Research Square

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miR-133a targets YES1 to reduce cisplatin resistance in ovarian cancer by regulating cell autophagy

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Abstract

Background

Accumulating evidences reveal that aberrant microRNAs (miRNAs) expression can affect the development of chemotherapy drug resistance by modulating the expression of relevant target proteins. Emerging evidences have demonstrated that miR-133a participates in tumorigenesis of various cancers. However, whether miR-133a is associated with cisplatin resistance in ovarian cancer remains unclear.

Objective

To investigate the role of miR-133a in the development of cisplatin resistance in ovarian cancer.

Methods

MiR-133a expression in cisplatin-resistant ovarian cancer cell lines was assessed by reverse-transcription quantitative PCR (RT-qPCR). Cell counting kit-8 (CCK-8) assay was used to evaluate cell viability of tumor cells treated with cisplatin in the presence or absence of miR-133a. Luciferase reporter assay was used to analyze binding of miR-133a with 3’ untranslated regions (3’UTR) of YES proto-oncogene 1 (YES1). The YES1 expression level was analyzed using the dataset from the international cancer genome consortiu (ICGC) and assessed by RT-qPCR and western blotting in vitro. The roles and mechanisms of YES1 on cell functions were further probed via gain- and loss-of-function analysis.

Results

The expression of miR-133a was significantly decreased in cisplatin resistant ovarian cancer cell lines (A2780-DDP and SKOV3-DDP), and the overexpression of miR-133a mimic reduced cisplatin resistance in A2780-DDP and SKOV3-DDP cells and the treatment of miR-133a inhibitor increased cisplatin sensitive in normal A2780 and SKOV3 cells. MiR-133a binds 3’UTR of YES1 and down-regulates its expression. Bioinformatics analysis revealed that YES1 expression was upregulated in recurrent cisplatin resistance ovarian cancer tissue and in vitro experiments also verified its upregulating in cisplatin resistance cell lines. Furthermore, we discovered that miR-133a down-regulated the expression of YES1 and thus inhibited the cell autophagy to reduce cisplatin resistance. Yes1 knockdown significantly suppressed the cisplatin resistance of ovarian cancer cells through inhibiting autophagy in vitro. Xenograft tumor implantation further demonstrated that Yes1 overexpression promoted ovarian tumor development and cisplatin resistance.

Conclusion

Our results suggest that miR-133a/YES1 axis plays a critical role in cisplatin resistance in human ovarian cancer by regulating cell autophagy, which might serve as a promising therapeutic target for ovarian cancer chemotherapy treatment in the future.

Background

Ovarian cancer is one of the most lethal gynecologic cancers that seriously threaten women's health[1]. In the past 30 years, comprehensive/systematic surgery followed by platinum-based chemotherapy are still the main treatment, but the overall prognosis of ovarian cancer has not improved significantly, and the 5-year survival rate of patients with advanced (FIGO 3–4) ovarian cancer is less than 30%[2]. Tumor relapses and acquired drug resistance are the treatment bottlenecks for improving the overall survival of ovarian cancer patients[3], and the molecular mechanism of drug resistance in ovarian cancer remains unknown. Thus, further understanding of the pathogenesis and mechanisms of ovarian cancer chemotherapy resistance are crucial to improve overall survival, identify therapeutic markers, and develop new efficient treatment strategies.

Aberrant microRNAs (miRNAs) expression plays critical roles in various types of cancers and some of them are considered ideal targets for tumor treatment [4].Emerging evidences have demonstrated that miR-133a participates in tumorigenesis of various cancers [5]. For examples, Wang reported that miR-133a expression was down-regulated in pancreatic cancer tissues and serum specimens of patients, and its expression is negatively correlated with the stage and prognosis of pancreatic cancer patients, which may be an ideal marker for early diagnosis of pancreatic cancer [6].Yuan et al. reported that nuclear paraspeckle assembly transcript 1(NEAT1)/miR133a axis promoted cervical cancer progression through regulating SRY-box transcription factor 4 (SOX4) [7]. In addition, miR-133a was low expressed in gastric cancer tissues and cells, and it inhibited tumor cell proliferation and metastasis by regulating the autophagy of gastric cancer tumor cells [8].Our previous research found that miR-133a/ Glycogen Phosphorylase B (PYGB) axis can inhibit the occurrence and development of ovarian cancer both in vitro and in vivo [9]. Moreover, miR-133a also plays a key role in tumor drug resistance. MiR-133a could reduce cisplatin resistance in Hep-2v cells through negatively regulating ATPase copper transporting beta (ATP7B) expression [10].Overexpression of miR-133a could decreased oxorubicin resistance in MCF-7/Dox breast cancer cell line via decreasing the expression of mitochondria uncoupling protein 2 (UCP-2).However, the function of miR-133a in ovarian cancer chemotherapy resistance has not been researched. YES1 function as tumor oncogenes and may be a potential therapeutic target in different types of cancers [11].Knockdown of YES1 led to the suppression of proliferation and cell cycle arrest in ovarian cancer cells [12]. A clinical retrospective study showed that overexpression of Yes1 indicated favorable prognosis and increased platinum-sensitivity in primary epithelial ovarian cancer patients [13].Moreover, the expression of YES1 was higher in recurrent post-chemotherapy high grade serous ovarian cancer. Overexpression of Yes1 decreased the rate of apoptotic cells in OVCAR8 cells with taxol treatment, suggesting a potential association between YES1 and taxol chemoresistance [14]. However, how YES1 regulates chemoresistance in recurrent ovarian cancer is not fully understood.

In this study, we aimed to explore the expression pattern and functional role of miR-133a in cisplatin resistance ovarian cancer and its potential mechanism in regulating cisplatin-resistance of ovarian cancer. We found that miR-133a is down-expressed in cisplatin resistance ovarian cancer and overexpression of miR-133a could reduce cisplatin resistance in ovarian cancer cell line. Mechanistically, miR-133a down-regulates YES1 through binding with 3'UTR of YES1. Knockdown of Yes1 inhibits cisplatin resistance in cisplatin resistance cell line and overexpression of Yes1 induces cisplatin resistance in cisplatin sensitive cells. Furthermore, we demonstrated that YES1 affects cisplatin resistance by regulating cell autophagy both in ovarian cancer cells and Xenograft model. Taken together, our findings suggest that miR-133a targets the 3'UTR of YES1 and reversely regulates the expression of YES1, which ultimately regulates cell autophagy in ovarian cancer with cisplatin resistance.

Materials And Methods

Cell culture and treatment

Human ovarian cancer cells (SKOV3 and A2780) were obtained from the Cell Bank, Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). The cisplatin-resistance cell line (SKOV3-DDP and A2780-DDP) were established in our lab. All cell lines were cultured in RPMI medium 1640 medium (Gibco) supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco),100 μg/ml streptomycin, and 100 U/ml penicillin in a humidified atmosphere at 37°C with 5% CO_2. For cisplatin treatment, ovarian cancer cells were cultured with 10–80 μM cisplatin (selleck) for 48 h. For autophagic promotion and inhibition, cells were treated with autophagic antagonist-chloroquine (50 μM, sigma) and autophagic agonist-rapamycin (100 nM, sigma), respectively.

qRT-PCR

Total RNA from tissues and cultured cells was extracted using TRIzol reagent(sigma). RNA concentration was measured and equal amount of mRNA and miRNA was reverse transcribed to cDNA using reverse transcription kits(Takara and TransGen) respectively. Quantitative realtimePCR (qPCR) was performed on ABI 7500 real-time PCR system using specific primers as following: YES1 forward primer: 5'-CTCAGGGGTAACGCCTTTTGG-3'; reverse primer: 5'-CACCACCTGTTAAACCAGCAG-3'; GAPDH forward primer: 5'-GGAGCGAGATCCCTCCAAAAT-3'; reverse primer: 5'-GGCTGTTGTCATGCTTCTCATGG-3'; miR-133a forward primer: 5'-CAGCTGGTTGAAGGGGACCAAA-3'; U6 forward primer: 5'-CTCGCTTCGGCAGCACA-3'; reverse primer: 5'-AACGCTTCACGAATTTGCGT-3'. GAPDH and U6 were used as internal control. Relative YES1 expression was normalized to GAPDH levels and relative miR-133a was normalized to U6 levels both using the $2^{-\Delta\Delta Ct}$ quantification method.

YES1 and miR-133a transfection

YES1-Overexpression (YES1-OE) vectors and a negative control and miR-133a mimics, miR-133a inhibitor and relative controls were purchased from Yazai Biotechnology Co Ltd. (Shanghai, China). The siYES1 and relative controls were purchased from Gene Pharma (Shanghai, China). Transfection was conducted

using Lipofectamine 3000 according to the manufacturer's manual. Lentiviruses-YES1-OE for stable overexpression of YES1 or the negative control construct was designed and constructed by Hanbio Biotechnology Co., Ltd. (Shanghai, China).

Western blot analysis

Tissue samples or cultured cells were lysed, and the protein concentration was measured by the BCA protein assay. Equal amounts of protein were resolved by 10% or 15% SDS-PAGE and transferred onto a PVDF (Polyvinylidene fluoride) membrane. The membranes were blocked at room temperature with 5% skimmed milk and then incubated with primary antibodies overnight at 4°C. After washing with TBST (tris buffered saline tween), the membranes were further incubated with fluorescent secondary antibodies at room temperature in the dark for 1–2 h. GAPDH was used as internal control. The signals were detected using an Odyssey detection system (Odyssey CLx, LI-COR biosciences, NE, USA). Quantification analysis of western blot was performed using software ImageJ (Bethesda, USA). Primary antibodies against YES1 (1:1000, ABclonal, A0628), LC3b (1:1000, Abcam, AB192890), Gapdh (1:5000, Proteintech, 51067-2-AP).

Cell proliferation

Cell proliferation was determined by Cell Counting Kit-8 assay (CCK-8)(Dojindo Laboratories, Kumamoto) according to the manufacturer's protocol. SKOV3 and SKOV3-DDP cells were seeded at a density of 3×10^3 cells/well and A2780 and A2780-DDP cells were seeded at a density of 8×10^3 cells/well into a 96-well plate. 10 μL CCK-8 working solution was added and cells were incubated for 2 h before the measurement of absorbance at 450 nm.

Luciferase reporter assays

WT or mutated 3'-UTR of YES1 sequences were cloned and constructed into the pGL3-Luc reporter vector. A2780 was transfected with the WT or mutated luciferase reporter vectors, together with miR-133a mimics or negative control. Relative luciferase activity was analyzed using a Dual-Luciferase Reporter Assay Kit 48h later.

GFP-RFP-LC3 assay

To monitor the autophagy, the tandem GFP-RFP-LC3 adenovirus construct obtained from Hanbio Inc (Shnaghai, China) which capitalizes on the pH difference between the acidic autolysosome and the neutral autophagosome and the pH sensitivity differences exhibited by GFP (green fluorescent protein) and RFP (red fluorescent protein) to monitor progression from the autophagosome to autolysosome was used. In brief, A2780 and A2780-DDP cells were infected with tandem GFP-RFP-LC3 adenovirus for 2 h and then were cultured with normal medium and 10 μM cisplatin for 48 h. Finally, cells were treated and imaged for GFP and RFP by using fluorescence microscopy.

The international cancer genome **c**onsortiu(ICGC)dataset analysis

The RNA sequencing data of ICGC OV-AU used for gene expression analysis. Genes with low read abundance were filtered, followed by the rlog transformation based on the R package DESeq2 (version 1.28.1). Then the principal component analysis (PCA) of the normalized expression matrix, relied on the FactoMineR package (version 2.3), was performed to detect the outlying samples. The remaining samples were randomly divided into primary sensitive (n = 12) and relapse resistant (n = 24) groups from 23 high-grade serous ovarian cancer (HGSOC) patients [15].

In vivo tumorigenicity

All the mice experiments were approved by the Experimental Animal Ethics Committee of the Tenth people's Hospital Affiliated of Tongji University. Female BALB/c nude mice (4–5 weeks old, 18–20 g) were purchased from Vital River Laboratory (Beijing, China). For xenograft model, 5×10^6 of A2780 ovarian cancer cells transfected with Lentiviruses-YES1-OE or the negative control were subcutaneously implanted into nude mice. Intraperitoneal injection with density of 5mg /kg cisplatin was conducted every 3 days in a week after all the tumor nodules in the same group were appeared. Tumor volume was calculated based on tumor sizes determined by a vernier caliper every week (length × width^2/2). The mice were euthanized 14 days later and tumor weights were determined.

Statistical analysis

All statistical analyses were conducted using a SPSS software (version23.0) and GraphPad Prism 6. Experimental results were presented as the mean ± standard deviation of the mean (SD) based on the results from three independent experiments. Unpaired two-tailed Student’s t-test and the one-way analysis of variance (ANOVA) were used where necessary for calculation of p values. Differences in clinical pathological factors between the YES1 high- or low-expression groups were analyzed via the Chi-square test. P values < 0.05 was considered statistically significant.

Resultes

MiR-133a can reduce cisplatin resistance in ovarian cancer cell lines.

To figure out the expression of miR-133a in cisplatin-resistant ovarian cancer cells, we examined the expression level of miR-133a in A2780-DDP and SKOV3-DDP compared with SKOV3 and A2780 by RT-qPCR, and the results show that miR-133a expression level was remarkably lower in cisplatin-resistant ovarian cancer cells lines(A2780-DDP,SKOV3-DDP) compared to parental cisplatin-sensitive ovarian cancer cells(A2780,SKOV3)(Fig. 1A). To further verify the function of miR-133a in the cisplatin sensibility, we disturbed the miR-133a function in ovarian cancer cell lines, and the results showed that the treatment of miR-133a inhibitor increased the cisplatin resistant in A2780 and SKOV3(Fig. 1B), and miR-133a mimic treatment increased the cisplatin sensibility in A2780-DDP and SKOV3-DDP(Fig. 1C).These findings suggested that miR-133a was downregulated in cisplatin-resistant ovarian cancer cells and may reduce the cisplatin resistant.

MiR-133a regulated YES-1 by binding with 3'-UTR of YES1.

To understand how miR-133a regulates cisplatin resistance in ovarian cancer, we performed bioinformatics analysis using a miRBase online tool to predict the potential genes regulated by miR-133a (Fig. 2A). There are 5 potential genes have the putative 3'-UTR binding sites that match the miR-133a sequences by an intersection analysis from 3 databases (miRDB, TargetScan, and microRNA). To further understand which target gene between the 5 potential genes affects chemotherapy resistance of ovarian cancer cells, we analyzed the gene expression level between primary ovarian cancer and recurrent ovarian cancer groups, and the results demonstrated that only the YES1 expression level was dramatically increased in recurrent ovarian cancer tissues compared with that in primary tissues (Fig. 2B). We next investigated the association between miR-133a and YES1 through the luciferase activity analysis in A2780 cells transfected with a luciferase vector embodying the predicted miR-133a binding sites (Fig. 2C). Our data demonstrated that the luciferase activity of the reporter including the WT YES1 3'UTR sequence was significantly reduced by miR-133a mimic treatment, mutation of this sequence abolished the negative roles (Fig. 2D).

Yes1 knockdown reduced cisplatin resistance in vitro.

To further understand how YES1 plays a role in cisplatin resistance of ovarian cancer, we firstly demonstrated that YES1 was significantly highly expressed in both A2780-DDP and SKOV3-DDP cells by RT-qPCR and WB (Fig. 3A and 3B), which is consistent with the results of bioinformatics analysis (Fig. 2B). To further explore the function of YES1 affecting cisplatin sensitivity in ovarian cancer, we screened for a highly efficient Yes1 knockdown siRNA via measuring the silencing efficiency of three YES1 siRNAs with different targeting sequences in SKOV3-DDP or A2780-DDP cells (Fig.S1), si-YES1#2 (referred as siYES1in the subsequent experiments) transfection in A2780-DDP and SKOV3-DDP ovarian cancer cells significantly down-regulated the YES1 expression both in mRNA and protein level (Fig. 3C and 3D). We found that knockdown of Yes1 significantly reduced cisplatin resistance in SKOV3-DDP and A2780-DDP cells (Fig. 3E). Meanwhile, Yes1-OE could enhance the cisplatin resistance in SKOV3 or A2780 cell lines (Fig. 3F-3H).

MiR-133a inhibit the expression of YES1.

To further evaluate the molecular mechanism, miR-133a was overexpressed by the treatment of miR-133a mimics, which significantly inhibited the YES1 expression in SKOV3-DDP or A2780-DDP cells both at mRNA and protein levels (Fig. 4A and 4B). Similarly, miR-133a inhibitor treatment could increase the YES1 expression in SKOV3 or A2780 cells both at mRNA and protein levels (Fig. 4C and 4D). Furthermore, co-transfection of miR-133a mimic and YES1-OE vector decreased the YES1 expression compared with miR-133a NC + YES-OE group but miR-133a inhibitor could not affect the YES1 expression (Fig. 4E). Functionally, we demonstrated that overexpression of YES1 together with miR-133a mimic transfection antagonized YES-OE-induced cisplatin resistant effects (Fig. 4F). Taken together, these findings suggested that miR-133a targets YES1 and downregulates its expression, thereby reducing the cisplatin resistance in ovarian cancer cells.

YES1 regulates cell autophagy in cisplatin resistant of ovarian cancer.

To further understand how YES1 functions in cisplatin resistant of ovarian cancer, we performed Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of top 500 genes with highest YES1 correlation coefficient using the gene expression data of ovarian cancer cohort from TCGA database (Fig. 5A). The results indicated that autophagy signaling pathway was markedly enhanced in ovarian cancer patients with high YES1 expression. To verify these results, we firstly confirmed that autophagy was enhanced both in A2780-DDP and SKOV3-DDP compared with A2780 and SKOV3 cells (Fig. 5B). In addition, inhibiting miR-133a in SKOV3 and A2780 cells can significantly enhance cell autophagy (Fig. 5C), and transfecting miR-133a mimic in A2780-DDP and SKOV3-DDP cells can both reduced autophagy (Fig. 5D). We further validated the relationship between Yes1 expression and autophagy. As shown in Fig. 5E, Yes1 knockdown remarkably reduced LC3B expression in A2780-DDP and SKOV3-DDP, and Yes1-OE remarkably increased LC3B expression in A2780 and SKOV3 (Fig. 5F). Autophagic flux determination test also verified that Yes1 overexpression could enhance autophagic flux in A2780 cells (Fig. 5G), and Yes1 knockdown could decreased autophagic flux in A2780-DDP cells (Fig. 5H). Furthermore, Yes1 overexpression induced cisplatin resistance effect could be reversed by an autophagic antagonist-chloroquine (Fig. 5I). Reversely, Yes1 knockdown could increase cisplatin sensitivity and this effect could be reversed by an autophagic agonist-rapamycin (Fig. J). Taken together, these findings suggest that YES1 might affect cisplatin resistance through regulating cell autophagy.

YES1 overexpression induces cisplatin resistance through regulating autophagy in vivo.

To further investigate the function of YES1 in cisplatin resistance of ovarian cancer in vivo, xenograft tumor model was established by implanting ovarian cancer cells A2780 transfected with YES1-OE or YES1-NC into nude mice. As shown in Fig. 6A, Yes1 overexpression significantly enhanced the ovarian tumor growth and induced cisplatin resistance, with slower tumor growth curve and slower tumor growth volume after cisplatin injected intraperitoneally in YES1-NC group in comparison with those in YES1-OE group (Fig. 6B). Furthermore, we examined that the LC3B expression in the mouse ovarian tumor tissues and the results showed increased expression of LC3B in YES1-OE group after cisplatin injecting (Fig. 6C). In summary, we demonstrated that YES1 overexpression enhanced cisplatin resistance through activating cell autophagy in vivo.

Discussion

Nowadays, the chemotherapy resistance in both primary and recurrent ovarian cancer contributes to the poor prognosis and high mortality. Recently, researches on tumor-associated miRNAs has been getting increasing attention. Emerging evidences have suggested that miR-133a plays a critical role in the different kinds of cancers [5]. Some researches have proved that miR-133a promoted tumor development in multiple cancers by regulating autophagy. For examples, miR-133a could target GABA Type A Receptor Associated Protein Like 1 (GABARAPL1) to inhibit autophagy-mediated glutaminolysis, repressing gastric cancer growth and metastasis [8]. MiR-133a could also regulate cell autophagy by binding to the 3'UTR

of forkhead box P3 (FOXP3) in gastric cancer [16]. Moreover, miR-133a also plays a key role in tumor drug resistance. MiR-133a could reduce cisplatin resistance inHep-2v cells through negatively regulating ATPase Copper Transporting Beta (ATP7B) expression [10]. Overexpression of miR-133a could decreased oxorubicin-resistance in MCF-7/Dox breast cancer cell line via decreasing the expression of mitochondria uncoupling protein 2 (UCP-2). However, the function of miR133a in ovarian cancer chemotherapy resistance has not been researched. Here we firstly demonstrated that miR-133a reduced cisplatin resistance in ovarian cancer cells. we revealed that miR-133a expression was significantly decreased in cisplatin resistance ovarian cancer cell lines. MiR-133a inhibitor increased the cisplatin resistant and miR-133a mimic treatment increased the cisplatin sensibility in vitro. Furthermore, we identified the miR-133a, as the potential YES1 posttranscriptional regulator, directly binds 3'UTR of YES1 and downregulates its expression in ovarian cancer. Multiple researches have demonstrated that YES1,function as a oncogene, is dysregulated in various carcinomas [11] .Previous research has reported miR-133a inhibited cell proliferation in non-small cell lung cancer by targeting YES1 [17]. In this study, we further investigated the roles of YES1, a protein highly expressed in cisplatin resistant tissue and cell lines, in cisplatin resistant of ovarian cancer. Our results also demonstrate that YES1 knockdown suppress cisplatin resistant in ovarian cancer in vitro and YES1 overexpression induce cisplatin resistant both in vitro and in vivo.

Previous studies have also confirmed that inhibition of autophagy could increase the sensitivity to cisplatin of ovarian cancer [18, 19]. Wang et al. reported that LncRNA-ATB regulates autophagy by activating Yes-associated protein and increasing ATG5 (Autophagy Related 5) expression. In this study We analyzed the TCGA ovarian cancer cohort using KEGG pathway and found that autophagy signaling pathway was significantly enhanced in ovarian cancer patients (Fig. 2A). We firstly verified that knockdown of Yes1 inhibited the expression of LC3B and decreased autophagic flux, and overexpression of Yes1 activated LC3B expression and increased autophagic flux in vitro. Moreover, rapamycin, an autophagic agonist, could reverse siYes1 induced cisplatin resistance and the and chloroquine, an autophagy antagonist,could reverse Yes1-OE induced cisplatin resistance (Fig. 5I and 5J), which further demonstrated that YES1 affects cisplatin resistance in ovarian cancer by regulating cell autophagy. Overexpression of Yes1 also increased LC3B expression and led to cisplatin resistance in a xenograft tumor model (Fig. 6).

Also, this study has some limitations. For example, we studied the YES1 regulates cisplatin resistance in ovarian cancer. Previous studies proved there may be some most important pathways, such as AMPK, Hippo and mTOR pathways, that participate in the regulatory roles of autophagy [20–22], but our study did not verify definitively whether YES1 plays a role by influencing these important pathways which are also worth studying. Additionally, in our study, we only demonstrate YES1 was upregulated in recurrent tissues resistance to platinum chemotherapy by bioinformatics analysis, did not prove the results by our own clinical specimens.

Taken together, our research suggested that miR-133a directly targets YES1 by binding its 3'UTR area and miR-133a/YES1 axis might regulate cisplatin sensitivity via cell autophagy in ovarian cancer. Our findings

provide insight into miR-133a/YES1/autophagy axis to be used as a novel diagnosis biomarker and potential gene therapeutic target for ovarian cancer chemotherapy.

Declarations

Acknowledgements

Not applicable.

Ethics declarations

Funding

Not applicable.

Conflict of interests

The authors Yang Zhou, Chunyan Wang, Jinye Ding, Yaoqi Sun, and Zhongping Cheng declare that they have no conflicts of interest that might be relevant to the contents of this manuscript.

Ethics approval

All the mice experiments were approved by the Experimental Animal Ethics Committee of the Tenth people's Hospital Affiliated of Tongji University.

Consent to participate

Not applicable.

Consent for publication

Consent to publish has been obtained from all authors.

Availability of data and materials

The datasets obtained and analyzed during the current study were made available from the corresponding authors through request.

Code availability

Not applicable.

Authors' contributions

ZC: Conception and design of the study, and manuscript writing; YZ: In vitro and vivo experiments and data analysis; CW: bioinformatics analysis and result interpretation; JD and YS: parts of vitro

experiments. All authors read and approved the final manuscript.

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Figures

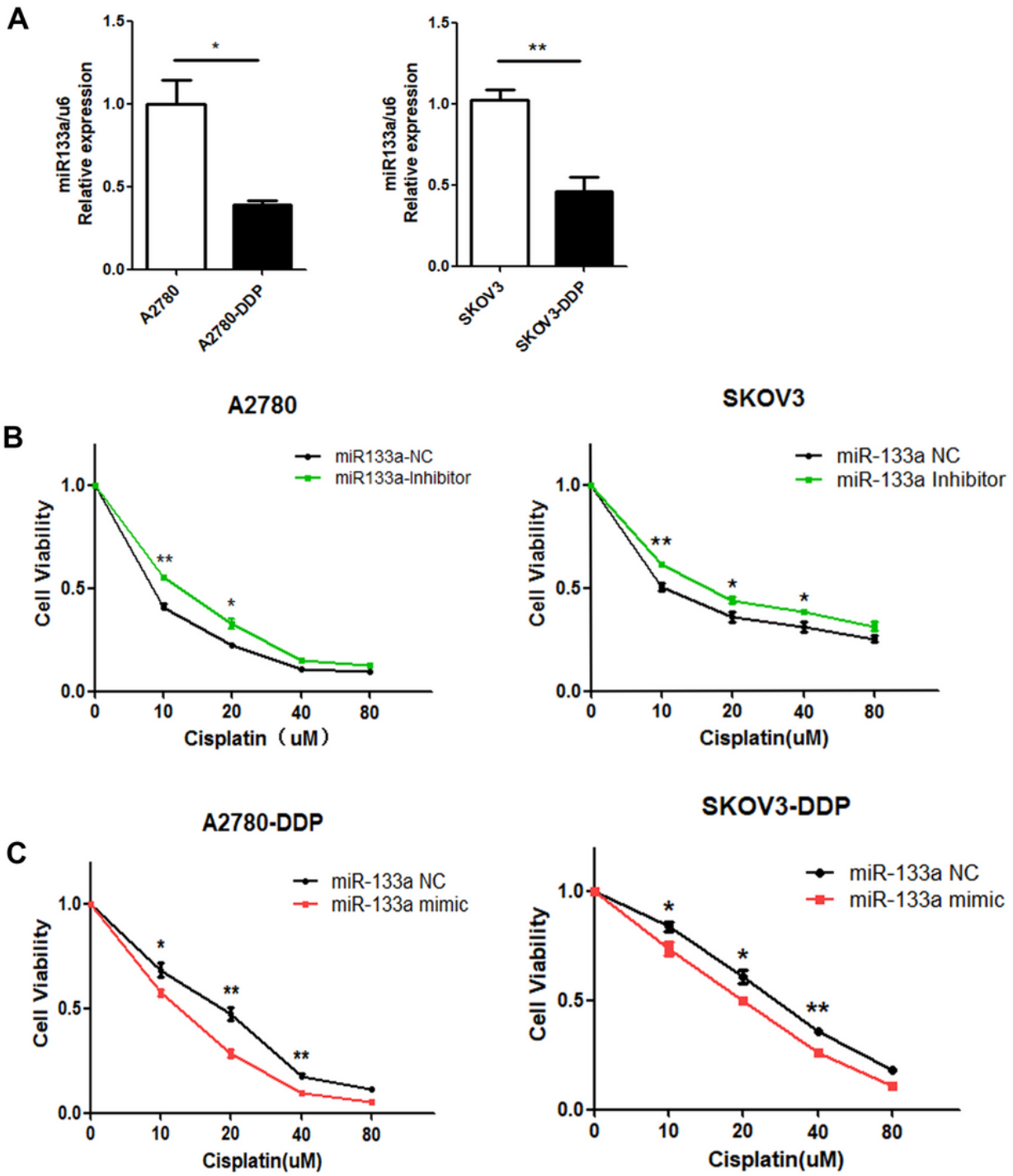

Figure 1

miR-133a can reduce cisplatin resistant in cisplatin-resistant ovarian cancer cells lines. (A) miR-133a is downregulated in SKOV3-DDP and A2780-DDP cell lines compared with SKOV3 and A2780, respectively. (B) miR-133a knockdown induce cisplatin-resistant in A2780 and SKOV3 cells. (C) miR-133a mimic increase cisplatin sensibility inA2780-DDP and SKOV3-DDP cell lines. For comparisons, the Student's t-test were performed; *p < 0.05, **p < 0.01, *** p < .001.

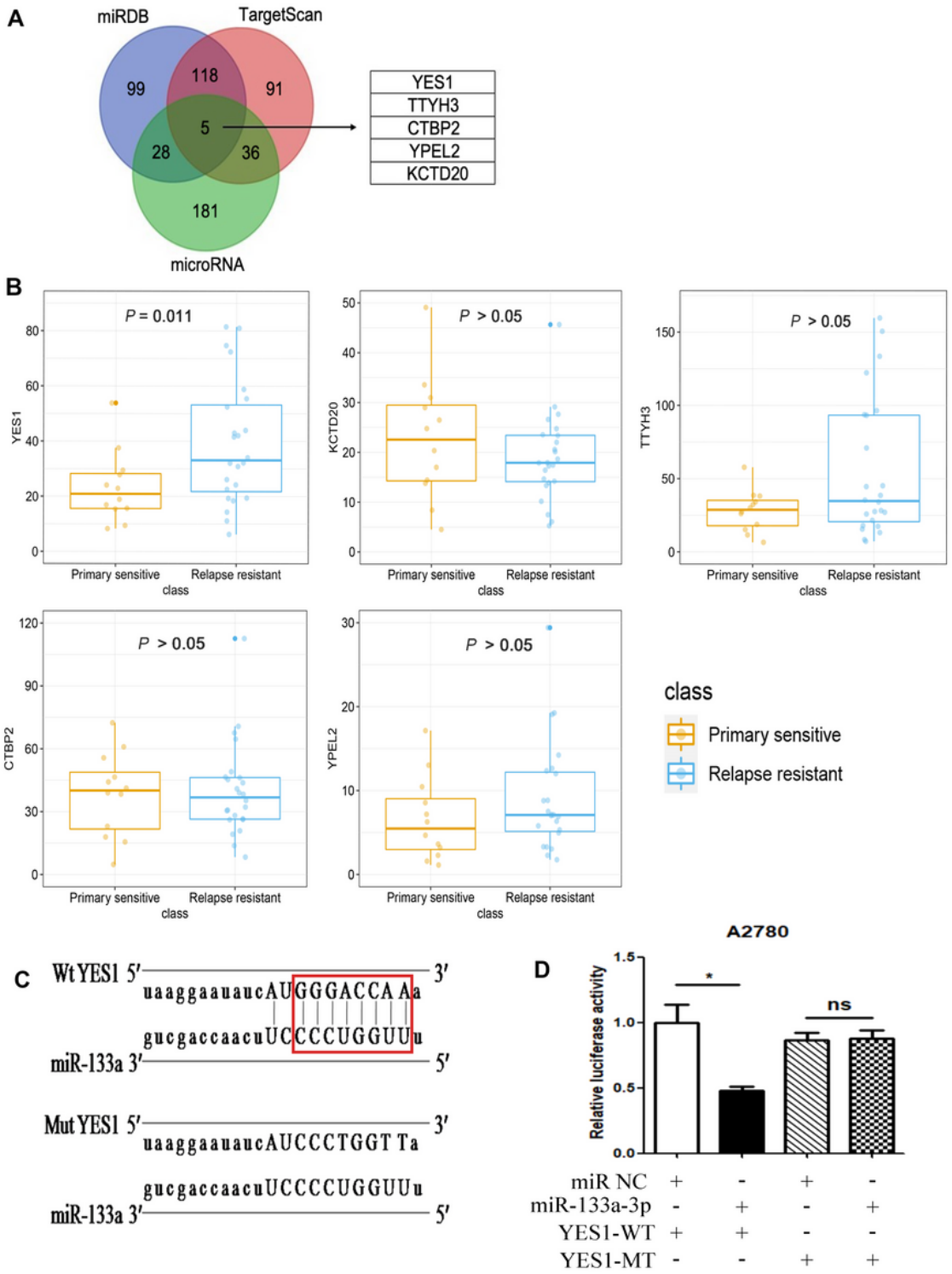

Figure 2

MiR-133a targets YES1 by binding with 3'-UTR of YES1. (A) YES1, TTYH3, CTBP2, YPEL2, and KCTD20 were selected as potential downstream genes regulated by miR-133a by an intersection analysis from 3 databases (miRDB, TargetScan, and microRNA). (B) YES1 expression level was dramatically increased in drug resistance ovarian cancer tissues compared with primary ovarian cancer tissues. (C) The predicted miR-133a binding sequence in 3′UTR of YES1 and the generation of dual-luciferase reporter plasmids of

YES1-Wt and YES1-Mut were shown. (D) Luciferase activity assays were carried out in A2780 cells co-transfected with miR-133a mimic or miR-133a NC and YES1-Wt or YES1-Mut. For comparisons, the Student's t-test were performed; *p < 0.05

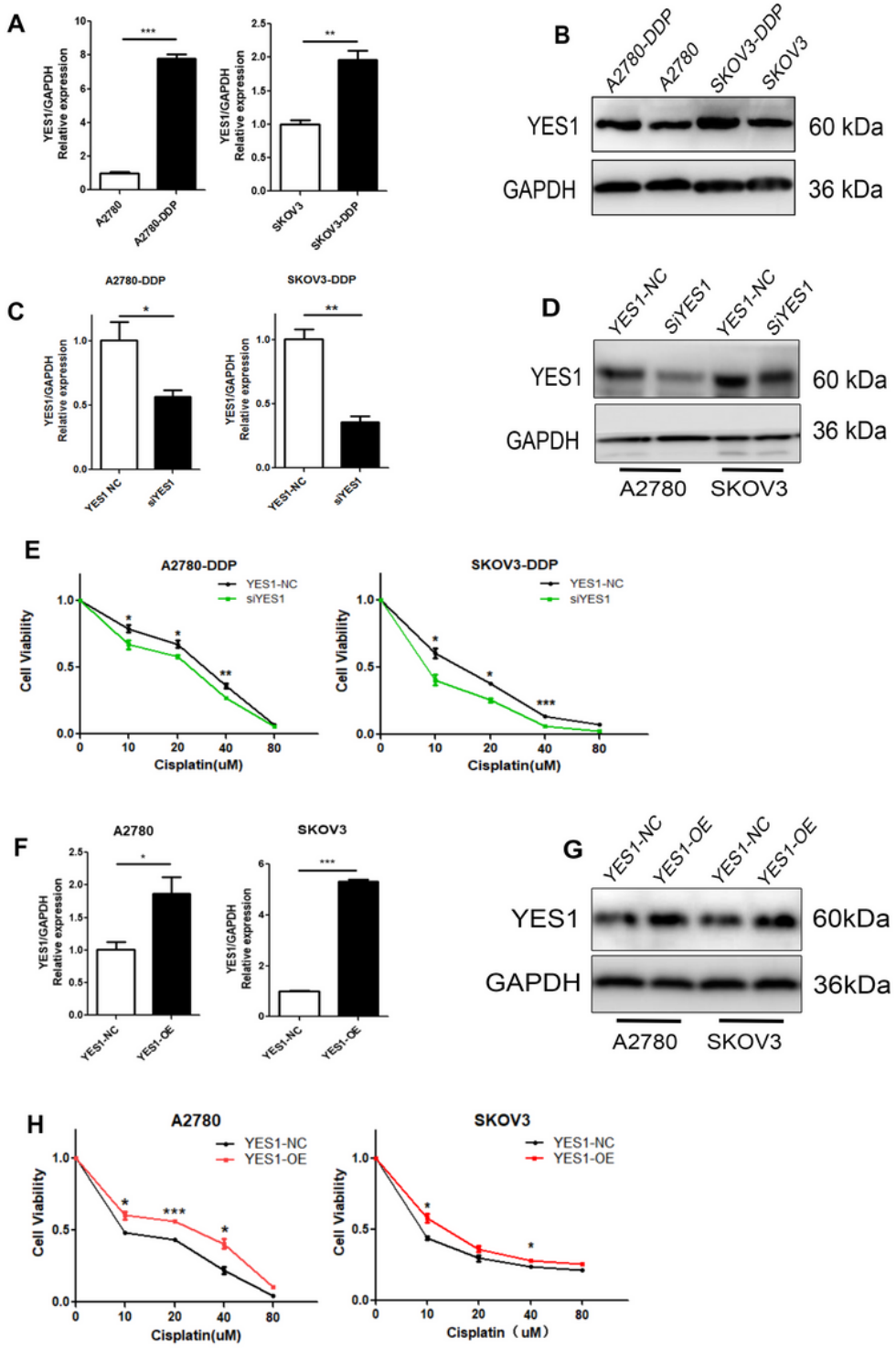

Figure 3

YES1 depletion decreases cisplatin sensitivity in ovarian cancer cell lines. (A and B) YES1 is significantly upregulated both in mRNA level (A) and protein expression (B) in A2780-DDP and SKOV3-DDP cell lines

compared with A2780 and SKOV3, respectively. (C and D) YES1 mRNA level (C) and protein expression (D) were reduced significantly after siRNA targeting YES1 transfection. (E) Yes1 knockdown increases cisplatin sensibility in A2780-DDP and SKOV3-DDP cell lines. (F and G) YES1 mRNA level (F) and protein expression (G) were increased significantly after YES1-OE vectors transfection. (H) Yes1 overexpression increases cisplatin resistant in A2780 and SKOV3 cells. For comparisons, the Student's t-test were performed; *p < 0.05, **p < 0.01

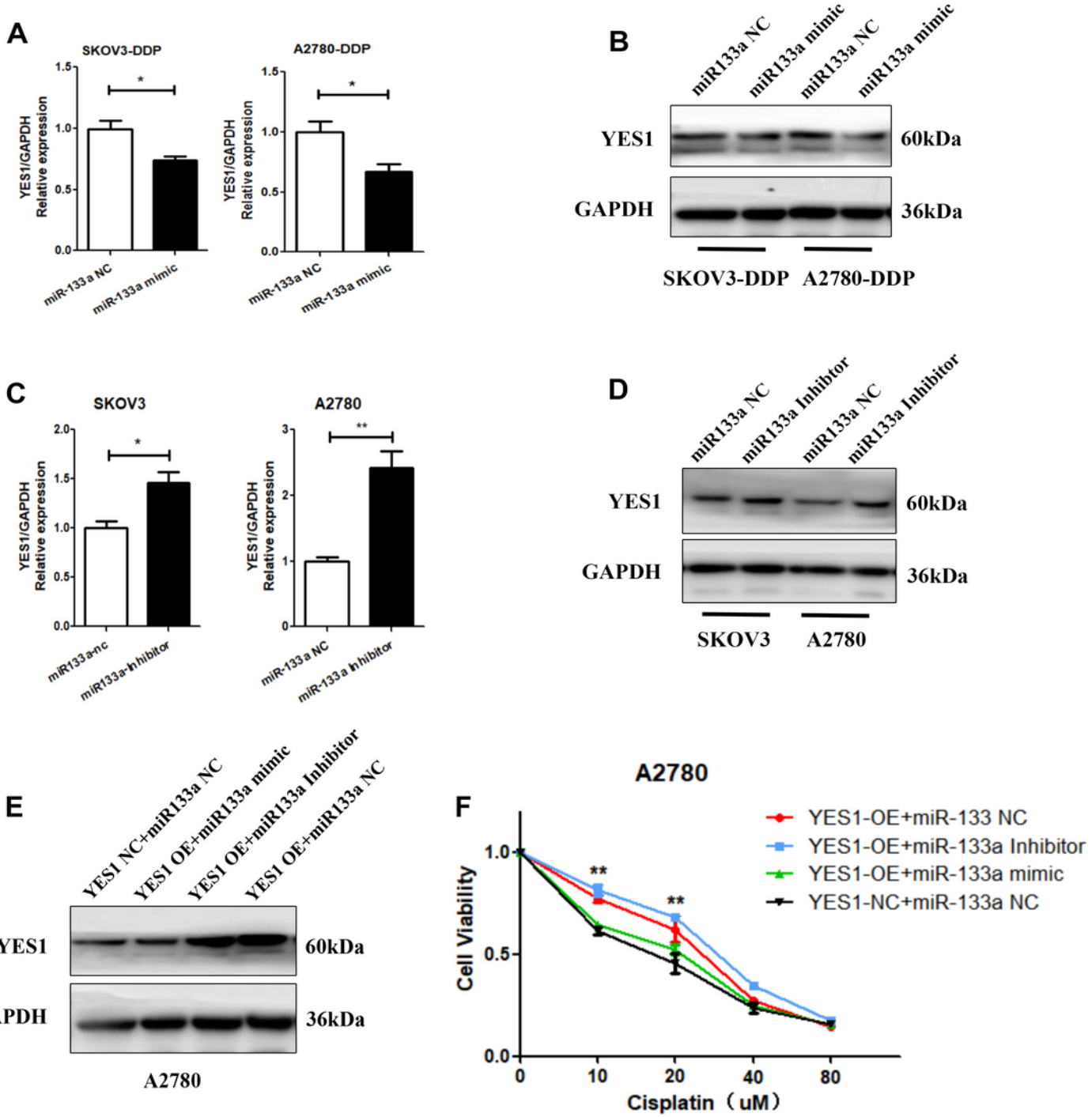

Figure 4

MiR-133a targets YES1 and downregulates its expression. (A and B) MiR-133a mimic inhibited the YES1 expression in SKOV3-DDP and A2780-DDP cells both at mRNA (A) and protein (B) levels. (C and D) MiR-133a inhibitor upregulated YES1 expression in SKOV3 and A2780 cells both at mRNA (C) and protein (D) levels. (E) MiR-133a mimic reduced the YES1 overexpression function induced by YES1-OE but MiR-133a inhibitor had no effect on the overexpression of YES1 compared with miR-133a NC + YES1-OE group. (F) Co-transfection of YES1-OE vector together with miR-133a mimics transfection antagonized YES1-OE-induced cisplatin resistant effects. For comparisons, the Student's t-test were performed; *$p < 0.05$, **$p < 0.01$

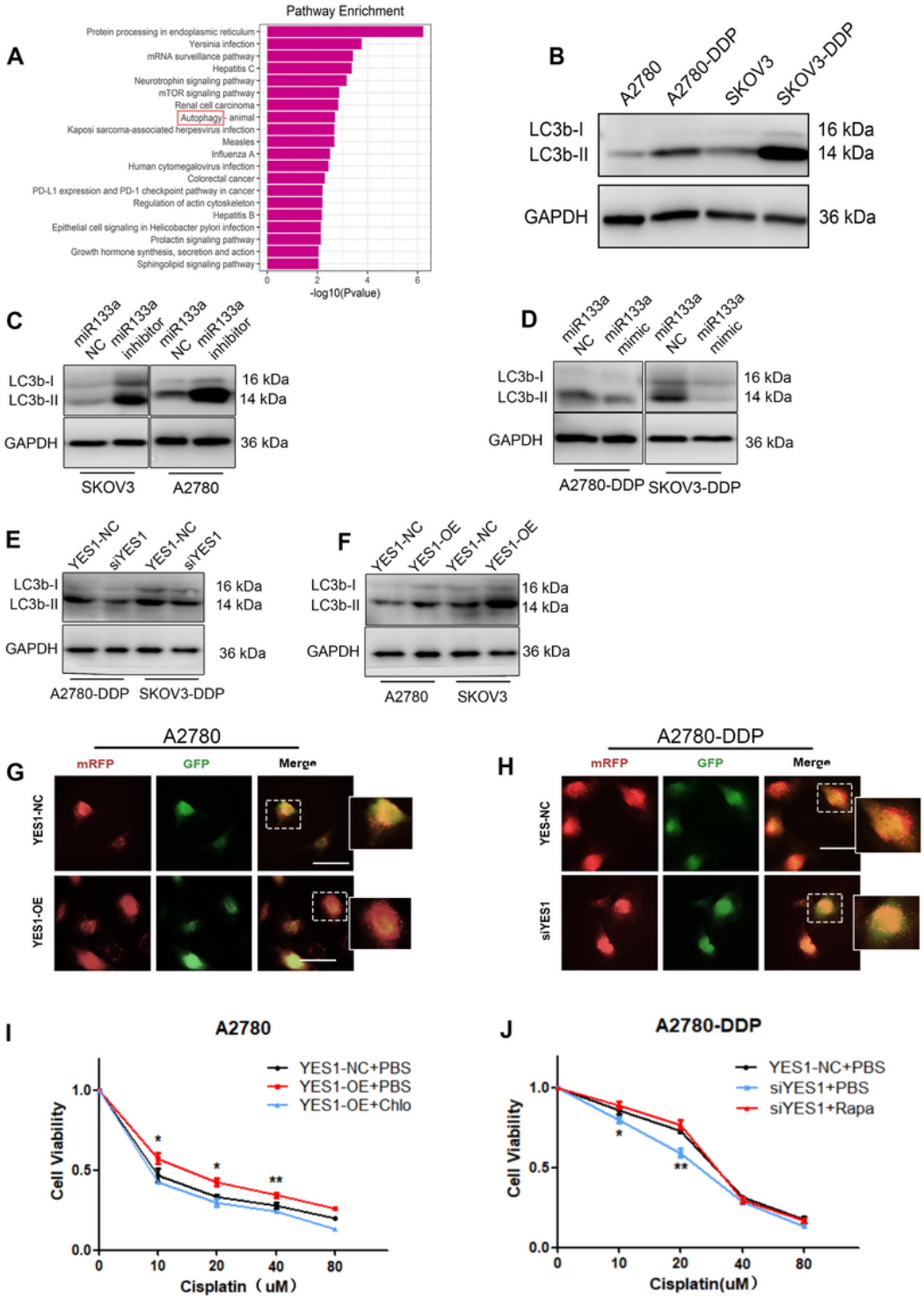

Figure 5

YES1 regulates cell autophagy in cisplatin resistance of ovarian cancer. (A) KEGG pathway enrichment analysis of the top 500 genes with high YES1 expression in ovarian cancer cohort from TCGA database. (B) LC3B expression were increased both in A2780-DDP and SKOV3-DDP compared wi th A2780 and SKOV3. (C) Inhibiting miR-133a significantly enhance LC3B expression in SKOV3 and A2780. (D) Transfection miR-133a mimic reduce LC3B expression in A2780-DDP and SKOV3-DDP. (E) YES1

knockdown reduces LC3B expression in SKOV3-DDP and A2780-DDP. (F) YES1-OE increases LC3B expression in SKOV3 and A2780. (G) Autophagic flux was enhanced after transfecting with mRFP-GFP-LC3 adenovirus in YES1-OE A2780(H)Autophagic flux was decreased after transfecting with mRFP-GFP-LC3 adenovirus in siYES1 A2780-DDP. (I)Autophagic antagonist-chloroquine reverses cisplatin resistance caused by YES1-OE in A2780. (J) Autophagic agonists-rapamycin weakens cisplatin sensitivity caused by siYES1 in A2780-DDP. For comparisons, the Student's t-test were performed; *p < 0.05, **p < 0.01

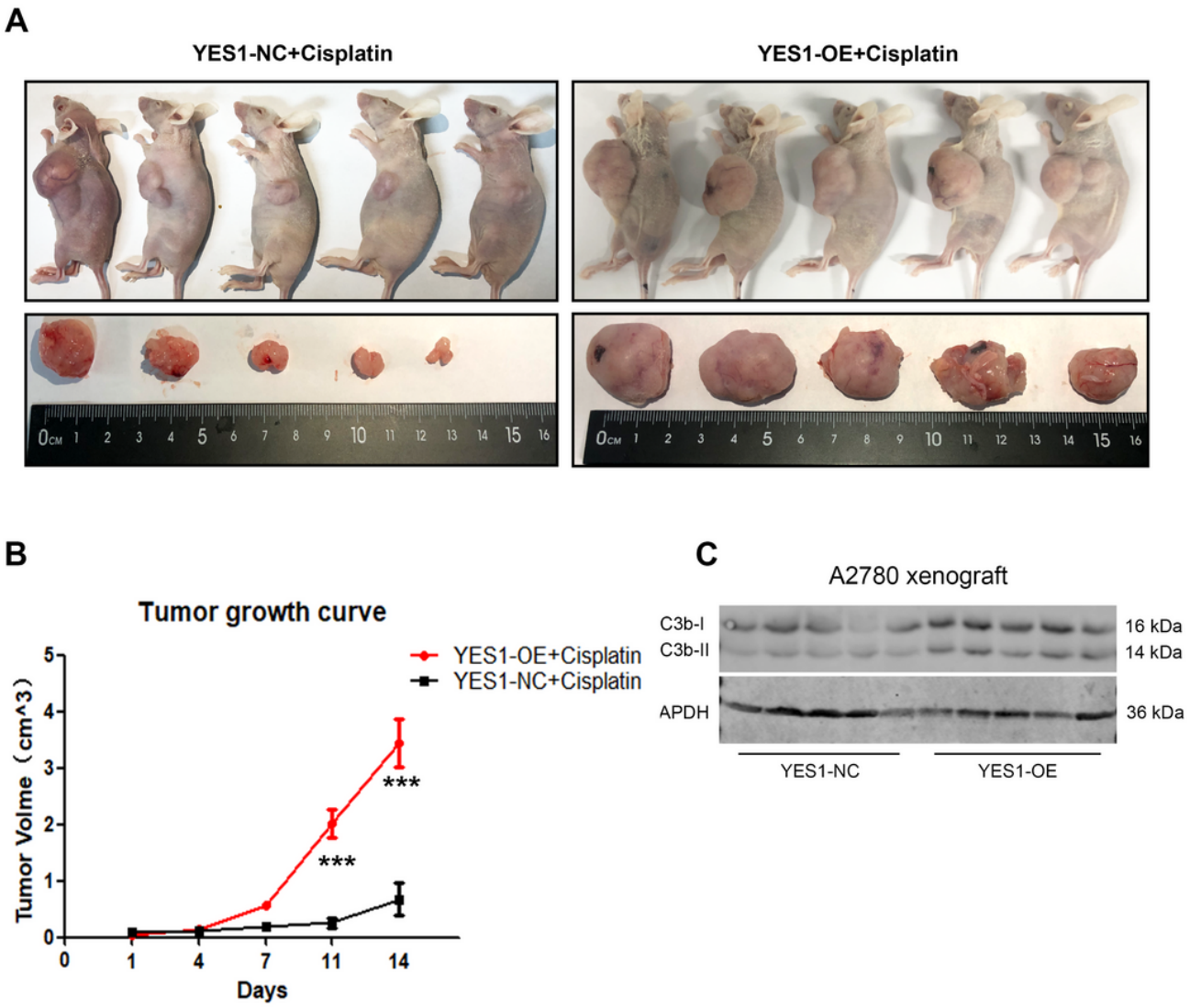

Figure 6

YES1 overexpression induces cisplatin resistance through regulating autophagy in vivo. (A) Nude mice and ovarian tumor tissues from YES1-NC and YES1-OE groups at day 14 after cisplatin injected intraperitoneally. (B) Growth curves of tumors were determined based on tumor size measured every 3 days (n = 5). (C) LC3B expression level of xenograft tumor in YES1-NC and YES1-OE groups after cisplatin treatment. For comparisons, the Student's t-test were performed; *p < 0.05, **p < 0.01