Integrin alpha subunit 2 (*ITGA2*) enhances cisplatin resistance in osteosarcoma

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Abstract

Osteosarcoma (OS) is the leading cause of cancer-related deaths in children and adolescents. Current standard treatment strategies, which combine chemotherapy and surgical resection, have significantly improved 5-year survival rates. However, overall survival rate has not dramatically improved over the past few decades. One of the critical factors affecting the overall survival of patients with OS is chemoresistance, either present in the primary tumor or acquired during chemotherapy. In the present study, we established cisplatin-resistant OS cell lines with continuous exposure to cisplatin and observed dysregulated wound healing signaling. Among the genes responsible for wound healing, we focused on *ITGA2* (integrin alpha subunit 2), and found that *ITGA2* was a critical determinant of cisplatin sensitivity in the OS cells in vivo. Depletion of *ITGA2* in cisplatin-resistant OS cells restored cisplatin sensitivity, with a concomitant decrease in previously reported cisplatin resistance signatures, including MYC and oxidative phosphorylation. Conversely, *ITGA2* overexpression leads to cisplatin resistance in OS cells. Consistent with these findings, OS patients with high *ITGA2* expression showed enhanced gene expression signatures of wound healing and worse prognosis compared to those with low *ITGA2* expression. There results suggest that *ITGA2* is a potential prognostic marker and therapeutic target in cisplatin-resistant OS.

Key words: Osteosarcoma, chemoresistance, cisplatin-resistant, *ITGA2* (integrin alpha subunit 2)

Significance Statement

Chemoresistance is a major obstacle in the treatment of osteosarcoma. Here, we showed that a dysregulated wound-healing pathway is commonly observed in OS cells with acquired cisplatin resistance. Depletion of *ITGA2* restored cisplatin sensitivity and led to marked growth suppression in cisplatin-resistant OS cells in vitro and in vivo. Consistently, patients with OS with higher *ITGA2* expression showed an enhanced wound healing gene expression signature and worse prognosis, rationalizing *ITGA2* as a potential prognostic marker and therapeutic target in cisplatin-resistant OS.

INTRODUCTION

Osteosarcoma (OS) is a common primary malignant bone tumor that causes cancer-related deaths in children and adolescents [1]. The standard therapeutic approach for OS involves a combination of tumor resection with a wide resection margin and chemotherapy to control tumor progression [2, 3]. Commonly used chemotherapeutic agents include methotrexate, cisplatin, and doxorubicin [4]. Among these drugs, cisplatin exhibits cytotoxic effects by intercalating DNA and is widely used for the treatment

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of various malignant tumors, including OS [5,6]. The above-mentioned multimodal treatment strategy significantly improved the 5-year survival from 15–20% to over 60% [7, 8, 9]; however, the survival rate of patients with OS has not reached satisfactory levels [10]. One of the reasons for this is that 35–45% of patients were reported to be refractory to chemotherapy [11], thus affecting overall survival. Thus, there is an urgent need to develop strategies to overcome chemoresistance in patients with OS. We previously reported that MYC activation and enhanced oxidative phosphorylation are significantly upregulated in OS cells with acquired cisplatin resistance [12]; however, the underlying molecular mechanisms are not fully understood.

The intimate link between wound healing and cancer was first proposed by Virchow R [13]. Accumulating evidence suggests that wound healing and cancer share common cellular and molecular processes [14, 15]. Essential events in wound healing, such as the inflammatory response and extracellular matrix (ECM) remodeling, are often dysregulated in cancer and affect tumor growth, invasion, and metastasis [16]. However, it is not well understood whether dysregulated wound healing contributes to cisplatin resistance.

Integrins form a large family of heterodimeric cell-surface receptors that play important roles in diverse cellular functions, including wound healing and ECM remodeling [17]. Integrins are involved in cancer progression and metastasis by accelerating cell survival, proliferation, and invasion [18, 19]. Integrins do not function as oncogenes in general, but cooperate with oncogenes and receptor tyrosine kinases to facilitate tumor development, including KRAS- and PI3K-mediated oncogenesis [18]. *ITGA2* encodes integrin subunit α2 (also called CD49b) and forms a heterodimer with β1 subunit, integrin α2β1. Integrin α2β1 functions as a receptor for collagens and their related proteins. Integrin α2β1 positively regulated tumor development by accelerating invasion, migration, and metastasis in multiple cancers, including OS [20,21,22]. Integrin α2β1 also enhanced angiogenesis in mouse melanoma models [23]. Conversely, integrin α2β1 has been reported to suppress breast cancer metastasis [24], suggesting a context-dependent function. Furthermore, single nucleotide polymorphisms (SNPs) in *ITGA2* are associated with multiple types of human cancers, such as breast, colorectal, and gastric cancers, and melanoma [25, 26, 27, 28, 29]. However, it is unclear whether an aberrant regulation of *ITGA2* contributes to cisplatin resistance in OS.

In this study, we established cisplatin-resistant U2OS and Saos-2 cells with continuous exposure to cisplatin and found that the genes involved in wound healing were commonly upregulated. Among the responsible genes, we focused on *ITGA2* and found that *ITGA2* depletion restored cisplatin sensitivity in the cisplatin-resistant U2OS cells in in vitro and mouse xenograft models. In contrast, *ITGA2* overexpression enhances cisplatin resistance. Furthermore, *ITGA2* depletion markedly reduces MYC expression and oxidative phosphorylation in cisplatin-resistant U2OS cells, which are significant signatures of cisplatin-resistant OS cells[12]. Consistent with these findings, patients with high *ITGA2* expression showed activation of wound healing-related pathways and a worse prognosis. In summary, *ITGA2* may serve as a novel therapeutic target for restoring cisplatin sensitivity, and as a prognostic marker for predicting cisplatin resistance.

MATERIALS AND METHODS

Cell culture

The human osteosarcoma (OS) cell lines U2OS and Saos-2 were obtained from the Riken Bioresource Research Center (Tsukuba, Ibaraki, Japan). U2OS cells were maintained in McCoy's 5A medium (Thermo Fisher Scientific, Waltham, MA, USA) and Saos-2 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; Thermo Fisher Scientific), which were cultured at 37°C in a humidified atmosphere containing 5% CO_2. All culture media were supplemented with 10% heat-inactivated fetal bovine serum (FBS; JRH, Nichirei Biosciences, Tokyo, Japan), penicillin (100 units/ml; Thermo Fisher Scientific), and streptomycin (100 μg/mL; Thermo Fisher Scientific). Cisplatin-less sensitive U2OS and Saos-2 cells (CisLS-U2OS and -Saos-2 cells, respectively) were established and maintained as previously described[12]. In brief, 1×10^6 U2OS and Saos-2 cells were cultured on a 10-cm dish in the presence or absence of cisplatin (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan) at concentrations ranging from 1 to 30 μM over 12 months. The medium was changed every other day with occasional passages to maintain cellular confluency. The cells resistant to 5 μM cisplatin were used for the following experiments.

RNA sequencing data analysis

RNA sequencing and data processing were performed as previously described [12]. Genes (Ensembl ENSG ID) with fragments per kilobase of exon per million mapped (FPKM) > 0.01 in both CisLS and the parental control were used for further analyses. The 500 genes upregulated and downregulated in

CisLS-U2OS and CisLS-Saos-2 cell lines were obtained from the ranked gene list generated by Gene Set Enrichment Analysis (GSEA), using log2_ratio_of_classes as metrics for ranking genes against the parental control U2OS and Saos-2 cells, respectively. Metascape pathway analysis was conducted using 'Express analysis' mode[30].

RNA interference and overexpression of *ITGA2*

To knock down *ITGA2*, lentiviral vectors containing shRNA against *ITGA2* (Merck, sh1: TRCN0000308081 and sh2: TRCN0000057731) were transduced into CisLS-U2OS cells (CisLS-U2OS-KD1 and -KD2, respectively). A lentiviral vector containing a scrambled shRNA sequence (Addgene, #162011) was used to establish the control CisLS-U2OS cells (CisLS-U2OS-SC). After the introduction, the cells were selected with 1 μg/mL of puromycin (Thermo Fisher Scientific) for four days. *ITGA2* knockdown was confirmed by qRT-PCR and western blotting. The target sequence of *ITGA2* sh1 is 5'-CCGGCCAGATAGTGCTATATA-3' and that of sh2 is 5'-ATGGCAATATCACGGTTATTC-3.'

For forced *ITGA2* expression, U2OS cells were transfected with *ITGA2* in a lentiviral vector (Sino Biological, HG13024-NM) (U2OS-*ITGA2*). U2OS cells with an empty lentivirus vector (U2OS-empty) were used as controls. After introduction, the cells were selected with 250 μg/mL of Hygromycin (Thermo Fisher Science) for one week. *ITGA2* protein expression levels were evaluated by western blot analysis.

Lentivirus production

293JD packaging cells (a kind gift from Dr. James Ellis, University of Toronto) were seeded on 6 cm dishes at 2×10^6 per dish in DMEM supplemented with 10% FBS and 100 μg/mL penicillin/streptomycin 24 hr before vector transfection. For packaged lentiviral production, 94 μL of OptiMEM (Thermo Fisher Scientific) and 6 μL of FuGENE HD Transfection Reagent (Promega, WI, USA), 1 μg plasmid vector, 0.75 μg of psPAX2 (Addgene, #12260), 0.5 μg of pMD2.G (Addgene, #12259) per 6 cm dish was used. The supernatant containing the viral particles was collected from the dishes 48 hr after transfection.

Real-time quantitative PCR (RT-qPCR)

Total RNA was extracted using a Monarch Total RNA Miniprep Kit (New England Biolabs). First-strand cDNA was synthesized using the iScript™ cDNA Synthesis Kit (BioRad), followed by PCR specific for the target genes. RT-qPCR was performed using SYBR premix Taq (ThermoFisher Scientific) with 35 cycles (1 cycle: 98°C for 10 sec - 54°C for 30 sec - 68°C for 15sec), and data were collected using Real-time PCR system (ThermoFisher Scientific). β-actin was used as the internal control. The PCR primers for *ITGA2* were 5'-CGGTTATTCAGGCT-CACCGA-3' (forward) and 5'- TTGGTGCACCTAC-CAAGAGAGC-3' (reverse) and those for beta-actin were 5'- CATGTACGTTGCTATCCAGGC-3' (forward) and 5'- CTCCTTAATGTCACGCACGAT-3' (reverse).

Live cell metabolic assay

Mitochondrial functions were measured as the extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) using a Seahorse Bioscience XFe96 Extracellular Flux Analyzer (Agilent Technologies, CA, USA). Cells were seeded in the wells ($1x10^4$ cells/well) of a standard XFe96 microplate (Agilent Technologies) and incubated at 37℃. Twelve hours later, cells were analyzed using flux analyzer. Analysis medium supplemented with 1-mM pyruvic acid, 10-mM glucose, and 2-mM glutamine (pH 7.4) was freshly prepared according to the manufacturer's protocol.

Data analysis from osteosarcoma patients and OS cell lines

Normalized gene expression data were obtained from the International Cancer Genome Consortium (ICGC) data portal for OS[31], and the Cancer Cell Line Encyclopedia (CCLE) project[32, 33] for the CADOES1, Saos-2, MHHES1, SKES1, HOS, G292CLONEA141B1, SKPNDW, SKNEP1, SJSA1, CAL78, MG63, and U2OS cell lines. GSEA analyses[34] were conducted to compare the following groups: the patient with high *ITGA2* expression (n = 14, log2 (RPKM (fragments per kilobase of exon per million reads mapped)) > 0.15) vs. those with low *ITGA2* expression (n = 21, log2(RPKM) < -0.15); the cell line with high *ITGA2* expression (n = 7, log2 (RPKM) > 0.05) vs. those with low *ITGA2* expression (n = 5, log2(RPKM) < -0.05). Analyses were performed using the HALLMARK, KEGG, and REACTOMEK gene sets. The normalized enrichment score (NES) was calculated using GSEA. A false discovery rate (FDR) q-value <0.25 was considered significant. Principal component analysis, pathway analysis, and heatmap preparation were performed using iDEP.96[35]. Kaplan-Meier survival curves were prepared and analyzed using Prism 9 (GraphPad, CA, USA).

Xenograft in mice

CisLS-U2OS-KD2 and -SCR (scramble) cells were dissolved in 150 μL of McCoy5 medium plus 50% Matrigel (Corning Inc.) at 1.5×10^6 cells per tube. The mixture was subcutaneously injected into the back of 5-week-old male nude mice. The tumor size and general condition of the mice were monitored on alternate days. Intraperitoneal cisplatin administration was initiated three weeks after transplantation at a concentration of 4 mg/kg three times a week. The tumor size, body weight, and general condition were monitored daily. Four weeks after cisplatin administration, the mice were euthanized and the tumor size was evaluated. Tumor volume was determined according to the equation $(LxW^2)/2$, where L is the tumor length and W is the tumor width. Mice were housed in isolated cages with individual ventilation and water supply on a 12-hr light/dark cycle in a 22°C temperature- and humidity-controlled SPF room. The protocol for the mouse experiments was approved by the Local Institutional Animal Care and Research Advisory Committee of Kindai University Faculty of Medicine. The animal facilities are committed to the highest ethical standards of care for animals used for the purpose of continued progress in the field of human medicine.

Statistical Analyses

Results are presented as mean ± standard deviation (SD). Analysis between two groups was conducted using an unpaired Student's t-test. The Kaplan-Meier survival curve was tested using Gehan-Breslow-Wilcoxon and Log-rank (Mantel-Cox) tests. $P < 0.05$ was considered significant.

RESULTS

Involvement of wound healing signals in cisplatin resistance of OS cells

To further investigate the mechanism of cisplatin resistance, we compared the gene expression profiles of CisLS-U2OS cells and the newly established CisLS-Saos-2 OS cell lines. Among the top 500 upregulated and downregulated genes in each CisLS cell line against its parental control, the 31 genes were found to be positively altered in CisLS-U2OS and CisLS-Saos-2 cells (Figure 1A). To explore the biological processes involving these shared genes, we conducted Metascape pathway analysis[30]. This analysis identified 'response to wounding (GO:0009611)' and 'wound healing (GO:0042060)' as significantly upregulated common pathways (Figure 1B and 1C), implicating the contribution of wound response genes in cisplatin resistance.

Next, we sought to examine the expression levels of eleven genes belonging to the 'response to wounding' upregulated in CisLS-U2OS and -Saos-2 cells (Figure 1C) with patient outcome under the treatment of cisplatin. *ITGA2* is the strongly involved in this wound healing pathway. We were unable to evaluate OS because of limited data availability. The expression levels of none of the other genes (*PAGE4, HEYL, HBE1, HBG, SPP1 and LCP1*) significantly correlated with the overall survival of the same cohort (data not shown). These results prompted us to investigate the role of *ITGA2* in cisplatin resistance of OS cells.

***ITGA2* expression levels in each OS cell**

To test the relevance of increased *ITGA2* expression to cisplatin resistance in CisLS-U2OS cells, we depleted *ITGA2* in CisLS-U2OS cells. Two shRNAs against *ITGA2*, shRNA1 and shRNA2, were introduced lentivirally into CisLS-U2OS cells (CisLS-U2OS -KD1 and -KD2, respectively). CisLS-U2OS cells expressing scrambled shRNA (CisLS-U2OS-SC) were also established as controls. qRT-PCR (Figure 2A) confirmed the decreased expression of *ITGA2* in CisLS-U2OS-KD1 and -KD2 cells. The expression level of CisLS-U2OS was set as 100, each CisLS-U2OS-KD1, -KD2 cells were 19 and 17. Thus, we decided to use U2OS-R-KD2 cells for subsequent analyses. To examine the contribution of *ITGA2* to cisplatin resistance in OS cells, we introduced exogenous *ITGA2* and an empty vector into U2OS cells (U2OS-OE and -EV, respectively). *ITGA2* transcript levels were significantly higher in U2OS-OE than in U2OS-EV cells, the detail value was 112 times (Figure 2B).

***ITGA2* depletion affected oxidative phosphorylation in cisplatin resistant OS cells**

The oxidative phosphorylation generates ROS as a byproduct. Increased ROS causes oxidative damage to cellular molecules. This damage can lead to genomic instability, that might enable cancer cells to survive despite chemotherapy. To examine *ITGA2* expression level effect on mitochondrial function. Compared to CisLS U2OS cells, CisLS-U2OS-KD cells were increased mitochondrial function (Figure 2C). And CisLS-U2OS-KD cells were also highly exhibited basal respiration, proton leak, and ATP production (Figure 2D). The data indicate that *ITGA2* knockdown inhibit oxidative phosphorylation and cause to decrease ROS generation.

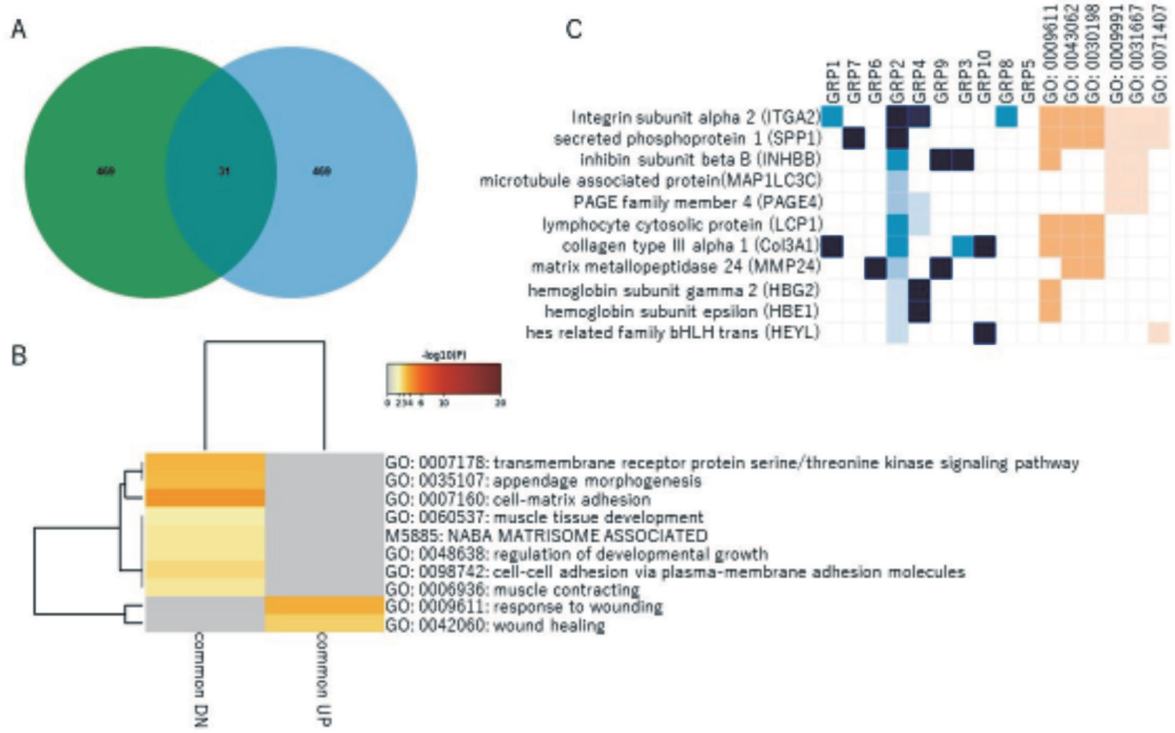

Figure 1. (A) The top 500 most highly expressed genes were extracted from the U2OS-R(green) and the Saos-2-R(blue) compared to each parental cells. Among these genes, we identified 31 genes that were commonly up-regulated. (B) The genes with commonly up-regulated were analyzed to determine which pathways were involved by using Metascape. (C) Among the commonly upregulated genes, those are particularly strongly involved in wound healing pathway by using Metascape. (GRP1; cell matrix adhesion GRP7; regulation of developmental growth GRP6; cell-cell adhesion via plasma-membrane adhesion molecules GRP2; response to wounding GRP4; wound healing GRP9; NABA matrisome associated GRP3; transmembrane receptor protein serine/threonine kinase signaling pathway GRP10; cell-matrix adhesion GRP8; muscle contraction GRP5; appendage morphogenesis)

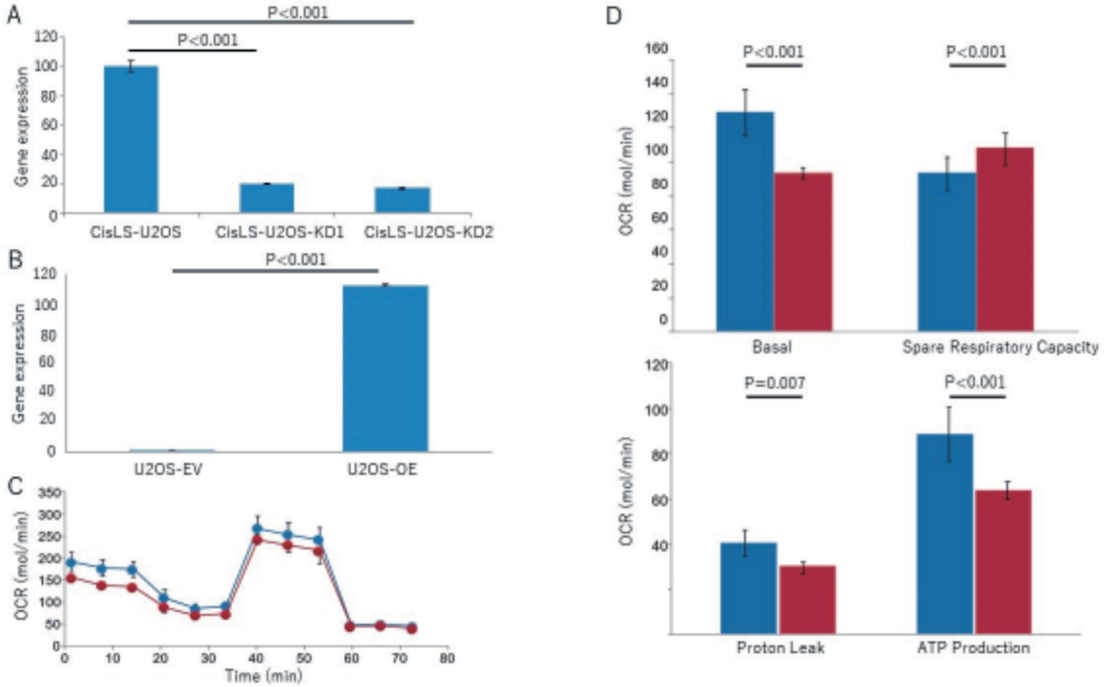

Figure 2. Results of Comparison Experiment between CisLS-U2OS and CisLS-U2OS KD cells

(A) RNA was extracted from each cell and the expression levels of *ITGA2* were compared by qPCR. The expression level of CisLS-U2OS was set as 100.

(B) The expression levels of *ITGA2* were compared by qPCR. The expression level of U2OS-EV was set as 1.

(C, D) The expression level of *ITGA2* was measured using a flex analyzer to determine whether it affects mitochondrial function, the major energy metabolic pathways in the cell.

ITGA2 depletion restored cisplatin resistance in vivo

To examine whether *ITGA2* depletion reduced tumor development in vivo, we established a mouse xenograft model. CisLS-U2OS-KD2 and control CisLS-U2OS-SC cells were suspended in a mixture of 50% McCoy's 5A medium and 50% Matrigel, and transplanted into the backs of nude mice using a 26G needle (n=5 for each cell line). Cisplatin treatment was started three weeks after the tumor became palpable and continued for four weeks (Figure 3A). After cisplatin treatment, the average size of the CisLS-U2OS-KD2 tumors was smaller than that of the CisLS-U2OS tumors. Notably, one CisLS-U2OS-KD2 tumor could not be recovered because of its significantly smaller size. Thus, CisLS-U2OS-KD2 tumors were more susceptible to cisplatin than the CisLS-U2OS control tumors (Figure 3B and 3D). On the other hand, the size of tumor without cisplatin treatment were compared. Both groups of cells were increased (Figure 3C and 3E). These data indicate that *ITGA2* knockdown increases sensitivity to cisplatin in vivo.

Patients with higher *ITGA2* expression showed worse prognosis

Our results using OS cell lines showed that *ITGA2* plays an important role in the chemoresistance of OS. Therefore, we analyzed the Cancer Cell Line Encyclopedia (CCLE) project[32, 33] and OS patient data obtained from the International Cancer Genome Consortium (ICGC) data portal for patients with OS[31]. First, we compared cell lines with high *ITGA2* expression (n = 7, log2 (RPKM) > 0.05) to those with low *ITGA2* expression (n = 5, log2 (RPKM) < -0.05). *ITGA2* transcript levels were positively correlated with the IC_{50} values of cisplatin treatment in OS cell lines, with the exception of Saos-2 in the CCLE data (Figure 4A). GSEA analysis of the CCLE data showed that HALLMARK_MYC_V1 and V2, KRAS_UP, and EWING_SARCOM_PROGENITOR_UP signatures were enriched in *ITGA2*-high compared to *ITGA2*-low cells (Figure 4B). These findings suggest that factors other than *ITGA2* contribute to cisplatin resistance in untreated Saos-2 cells. However, *ITGA2* was significantly upregulated in CisLS-Saos-2 cells (Figure 1), further demonstrating the importance of *ITGA2* upregulation in

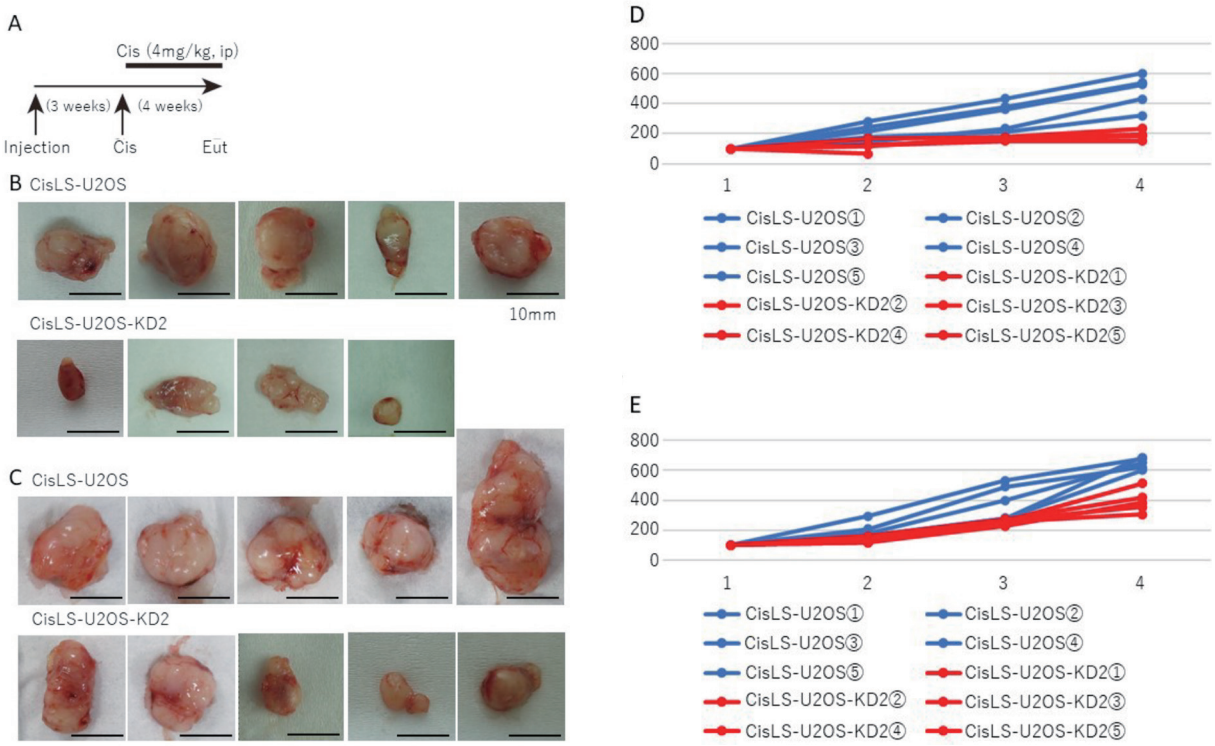

Figure 3. Effect of cisplatin treatment on each U2OS cells. Tumor xenografts were formed by two groups.
(A) The protocol of the xenograft experiment. Representative photographs of tumors treated with cisplatin (B) and without cisplatin (C) are shown. Each bar indicates 10 mm. Tumor volume was calculated with cisplatin treatment (D) and without treatment (E). At three weeks after injection was set as 100.

establishing acquired resistance to cisplatin. Next, we analyzed the human sample data. Principal component analysis of patients with high *ITGA2* expression (n = 14, log2 (RPKM) > 0.15) and those with low *ITGA2* expression (n = 21, log2 (RPKM) < -0.15) revealed that patients with OS with high *ITGA2* and those with low *ITGA2* were segregated into two different groups (Figure 4C). Differential gene expression analysis revealed that gene expression signatures related to wound healing were significantly upregulated in *ITGA2*-high patients, including immune system process, ECM organization, vascular development, and cell migration (Figure 4E). In addition, GSEA revealed that signatures related to KRAS (HALLMARKS_KRAS_SIGNALING_UP, NES=2.30), integrin (INTEGRIN_CELL_SURFACE_NETRACTIONS, NES=2.39), and EMT (HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION, NES=2.60) were enriched with high NES scores (Figure 4F and 4G). Furthermore, we analyzed patient survival using donor vital status data (n = 26) and found that patients with OS with high *ITGA2* expression had a worse prognosis (Figure 4H). These findings strongly suggest that high *ITGA2*

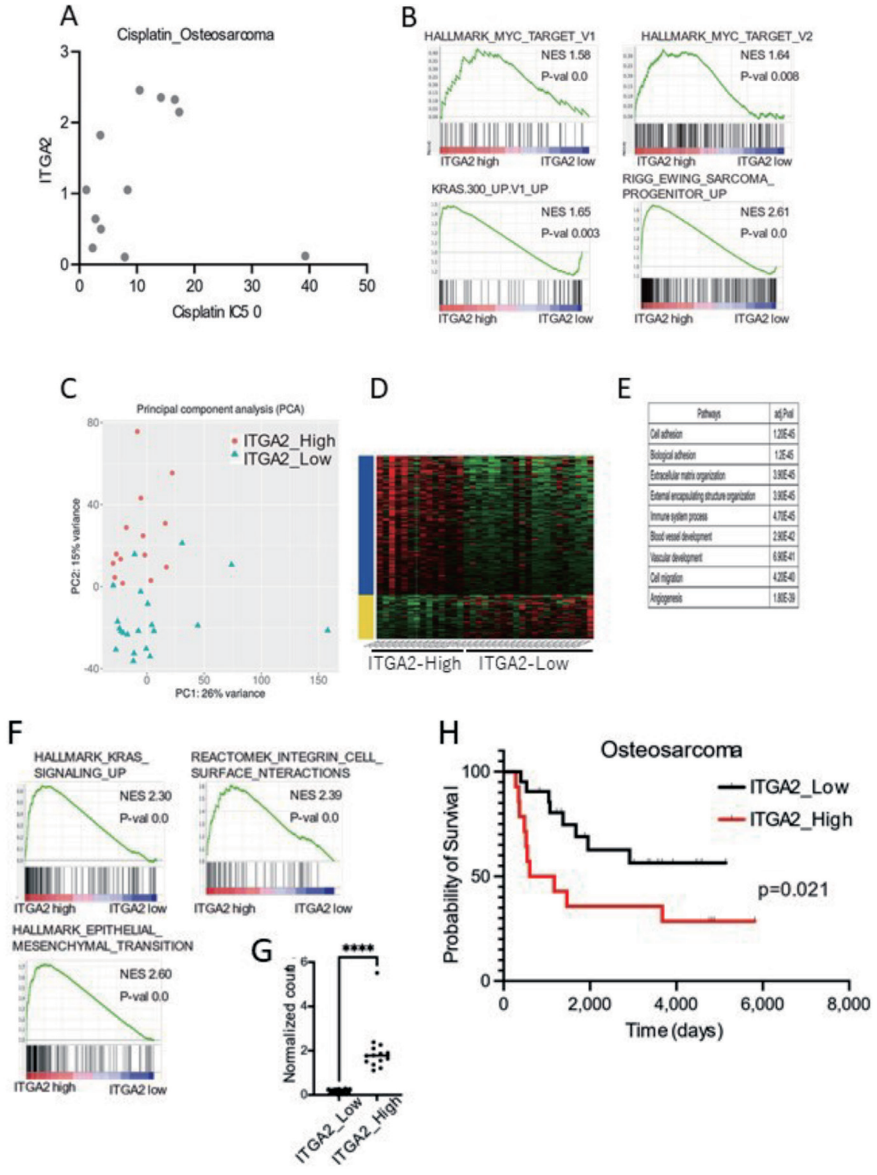

Figure 4. High *ITGA2* in OS patients is associated with worse prognosis.
(A) *ITGA2* transcript levels were positively correlated with the IC_{50} values of cisplatin treatment in OS cell lines. (B) GSEA analysis showed those signatures were enriched in *ITGA2*-high cells. (C) OS patients were divided into two groups by *ITGA2* expression levels in principal component analysis. (D,E) Wound healing signatures were significantly upregulated in *ITGA2* high patients. (F,G) GESA revealed those signatures were enriched with high NES scores. (H) High *ITGA2* expression OS patients are possibly aggressive.

expression negatively impacts the prognosis of patients with OS by activating wound healing and related signaling pathways.

DISCUSSION

MAP-based chemotherapy for OS is well established, but response rates (26–43%) for each agent are unsatisfactory[36, 37]. There are also osteosarcomas that are resistant to cisplatin[38]. Therefore, we investigated the effect of *ITGA2* on cisplatin resistance in OS.

ITGA2 encodes an α2 integrin that forms a heterodimer with the β1 subunit of α2β1 integrin and acts as a transmembrane receptor for cell adhesion to the ECM[39]. As a collagen receptor, α2β1 integrin can also bind laminin, fibronectin, and E-cadherin protein[40, 41]. Furthermore, it has been reported that α2β1 integrin is expressed primarily in neovascular vessels, activated endothelial cells, and immune cells in vivo[42, 43]. In addition, α2β1 integrin is absent in most normal organs and resting endothelial cells, but overexpressed in a variety of cancer cells[42, 44]. Furthermore, α2β1 promotes cancer cell migration and invasion and angiogenesis[45, 46]. In addition, some studies have investigated the expression profile of α2β1 integrin in cancer cells, which positively correlates with aggressive behavior in cancer pathology[47, 48].

In OS, high expression of miR-128 has been reported to suppress OS cell migration, invasion, and epithelial-mesenchymal transition development by targeting *ITGA2*[49]. Decreased expression of miR-548c-3p in OS contributes to cell proliferation by targeting *ITGA2*[50]. The results of the current study suggest that *ITGA2* mitigates cisplatin resistance in OS.

There are numerous reports on the downstream pathways of *ITGA2* in other malignancies. The PI3K/AKT signaling pathway has been reported as a downstream pathway in uterine leiomyomas[51]. HMGA2-FOXL2 is a downstream target in gastric cancer[52]. This is a downstream pathway of *ITGA2* in chemotherapy resistance, and is also a possible downstream pathway in OS. FAK-RAC1-PAK is a downstream pathway involved in lung cancer[53]. In addition, the Yes-associated protein (YAP) is thought to be downstream of hepatocellular carcinoma[54]. Several of these pathways have been reported as downstream pathways of *ITGA2* in other malignancies, and any one of these pathways could be a possible downstream pathway for the effect of *ITGA2* on cisplatin resistance in OS. Although downstream pathways were not examined in this study, they are possible. We believe that additional experiments are required in the future.

Limitations

This study had some limitations. First, it is an in vivo experiment using a limited number of OS cell lines. Second, downstream pathways were not investigated. Last, this study did not test for cisplatin resistance in human OS cells. However, the fact that *ITGA2* alleviates cisplatin resistance has been clarified, and further studies are required to confirm this.

Conclusion

The results of this study indicate that the addition of *ITGA2* alleviates cisplatin resistance. This suggests that the addition of *ITGA2* drugs to OS chemotherapy may enhance the efficacy of cisplatin.

AUTHOR CONTRIBUTIONS

Analyzed the data and wrote the manuscript. All authors have read and approved the final manuscript.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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