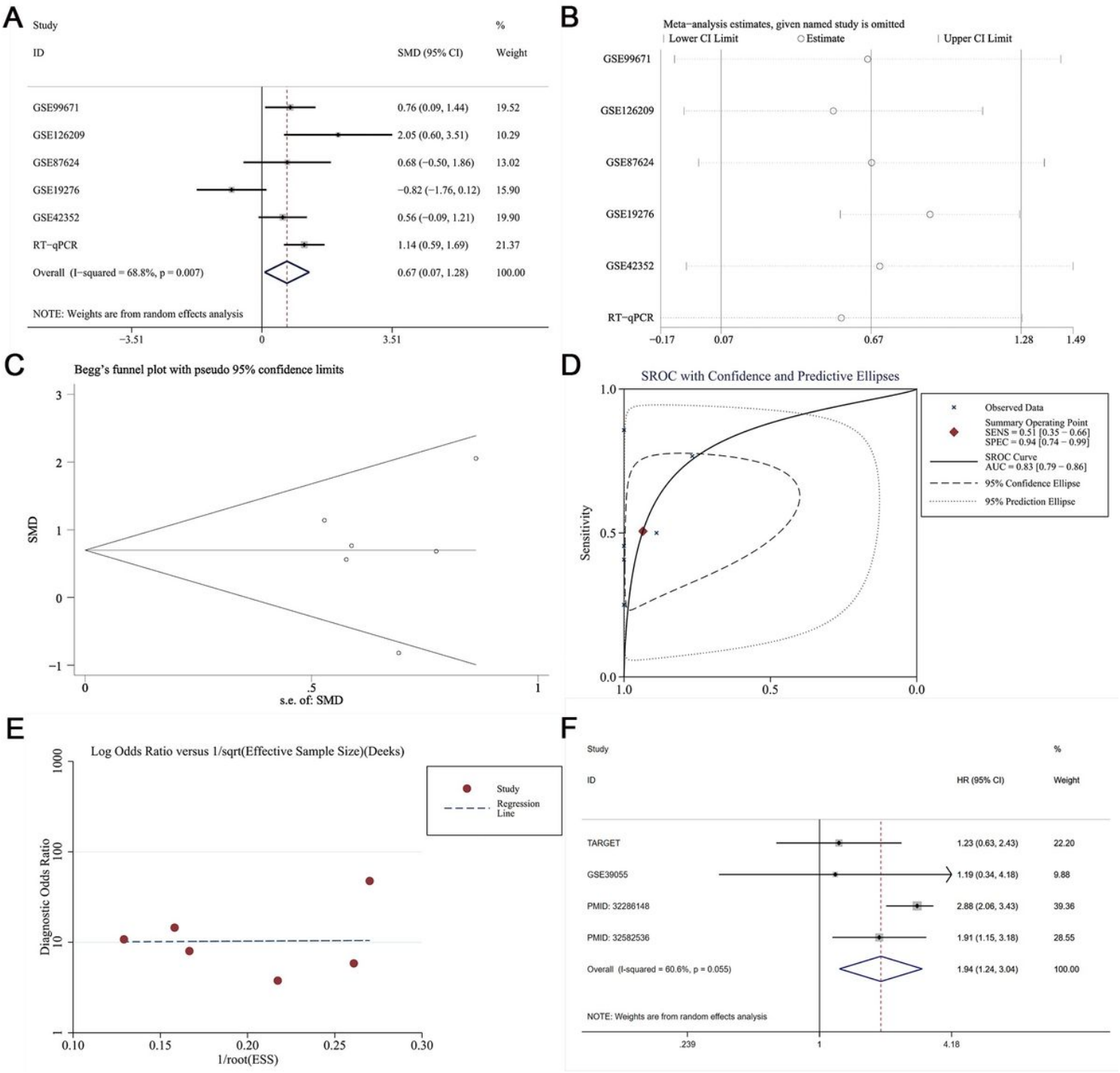

Figure 4

Clinical significance of KIAA1429 in OS was evaluated by meta-analysis. (a) Forest diagram of KIAA1429 expression in OS. (b) Sensitivity analysis of KIAA1429 expression in OS. (c) Begg's funnel diagram, which suggested no publication bias. (d) The SROC curve showed that KIAA1429 had a good capability to discriminate OS samples from non-cancer samples. (e) Deek's funnel diagram, which suggested no publication bias. (f) Forest plot of the effects of KIAA1429 expression on disease prognosis in OS. OS: osteosarcoma; SROC: summarized receiver operating characteristic.

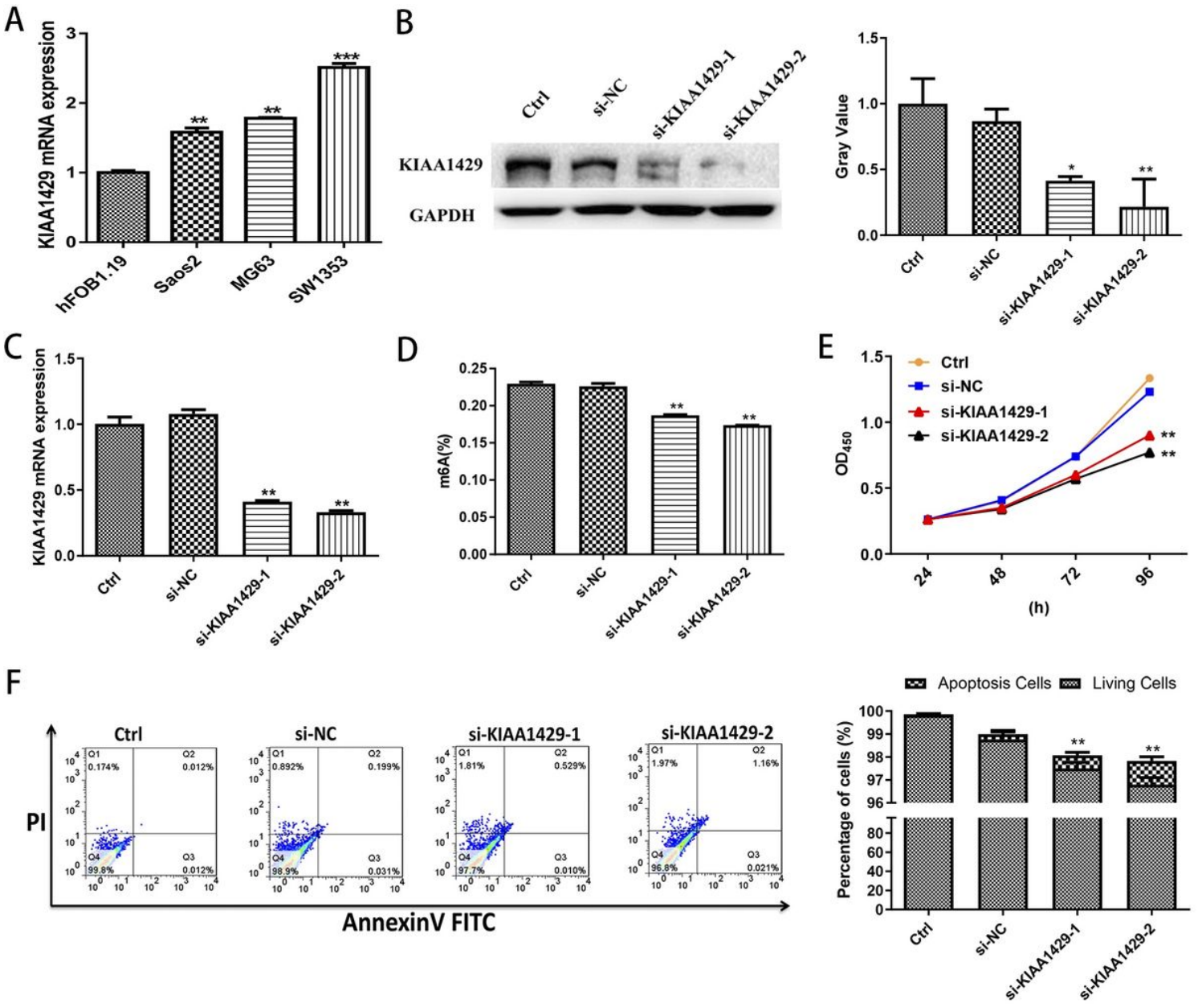

Figure 5

Biological function of KIAA1429 in OS. (a) The expression of KIAA1429 in OS cells and osteoblasts. (b) The knockdown efficiency of KIAA1429 in SW1353 cells was confirmed by western blot. (c) The knockdown efficiency of KIAA1429 in SW1353 cells was confirmed by RT-qPCR. (d) EpiQuik m6A RNA Methylation Quantification Kit was used to evaluate the content of m6A in SW1353 cells after silencing KIAA1429. (e) Cell counting kit-8 assay was performed to assess KIAA1429-knockdown on cell proliferation in SW1353 cells. (f) Flow cytometry assay was applied to determine the effects of KIAA1429-knockdown on cell apoptosis in SW1353 cells. OS□osteosarcoma. *P <0.05; **P <0.01.

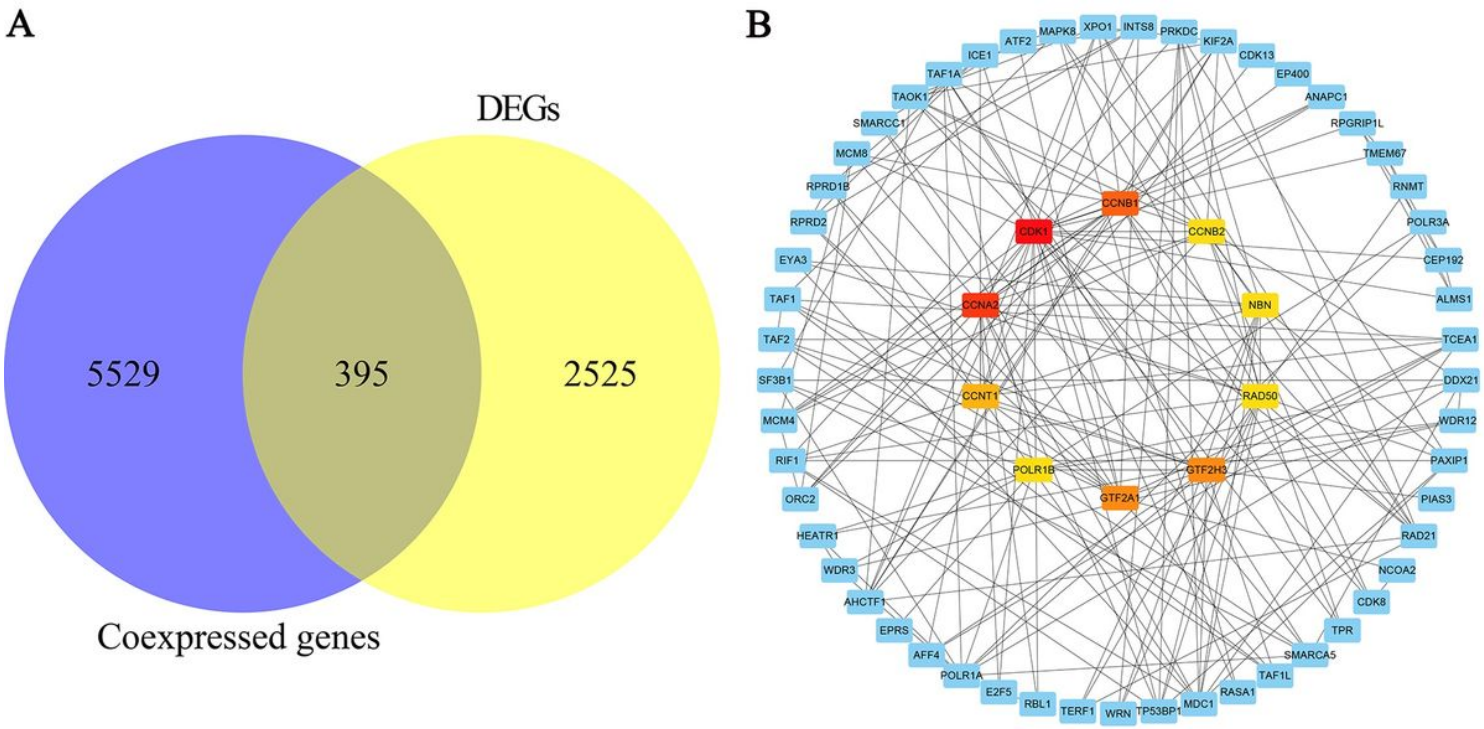

Figure 6

The screening and PPI network analysis of KIAA1429-related genes. (a) A total of 395 KIAA1429-related genes were identified from CEGs and DEGs in OS. (b) PPI network analysis showed that CDK1, CCNA2, and CCNB1 had the top three degrees of connection of the KIAA1429 related genes. PPI: protein–protein interaction; CEGs: co-expressed genes; DEGs: differentially expressed genes; OS: osteosarcoma.

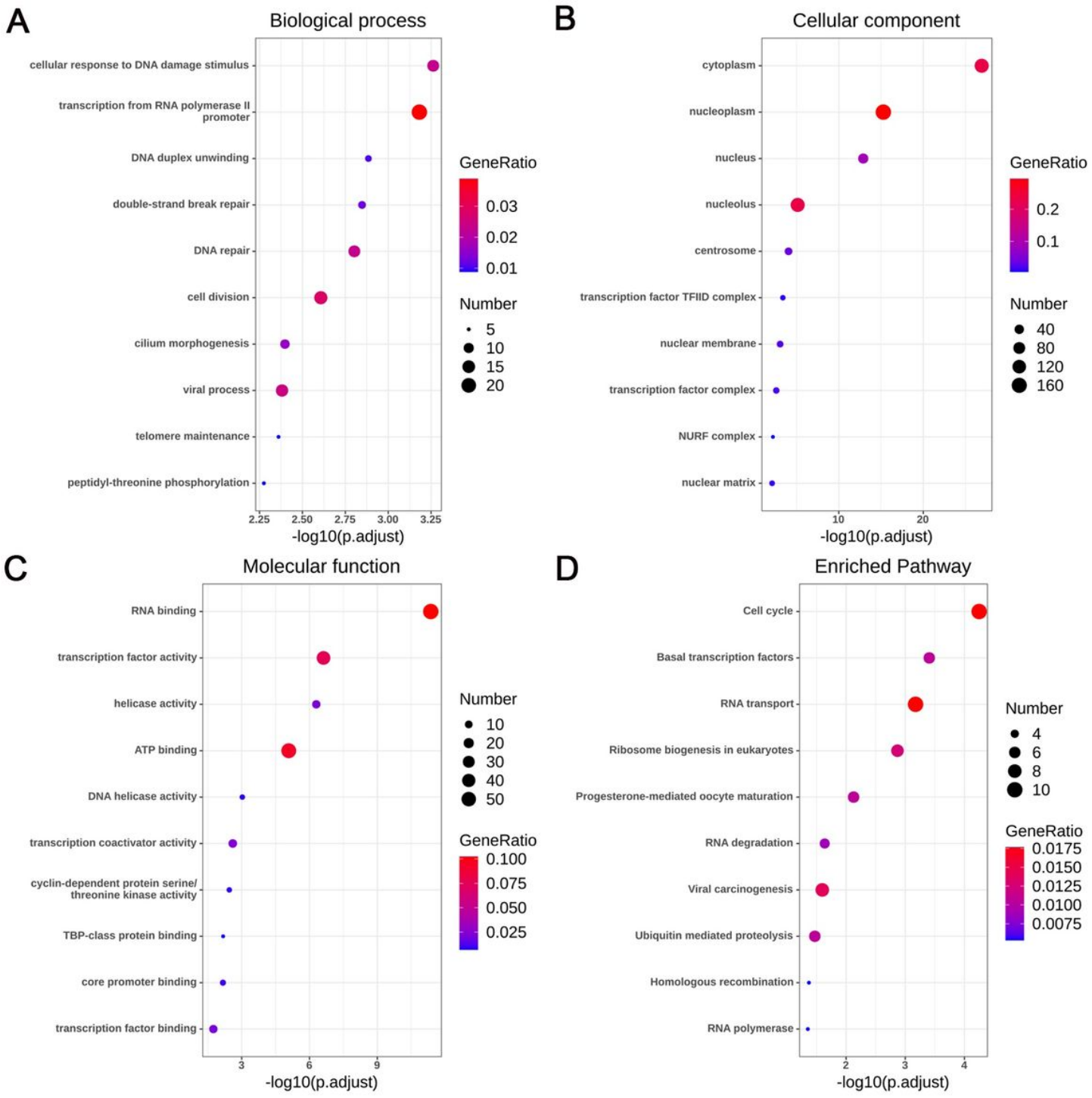

Figure 7

Functional and pathway enrichment analyses of the KIAA1429-related genes. (a) Biological process. (b) Cellular component. (c) Molecular function. (d) KEGG pathways. KEGG: Kyoto Encyclopedia of Genes and Genomes.

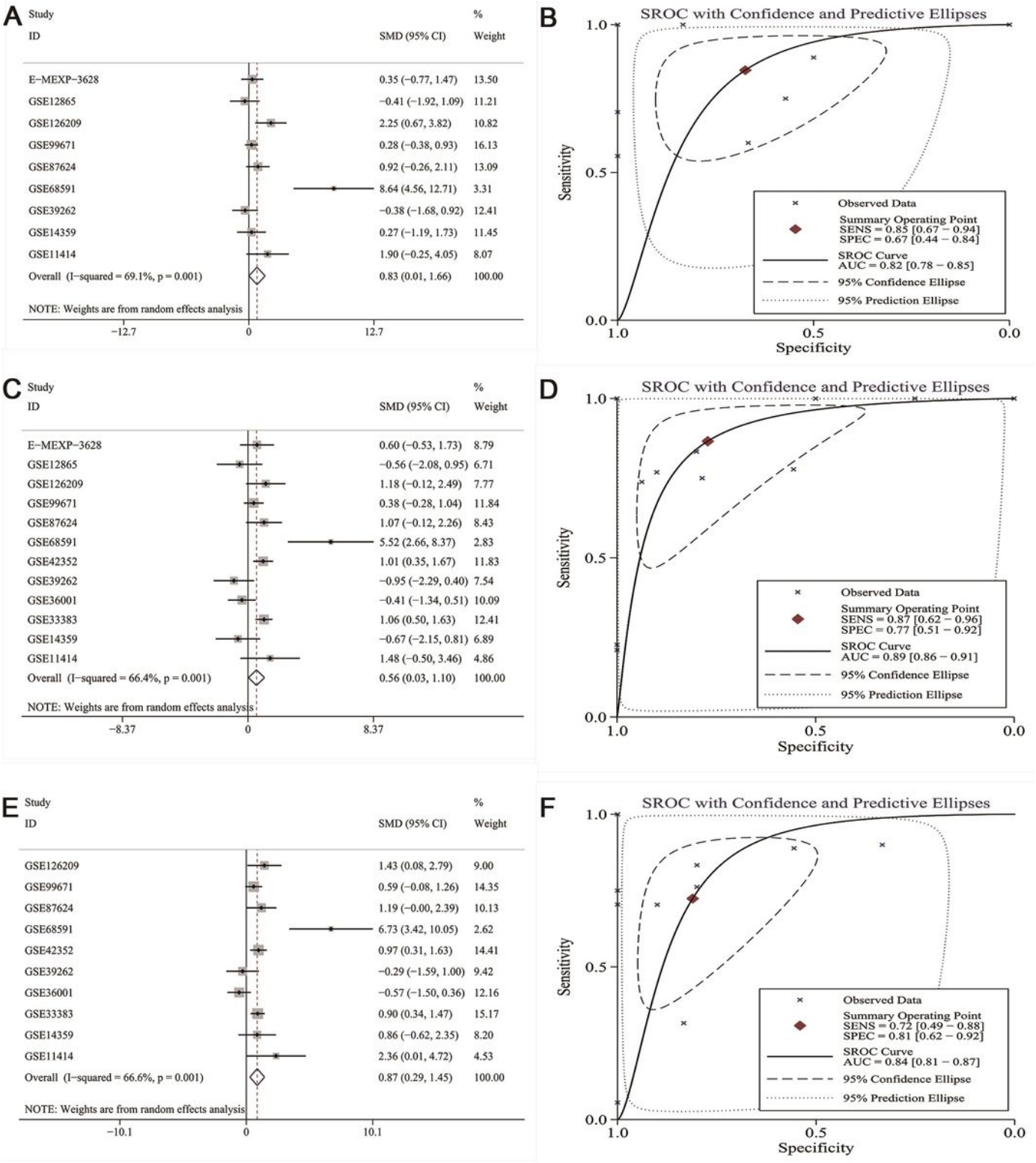

Figure 8

The expression and diagnostic capability of three hub related genes (CDK1, CCBA2 and CCNB1) in OS based on microarray and RNA sequencing data. (a, c and e) Three hub related genes were significantly overexpressed in OS samples compared with control samples. (b, d, and f) Three hub related genes demonstrated strong diagnostic potential between OS and control samples. OS: osteosarcoma.

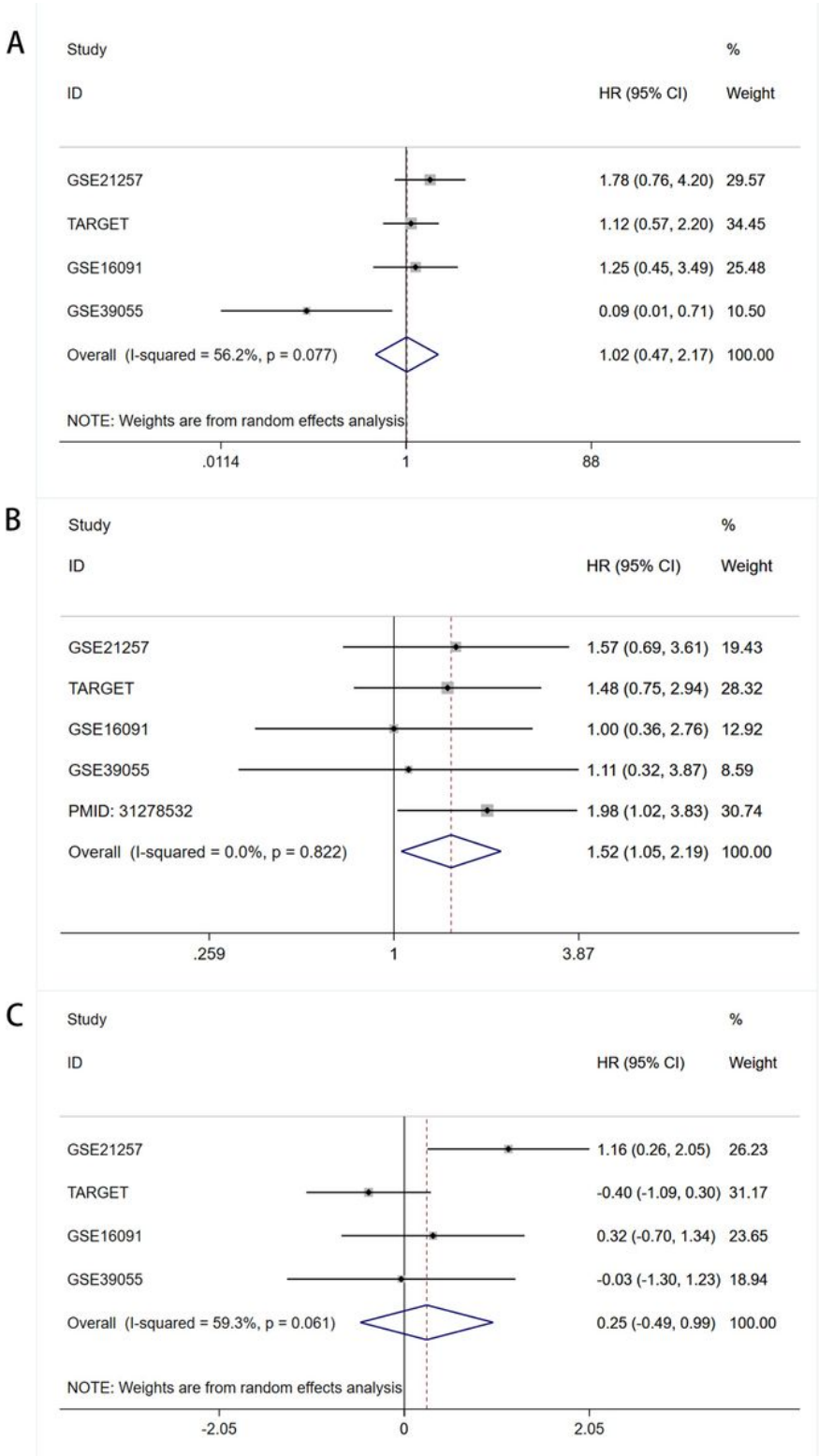

Figure 9

Survival analysis of three hub related genes (CDK1, CCBA2 and CCNB1) in OS based on microarray, RNA sequencing, and published data. (a) CDK1 expression was not associated with overall survival of OS patients. (b) Overexpression of CCNA2 associated with poorer prognosis among OS patients. (c) CCNB1 expression was not associated with overall survival of OS patients. OS: osteosarcoma.

Supplementary Files

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

- westernblotimagesofKIAA1429.docx
- westernblotimagesofGAPDH.docx