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Research Article
USP9X/HOXA1 Contributes to the Cisplatin-Resistentance of Oral Squamous Cell Carcinoma *in vitro* and *in vivo*

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Abstract

Background and Objective: The acquisition of Chemoresistance to Cisplatin (CDDP) is known as a challenge in Oral Squamous Cell Carcinoma (OSCC) therapy; but causative factors responsible for CDDP resistance in OSCC are largely ill-defined. Thus, the role and underlying mechanism of HOXA1 was explored in this study. **Materials and Methods:** The mRNA expression levels in normal and CDDP-resistant OSCC cells, were detected by qRT-PCR. The CCK-8, TUNEL and colony formation assays were performed to elevate the effect of HOXA1 on CDDP-resistance, cell viability and apoptosis of OSCC and CDDP-resistant OSCC (OSCC/R) cells. Western blot, Co-IP, ubiquitination and CHX analyses were carried out to uncover the regulation of USP9X on HOXA1 in CDDP-resistant OSCC, as finally confirmed by rescue experiments. The HOXA1 was highly expressed after developing into of CDDP resistance in OSCC cells. **Results:** A positive correlation was investigated between the expression level of HOXA1 and the IC_{50} value of CDDP in OSCC cells. The ability against CDDP-induced cell viability impairment and apoptosis was enhanced by overexpressing HOXA1 in OSCC cells; while was impaired by silencing HOXA1 in OSCC/R cells. Mechanically, the expression of HOXA1 was regulated by the de-ubiquitination role of USP9X. Silencing USP9X led to an impairment in the CDDP resistance of OSCC cells, while this effect was reversed by overexpressing HOXA1. **Conclusion:** The HOXA1 contributes to the resistance of OSCC cells to CDDP, which is regulated by the deubiquitination function of USP9X.

Key words: Chemoresistance, cisplatin, HOXA1, oral squamous cell carcinoma, USP9X

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

As a type of highly aggressive malignancy with a poor prognosis, Oral Squamous Cell Carcinoma (OSCC) accounts for about 2% of all cancer new cases worldwide in 2020[1]. Despite recent advances, the mortality of OSCC is still alarmingly high[2]. Till date, the commonly used treatment for patients with advanced-stage OSCC is chemotherapy. Cisplatin (CDDP) is one of the standard chemotherapeutic drugs applied for OSCC, of which treatment efficacy was impaired by the emergence of chemoresistance that seriously limits its clinical application[3]. Hence, to improve the treatment prognosis of OSCC, it's an urgent need to further elucidate the molecular pathways behind the development of CDDP resistance.

Over the past few decades, a large number of aberrantly expressed genes in OSCC exhibited a significant correlation with the poor prognosis of OSCC[4]. A recent report revealed that the up-regulation of PD-1 is positively correlated with nodal metastasis in OSCC[5]. The GLI3 contributes to the stemness and malignant behaviors of OSCC cells, which could be a possible target for OSCC therapy[6]. Omori *et al.*[7] revealed that YAP1 may be a critical regulator of the occurrence and progression of OSCC. The YAP1 has also been proven as an important therapeutic target in CDDP-resistant OSCC in a recent study[8]. Moreover, Shriwas *et al.*[9] demonstrated that RRBP1 is a putative cancer marker that can activate YAP1 to confer CDDP resistance in OSCC based on both *in vitro* and *in vivo* experiments.

The HOXA1 (Homeobox A1) is a potential oncogene, which was identified in many cancers, including breast cancer[10], gastric cancer[11] and glioma[12]. Notably, HOXA1 may also contribute to oral carcinogenesis because of its role in facilitating the proliferation and invasion of OSCC cells[13,14]. In a recent report, high HOXA1 expression was discovered to be closely related to poor clinicopathological characteristics in OSCC[15]. Previous studies revealed the contribution of HOXA1 in the chemoresistance of several cancers[16,17]. Nevertheless, whether HOXA1 contributes to the development of CDDP resistance in OSCC remains enigmatic.

This study investigated the effect of HOXA1 on the enhancement of OSCC cell resistance to CDDP by the detection of cell viability and apoptosis in OSCC cells treated with CDDP. Besides, our experiments also preliminarily uncovered the upstream regulator of HOXA1 in CDDP-resistant OSCC.

MATERIALS AND METHODS

Study area: This study was conducted at Baicheng Medical College from July 2022 to June 2023.

Cell culture: The normal human oral keratinocyte cell line (NHOK) and a human OSCC cell line (TSCCA) was supplied from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China); other human OSCC cell lines, which included SCC-25, SCC-9, SCC-15 and CAL-27 were supplied by the ATCC (Manassas, USA).

The CDDP-resistant OSCC cell lines (CAL-27/R and SCC-9/R) were established as previously described by Choi *et al.*[18]. To develop CDDP resistant cell lines, original OSCC cell lines were exposed to a gradually incremental dose of CDDP over a one-year period. As 2 μmol/L of CDDP was exploited to keep the CDDP resistance of CAL-27/R cells.

All cells were cultured in DMEM containing 10% FBS and 100 U/mL of penicillin/streptomycin at 37°C with 5% CO_2.

qRT-PCR: The RNA isolation from cells was performed using TRIzol reagent (Thermo Fisher, Massachusetts, USA). The cDNA synthesis for HOXA1 and USP9X was carried out using PrimeScript RT reagent kit (Takara, Tokyo, Japan). Using a SYBR Premix EX Taq™ II kit (Takara), qRT-PCR was performed on a Bio-Rad system to detect the expression of HOXA1 (primer sequence: 5'-TCATATGGACAGGAGCACCA-3' (forward) and 5'-TGACCCAGGTAGCCGTACTC-3' (reverse) and USP9X (primer sequence: 5'-TGTTACACCCACTGCACTCT-3' (forward) and 5'-TGACATGGATGGGCTCTGTT-3' (reverse). The β-actin (Primer sequence: 5'-GCGGACTATGACTTAGTTGCGTTACA-3' (forward) and 5'-TGCTGTCACCTTCACCGTTCCA-3' (reverse) was used as an internal control. The relative expression of HOXA1 and USP9X was calculated based on $2^{-\Delta\Delta CT}$ method[19].

Cell transfection: The HOXA1 overexpressing vector (HOXA1 OE) was established by inserting HOXA1 cDNA sequence into pcDNA3.1 (Thermo Fisher) and the pcDNA3.1 empty vector (Vector) was acted as a negative control. Short hairpin (sh) RNA against HOXA1 (sh-HOXA1: 5'-CCCACTGAA GATAGATCGGA-3') and USP9X (sh-USP9X: 5'- CGACCCTAAA CGTAGACATTA-3') and their scrambled negative control (sh-NC: 5'-CCTTCCCTGAAGGTTCCTCC-3') were synthesized by Genepharma (Shanghai, China).

By using Lipofectamine 2000 (Invitrogen, California, USA), cell transfections were performed. When cell confluency was about 60%, CAL-27 cells were transfected with HOXA1 OE or sh-USP9X or their combination, while CAL-27/R cells with sh-HOXA1. While 48 hrs transfection later, cells were harvested and transfection efficiency was analyzed.

CCK-8 assay: The cell viability of CAL-27 and CAL-27/R cells with transfection was detected using CCK-8 reagent (Dojindo, Japan) upon CDDP treatment. In brief, transfected cells were seeded into 96-well plates and subsequently treated with

diverse concentrations of CDDP. The CCK-8 reagent was added into the plates at designed time points (0, 24, 48 and 72 hrs), followed by 2 hrs additional incubation. Finally, the optical density (OD) at 450 nm was monitored with a microplate reader (Bio-Rad, California, USA) and the IC_{50} value was calculated.

TUNEL assay: After transfection, cell apoptosis of CAL-27 and CAL-27/R cells was detected by the TUNEL assay with the *in situ* Apoptosis Detection kit (Takara) upon CDDP (CAL-27, 2 µmol/L; CAL-27/R, 10 µmol/L). After 24 hrs cultivation, the cells were rinsed, fixed with 4% paraformaldehyde, followed by permeating with 0.3% Triton-x-100. Then, TUNEL reaction mixture was added to cells for 1 hr incubation, followed by 5 min counterstain with DAPI. Finally, TUNEL-positive cells were counted to analyze the proportion of apoptotic cells under a fluorescence microscope (Nikon, Tokyo, Japan).

Colony formation assay: The clonogenic abilities of CAL-27 and CAL-27/R cells with transfection were was determined by colony formation assay upon CDDP (CAL-27, 2 µmol/L; CAL-27/R, 10 µmol/L). Briefly, 1200 transfected cells were evenly seeded into 6-well plate and incubated for 2 weeks. After finishing incubation, cells were subjected to fixation with 4% paraformaldehyde and the colonies of each well were stained with 2% crystal violet for final counting.

Western blot: Total protein isolated from cells by using RIPA buffer (10X) (Cell signaling, Massachusetts, USA) was subjected to quantification with a BCA protein assay kit (Thermo scientific). After separating the total protein through SDS PAGE gel, proteins were transferred onto PVDF membranes. Afterward, membranes were blocked for 2 hrs and subsequently incubated overnight with primary antibodies against HOXA1 (ab230513; Abcam, Cambridge, UK), USP9X (ab19879; Abcam) and β-actin (ab8226; Abcam). Thereafter, membranes were rinsed and further incubated with HRP-conjugated secondary antibody (ab7090; Abcam) for 2 hrs. Finally, protein bands were visualized with an ECL Western Blotting Substrate (Thermo Scientific).

Co-Immunoprecipitation (Co-IP): After finishing transfection, the cells were lysed and incubated with bead-conjugated FLAG or the specific antibody with gentle rotation overnight at 4°C, followed by incubation with antibody-conjugated beads for 2 hrs. Next, after washing immuno-complexes that were coupled by beads four times with lysis buffer, the precipitated proteins were eluted using SDS-PAGE buffer. Finally, the interacting proteins were detected by western blot based on the eluted proteins.

***In vitro* ubiquitination assay:** After transfection of sh-USP9X or sh-NC for 6 hrs, cells were co-transfected with Flag-HOXA1 and HA-ubiquitin (HA-ub). After48 hrs transfection later, cells were administrated with 10 µmol/L MG132 for 6 hrs. Finally, cells were harvested, lysed, then subjected to western blot to observe the level of ubiquitination for HOXA1.

Cycloheximide (CHX) half-life assay: After transfection 48 hrs, the cells were subjected to incubation with cycloheximide (CHX, 20µg/mL). At the incubation time of 0, 2, 4 and 8 hrs, cells were harvested and lysed to obtain total protein. Finally, the protein levels of HOXA1 and USP9X from each sample were determined by western blot.

***In vivo* experimental validation:** Initially, CAL27/R cells were subjected to stable transfection with either sh-NC or sh-USP9X lentivirus before being subcutaneously injected into mice to establish the xenograft model. Mice supplied by the animal centre of The First Hospital of Jilin University were randomized into three groups (n = 5): sh-NC, sh-USP9X and sh-USP9X+AAV-HOXA1 groups. Mice from both the sh-NC group were injected with 1×10^5 sh-NC transfected cells, while those from both the sh-USP9X and sh-USP9X+AAV-HOXA1 groups were injected with the same density of USP9X-silencing CAL27/R cells. When the tumor volume reach 150 mm^3 (record as day 0), all mice were intraperitoneally injected with 5 mg/kg CDDP once a week and mice from the sh-USP9X and sh-USP9X+AAV-HOXA1 groups, respectively received the intratumoral injection of vector and AAV-HOXA1 twice a day. Tumor formation was monitored began at day 3 every three days until day 21 and the growth curve of tumors was plotted. Tumor volume was calculated using the following equation:

$$\text{Tumor volume} = \frac{L \times W^2}{2}$$

where, W represents the width and L represents the length. On the day 21, mice were sacrificed to collect xenografts for weighing and protein extraction.

Ethical consideration: This study was approved by Baicheng Medical College. All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

Statistical analysis: Data were represented as the Mean±SD and analyzed using the student t-test or one-way analysis of variance followed by Tukey's *post hoc* test on GraphPad Prism software. The $p<0.05$ means that the difference is significant.

RESULTS

HOXA1 is highly expressed and closely related to CDDP resistance in OSCC cells: The expression of HOXA1 was significantly increased in OSCC cells, when compared with non-tumorous oral cells (NHOK) (Fig. 1a). For investigating the correlation between HOXA1 expression with CDDP resistance, CDDP-Resistant OSCC (OSCC/R) cell lines were established to analyze the expression level of HOXA1 among them. It was observed that the HOXA1 expressions in all OSCC/R cell lines were higher than their parental cell lines (Fig. 1b). Moreover, a positive correlation between HOXA1 expression and IC_{50} value of CDDP was observed (Fig. 1c), which suggested HOXA1 might be closely related to the development of CDDP resistance in OSCC cells.

HOXA1 contributes to CDDP resistance in OSCC cells: To explore the role of HOXA1 in the development of CDDP resistance in OSCC cells, HOXA1 was overexpressed in CAL-27 cells. The qRT-PCR analysis showed that the expression of HOXA1 was dramatically increased in CAL-27 cells after the HOXA1 OE transfection, which verified the successful transfection of HOXA1 OE (Fig. 2a). The result demonstrated that up-regulation of HOXA1 effectively increased the resistance of CAL-27 cells to CDDP, as revealed by the significant increase in IC_{50} value (Fig. 2b). Then, we further explored the effect of Vector or HOXA1 OE transfection on the cell proliferation and apoptosis of CAL-27 cells upon CDDP using CCK-8, TUNEL and colony formation assays. Overexpression of HOXA1 led to an increase of

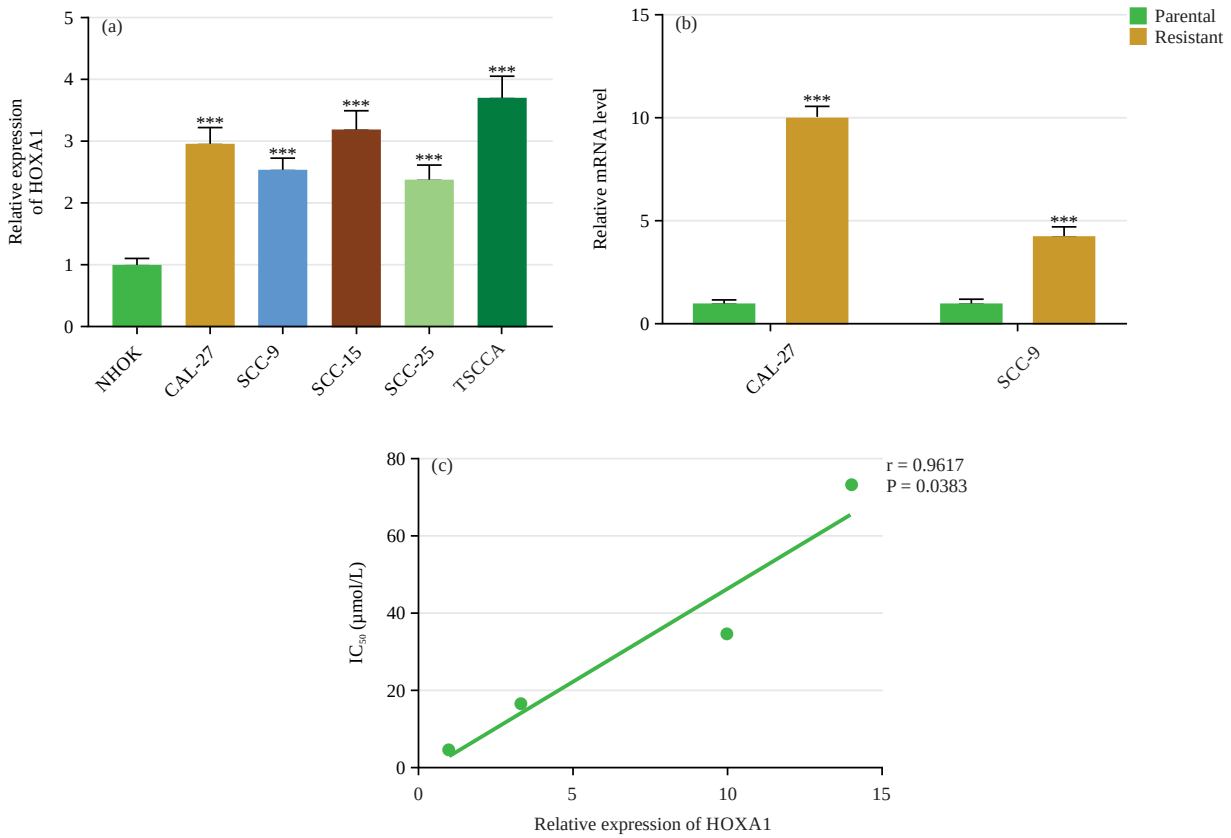

Fig. 1(a-c): HOXA1 is highly expressed and closely related to CDDP resistance in OSCC cells, (a) Expression levels of HOXA1 in different oral cell lines (NHOK, TSCCA, SCC-15, SCC-9, SCC-25 and CAL-27), (b) Comparison of HOXA1 expression between CDDP-resistant and the parental OSCC cell lines (SCC-9 and CAL-27) and (c) Correlation analysis for HOXA1 expression and IC_{50} value of CDDP-resistant OSCC cells

**p<0.01 and ***p<0.005, compared with the NHOK or normal group

cell viability and colony formation and a decrease of apoptotic ratio in CAL-27 cells (Fig. 2c-e), suggesting that the abilities of CAL-27 cells withstanding CDDP on cell viability and apoptosis were strengthened by overexpressing HOXA1.

Meanwhile, the role of HOXA1 in regulating CDDP resistance was verified by altering the expression of HOXA1 in CAL-27/R cells via transfection of sh-HOXA1. The transfection of sh-HOXA1 effectively down-regulated HOXA1 in CAL-27/R cells (Fig. 2a). As expected, the IC_{50} value for CDDP in CAL-27/R cells with sh-HOXA1 transfection was significantly lower than those with sh-NC transfection (Fig. 2b), signposting that silencing HOXA1 could increase the sensitivity of the OSCC/R cells to CDDP. The following experiments, such as CCK-8, TUNEL and colony formation assays, verified this point. Silencing HOXA1 enhanced the impairment induced by CDDP in CAL-27/R cells, as evidenced by the reduction of cell viability and clonogenic ability and the increase of cell apoptosis (Fig. 2c-e).

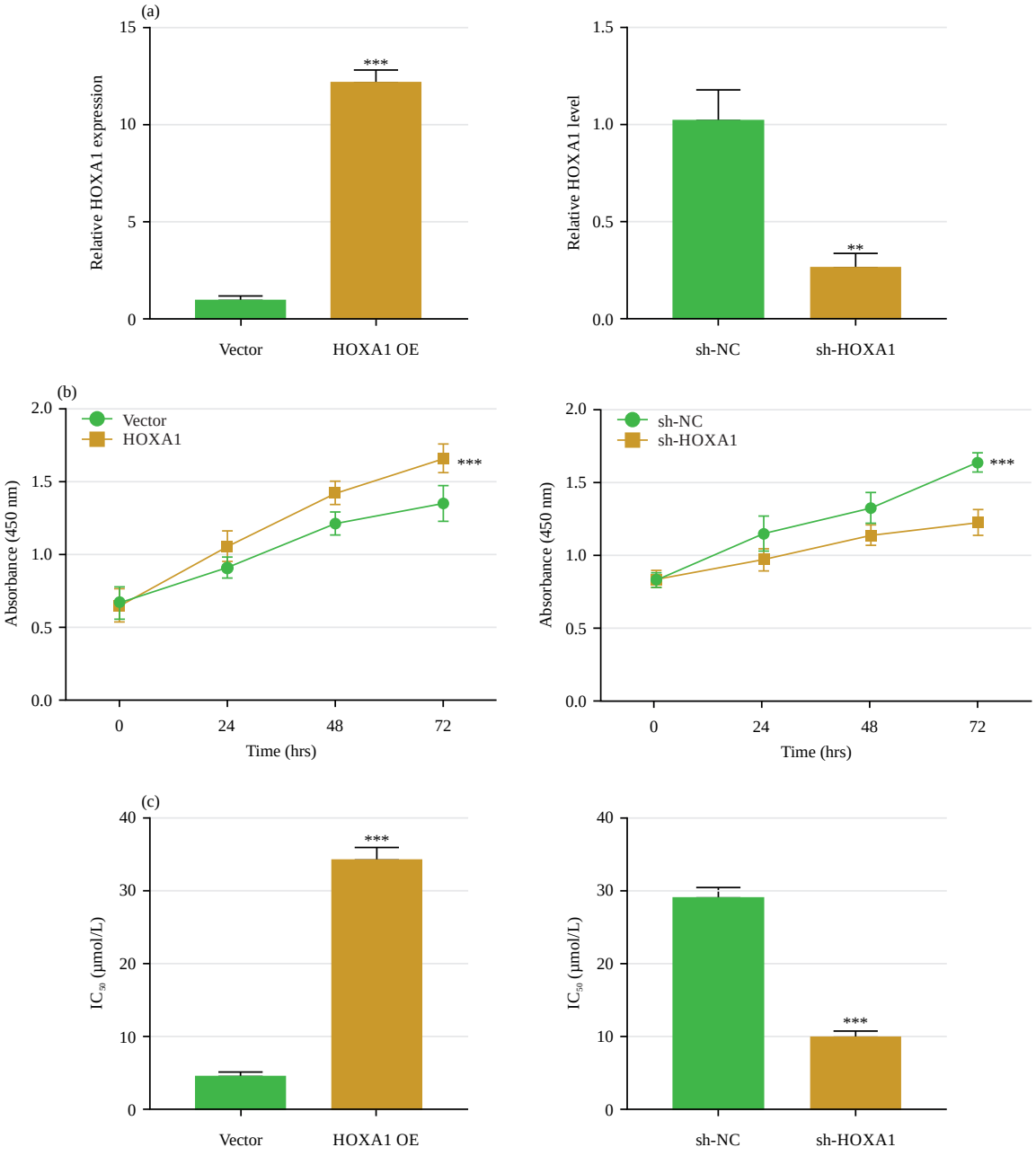

Fig. 2(a-e): Continue

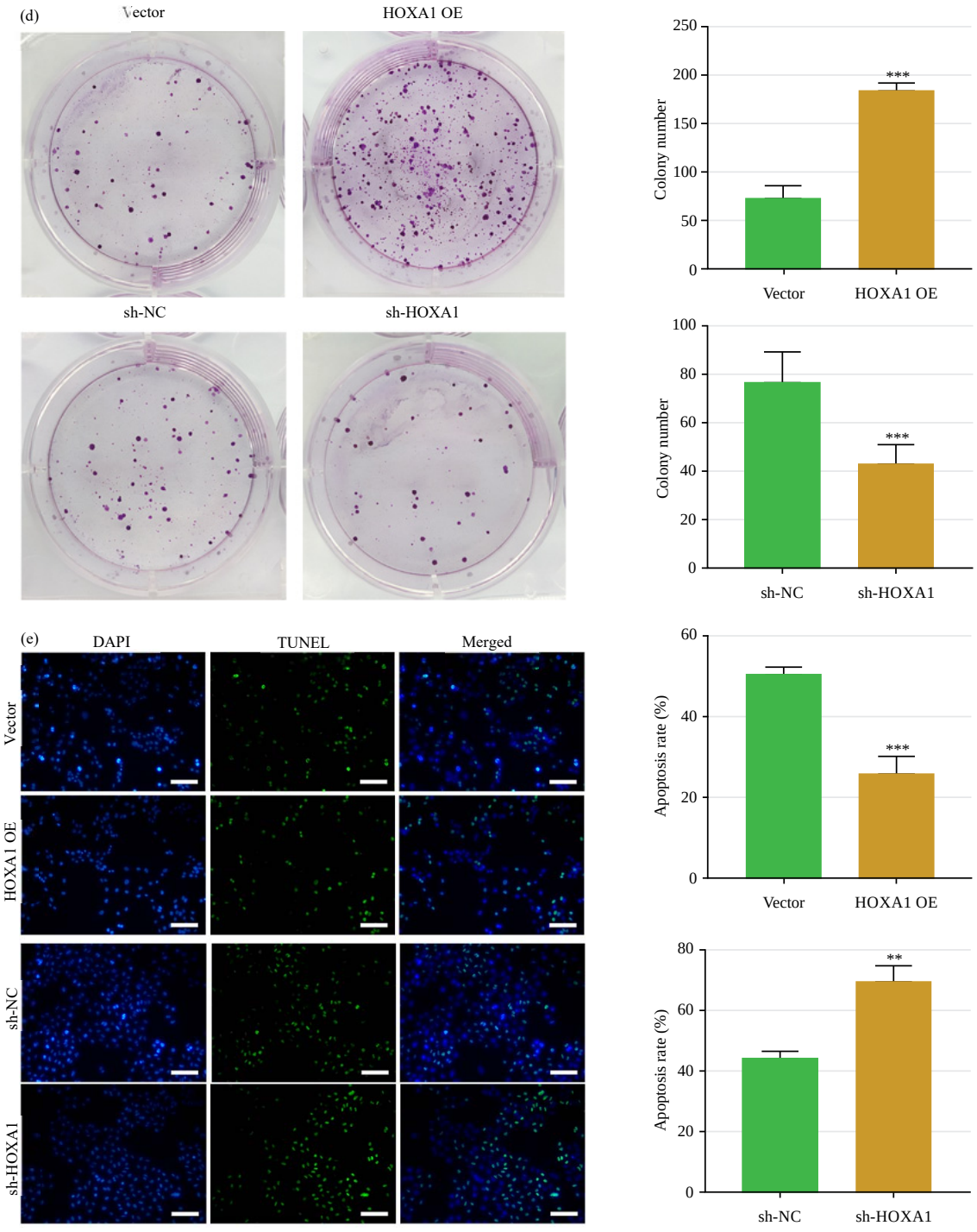

Fig. 2(a-e): Up-regulation of HOXA1 enhanced CDDP resistance in CAL-27 cells, CAL-27 cells were transfected with empty vector (Vector) or HOXA1 overexpression vector (HOXA1 OE); while CDDP resistant cell line, CAL-27/R, was subjected to transfection with short hairpin (sh) RNA against HOXA1 (sh-HOXA1 or the scrambled negative control (sh-NC), (a) Expression level of HOXA1, (b) IC_{50} value of CDDP in CAL-27 and CAL-27/R cells with different transfections, (c) Cell viability of CAL-27 and CAL-27/R cells with different transfections after treating with of CDDP for 24, 48 and 72 hrs, (d) Cell apoptosis and (e) Colony formation of CAL-27 and CAL-27/R cells with different transfections after treating with CDDP for 24 hrs

**p<0.01 and ***p<0.005, compared with the vector group or sh-NC group

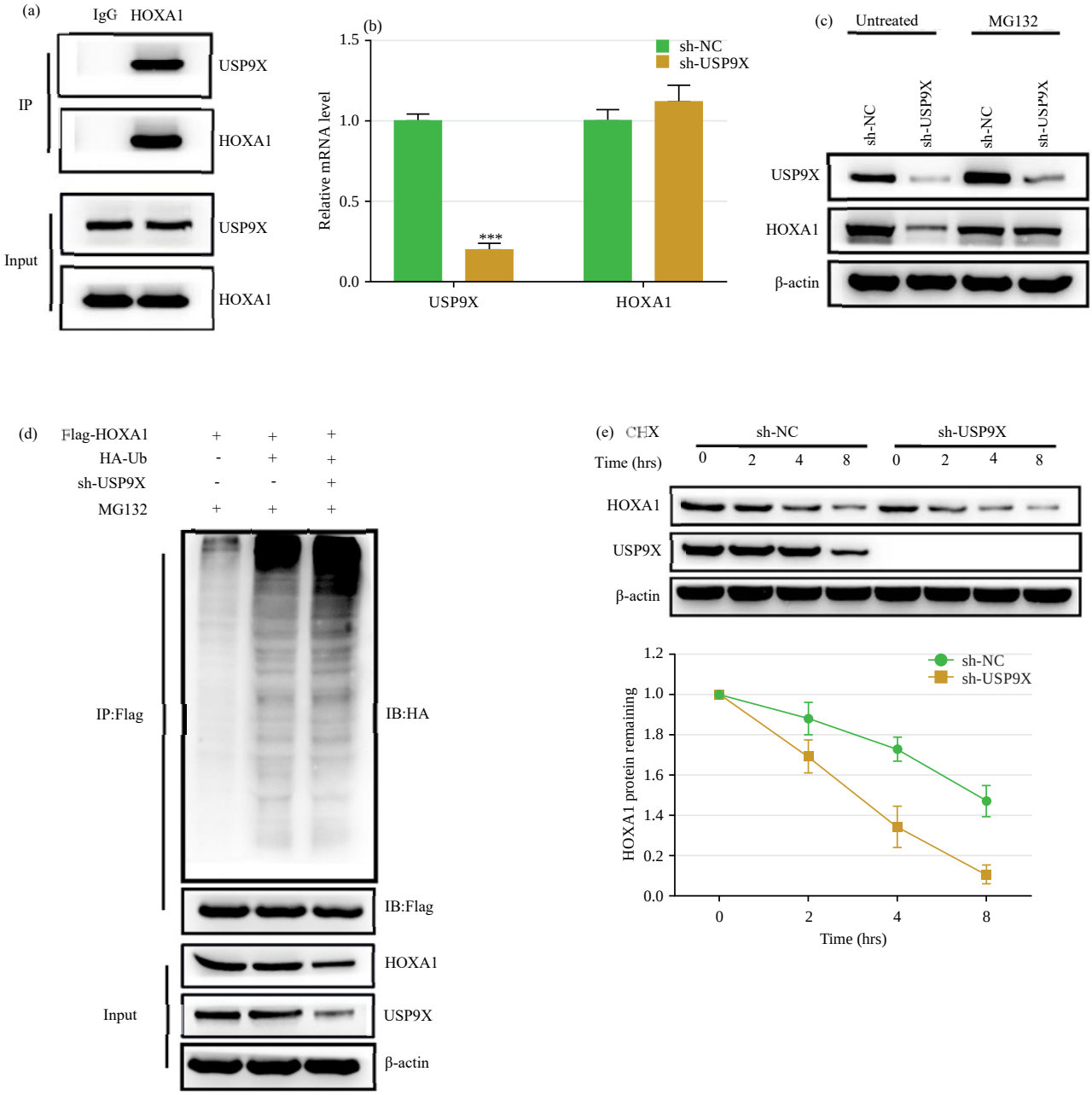

Fig. 3(a-e): USP9X up-regulated HOXA1 expression via deubiquitination in OSCC cells, (a) CAL-27 cells were lysed and immunoprecipitation was carried out with IgG or HOXA1 antibodies. The immunocomplexes were subjected to detect HOXA1 and USP9X expression, (b) The mRNA expression of HOXA1 in CAL-27 cells transfected with sh-USP9X or sh-NC, (c) Protein expression of HOXA1 and USP9X in CAL-27 cells with sh-USP9X or sh-NC transfection was detected after treatment with or without MG132, (d) CAL-27 cells were co-transfected Flag-HOXA1 with sh-USP9X or sh-NC, followed by subjecting to ubiquitination assay and (e) After treating with CHX 0, 2, 4, and 8 hrs, the protein expression levels of HOXA1 and USP9X in CAL-27 cells transfected with sh-USP9X or sh-NC were analyzed

***p<0.005, compared with the sh-NC group

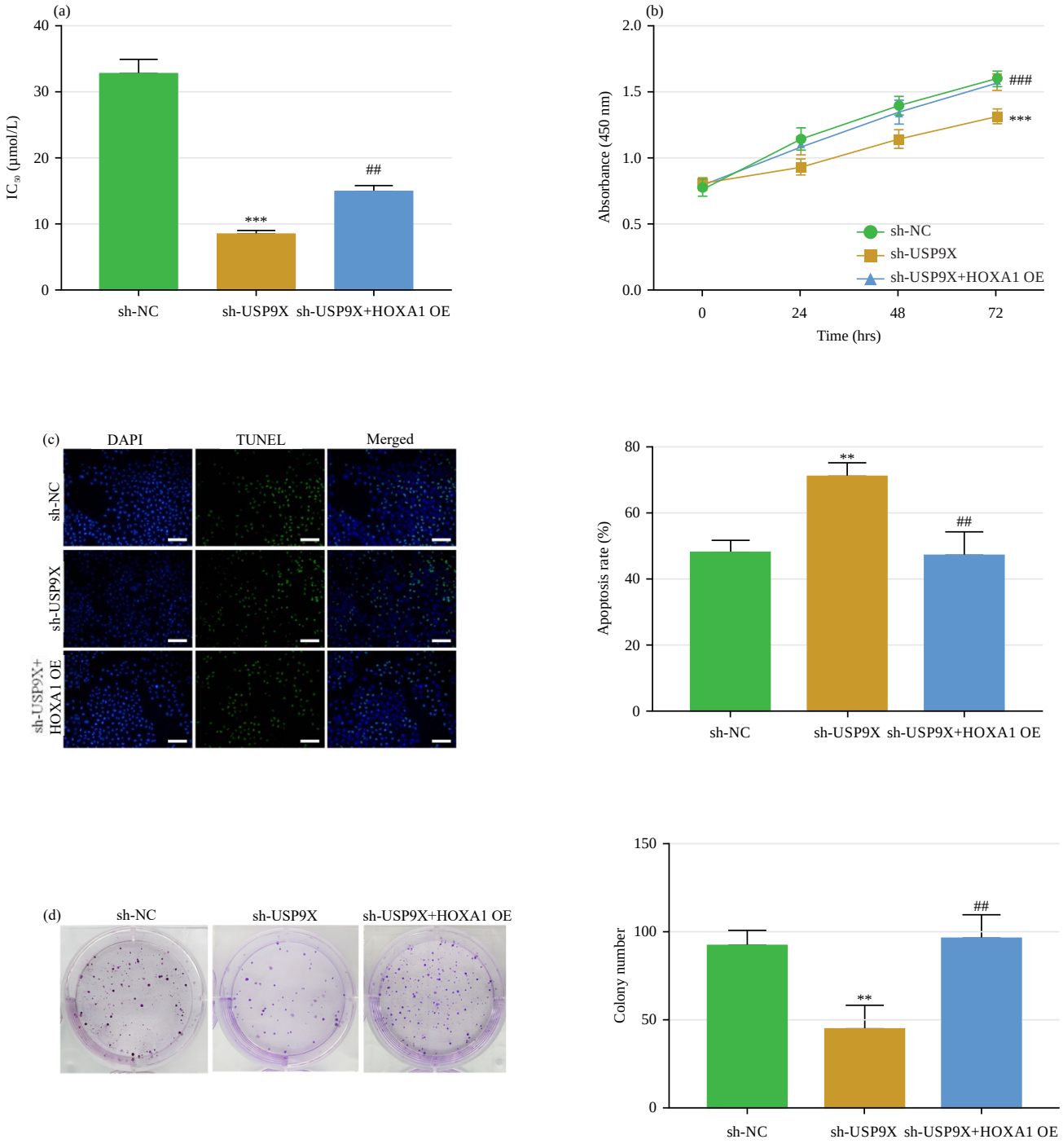

Fig. 4(a-d): USP9X knockdown enhances the sensitivity of CAL-27/R cells to CDDP via HOXA1, CAL-27/R cells were transfected with sh-USP9X or sh-NC or the combination of sh-USP9X and HOXA1, (a) IC_{50} value of CDDP in CAL-27/R cells with different transfections, (b) Cell viability of CAL-27/R cells with different transfections after treating with 2 μmol/L of CDDP for 24, 48 and 72 hrs, (c) Cell apoptosis and (d) Colony formation of CAL-27/R cells with different transfections after treating with 10 μmol/L of CDDP for 24 hrs

**p<0.01 and ***p<0.005, compared with the sh-NC group; #p<0.05, ##p<0.01, and ###p<0.005, compared with the sh-USP9X group

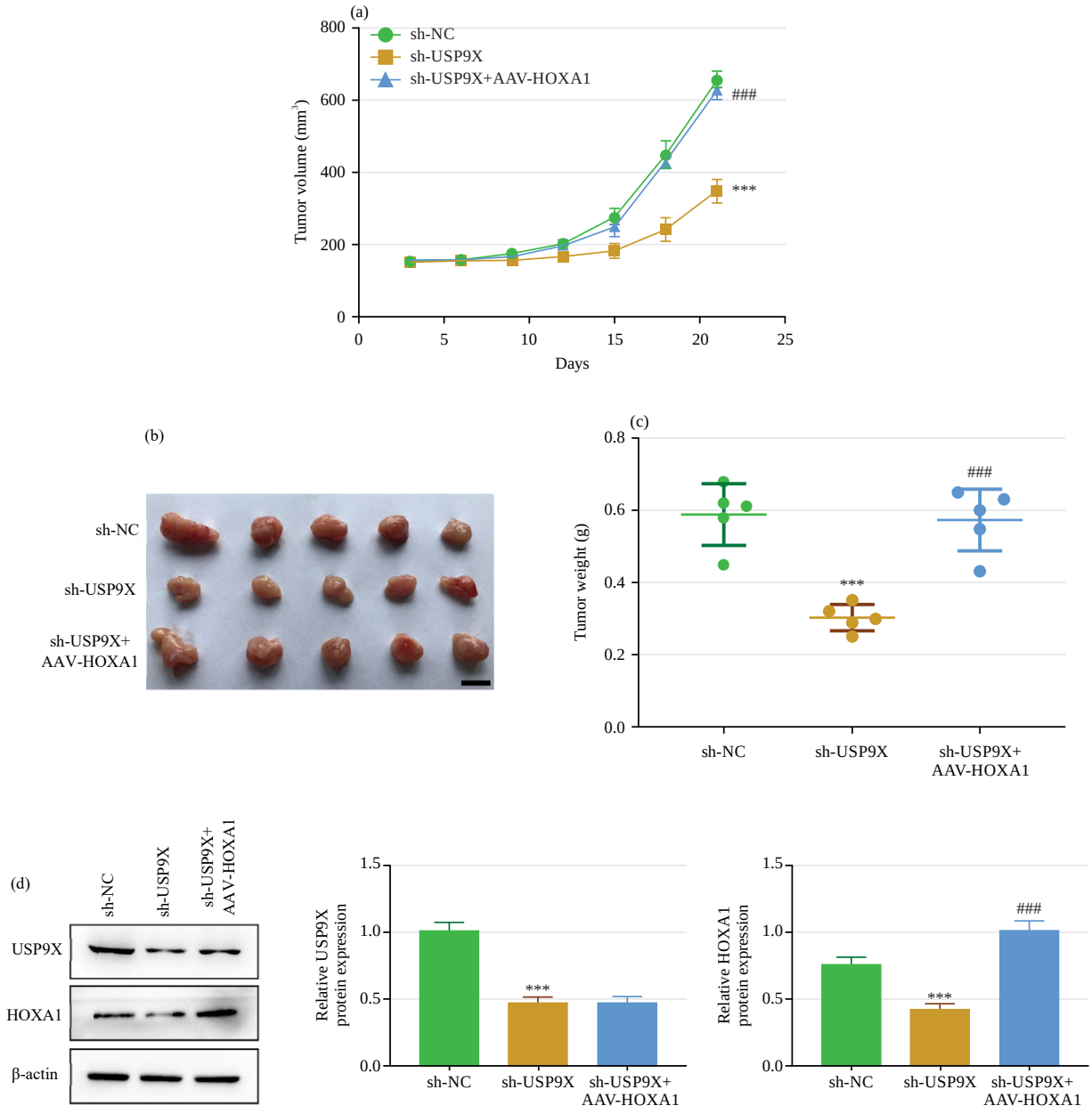

Fig. 5(a-d): USP9X knockdown reverses the CDDP resistance via HOXA1 in the OSCC/R xenograft model. Mice from both the sh-NC group were injected with sh-NC transfected cells, while those from both the sh-USP9X and sh-USP9X+AAV-HOXA1 groups were injected with the same density of USP9X-silencing cells. All mice were intraperitoneally injected with 5 mg/kg CDDP, and mice from the sh-USP9X and sh-USP9X+AAV-HOXA1 groups respectively received the intratumoral injection of vector and AAV-HOXA1, (a) Tumor growth curve, (b) Tumor was harvested from each group on Day 21, (c) Weight of the harvested tumor and (d) Expression level of USP9X and HOXA1 in the harvested tumor

***p<0.005, compared with the sh-NC group and ###p<0.005, compared with the sh-USP9X group

USP9X up-regulated HOXA1 expression via deubiquitination in OSCC cells: The USP9X is a deubiquitinase (DUB), which has been implicated in the CDDP resistance of several cancers[20,21]. Question arises whether USP9X participates in the regulation of HOXA1 in the CDDP resistance of OSCC. The Co-IP experiments were performed to examine if HOXA1 interacts with USP9X and demonstrated that endogenous HOXA1 immunoprecipitated with endogenous USP9X in CAL-27 cells (Fig. 3a). This interaction promoted us to further explore the potential role

of USP9X in regulating HOXA1 stabilization since its deubiquitination function. Results showed that silencing USP9X in CAL-27 cells remarkably reduced the expression of HOXA1 at protein level without affecting mRNA level (Fig. 3b-c). Meanwhile, we found that the decrease in HOXA1 protein levels was rescued by the treatment of MG132 (a proteasome inhibitor) (Fig. 3c). These results revealed that USP9X could modify HOXA1 in a post-transcriptional manner, probably through modulating the proteasomal degradation of HOXA1. *In vitro* ubiquitination assay was subsequently carried out to verify whether USP9X acts as a de-ubiquitinase for HOXA1 protein, which showed that polyubiquitylated HOXA1 protein levels were significantly increased after the transfection of sh-USP9X (Fig. 3d). Moreover, the CHX assay showed that the knockdown of USP9X caused a faster degradation in HOXA1 protein (Fig. 3e), further confirming the promotive role of USP9X in the stabilization of HOXA1 protein.

USP9X knockdown reversed CDDP resistance via HOXA1 in OSCC: Based on the interaction between USP9X and HOXA1 verified in the above experiments, whether the USP9X/HOXA1 axis contributes to the CDDP resistance of OSCC was subsequently explored. The IC_{50} value of CDDP for CAL-27/R cells was decreased by knocking down USP9X (Fig. 4a). However, overexpressing HOXA1 in USP9X-depleted CAL-27/R cells almost rescued their resistance to CDDP (Fig. 4a). In addition, the depletion of USPX9 sensitized CAL-27/R cells to CDDP, thereby enhancing the cytotoxicity of CDDP on cell proliferation and apoptosis of CAL-27/R cells (Fig. 4b-d). Conversely, overexpressing HOXA1 in USP9X-depleted CAL-27/R cells efficiently reversed the effect of USP9X silence on cell proliferation and apoptosis of CAL-27/R cells (Fig. 4b-d). These data collectively demonstrated a crucial role of USP9X in regulating the response of OSCC/R cells to CDDP via promoting HOXA1 stabilization.

Furthermore, *in vivo* studies were performed to verify the role of USP9X/HOXA1 in CDDP resistance of OSCC. The tumor growth curve showed that the sh-USP9X group developed significantly smaller tumors than the sh-NC group (Fig. 5a-b), verifying that USP9X silence effectively reverse CDDP resistance, which was corroborated by the tumor weight result (Fig. 5c). Consistent with the results of *in vitro* analyses, overexpressing HOXA1 could block the effect of USP9X silence in OSCC tumors (Fig. 5a-c). Western blot analysis confirmed that USP9X was knocked down in the sh-USP9X and sh-USP9X+AAV-HOXA1 groups, while the intratumoral injection of AAV-HOXA1 successfully caused the overexpression of HOXA1 in tumors (Fig. 5d).

DISCUSSION

The OSCC is one of the common fatal malignancies, accounting for a major cause of health burden worldwide. As an optional treatment for OSCC, chemotherapy of CDDP has been reported to prolong the survival time of patients with advanced-stage[22]. Since acquired drug resistance is substantially hindering the therapeutic efficiency of CDDP; understanding the molecular mechanisms behind CDDP resistance in OSCC is of great significance. It has been reported that the expression level of HOXA1 was positively correlated with poor prognosis in OSCC[15]. Given that HOXA1 has been implicated in CDDP resistance in several cancers, we assumed that HOXA1 might be a critical regulator during the development of CDDP resistance in OSCC.

Initially, a significant difference in the expression levels of HOXA1 between normal oral cells and OSCC cells was observed, which was consistent with previous research by Bitu *et al.*[13]. In order to investigate the correlation between CDDP resistance and HOXA1 expression in OSCC and CDDP-Resistant OSCC (OSCC/R) cell lines were established by exposing non-resistant OSCC cell lines to gradually increasing concentrations of CDDP. Interestingly, all CDDP-resistant cell lines exhibited a significant elevation of HOXA1 expression compared with their parental cell lines. Current study also uncovered a positive correlation between the IC_{50} value of CDDP and HOXA1 expression in OSCC/R cells. To confirmed the biological function of HOXA1 on CDDP resistance in OSCC, we delivered HOXA1 overexpression vector in CAL-27 cell and stably transfected CAL-27/R cells with shRNAs against HOXA1. Functional analyses revealed that HOXA1 overexpressing CAL-27 cells displayed stronger resistance against CDDP in comparison to CAL-27 cells transfected with empty vector. Conversely, HOXA1 knockdown could restore the sensitivity of CAL-27/R cells to CDDP, as revealed by decreased IC_{50} value of CDDP, cell proliferation, as well as colony formation and increased cell apoptosis. Hence, it was suggested that the development of CDDP resistance in OSCC cells might be attributed to its abnormal expression of HOXA1.

As known, ubiquitination and deubiquitination are two mutually reversible post-translational modifications of proteins, which participate in a series of biological processes[23]. The DUB is a key regulator in deubiquitination, which can cleave ubiquitin from target proteins to improve their stabilization, thus regulating their function and subcellular localization[24]. As the largest subfamily of DUB, ubiquitin-specific proteases (USPs) have arisen immense interest by researchers as their functional diversity[25]. In multiple research, USPs are implicated in the occurrence, progression and even

resistance development of numerous cancers, such as breast cancer[26], colorectal cancer[27], as well as OSCC[28]. For example, CYLD could stabilize ALK5 expression to regulate TGF-β signal transduction, thereby blocking the enhanced invasive behavior and acquisition of mesenchymal features in OSCC cells[29]. The USP9X interacts with PD-L1 to enhance its stabilization, leading to the suppression of tumor growth in OSCC[30]. Several reports indicated that USP9X is also responsible for the resistance development of multiple cancers[31]. In the current study, results found that USP9X silence led to a decrease in the ubiquitination of HOXA1, accompanied by an increase in its half-life. Moreover, USP9X knockdown impaired the resistance of CAL-27/R cells to CDDP, which could be partially rescued by the reintroduction of HOXA1 into USP9X-depleted cells. This finding was finally confirmed by the xenograft model. These data revealed that USP9X confers CDDP resistance to OSCC cells in a HOXA1-dependent way by enhancing HOXA1 stability via deubiquitination.

CONCLUSION

Current study identified that HOXA1 conferred CDDP resistance to OSCC cells and clarified that USP9X was an upstream regulator of HOXA1 function, suggesting that the therapeutic approach targeting the HOXA1/USP9X axis may be a potential route to overcoming CDDP resistance in OSCC.

SIGNIFICANCE STATEMENT

The acquisition of Chemoresistance to Cisplatin (CDDP) is a challenge in Oral Squamous Cell Carcinoma (OSCC) therapy. The present study aimed to explore the role and underlying mechanism of HOXA1 in CDDP resistance of OSCC. Our data revealed that HOXA1 conferred CDDP resistance to OSCC cells and clarified that USP9X was an upstream regulator of HOXA1 function *in vitro* and *in vivo*. This finding suggested that the therapeutic approach targeting the HOXA1/USP9X axis may be a potential route to overcoming CDDP resistance in OSCC.

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