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Cancer stemness of CD10-positive cells regulated by Hedgehog pathway promotes the resistance to cisplatin in oral squamous cell carcinoma

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Abstract

Objective: To explore the role of CD10 in cisplatin resistance of oral squamous cell carcinoma (OSCC) and its association with the Hedgehog (Hh) signaling pathway and cancer stem cells (CSCs).

Methods: The correlation between cell viability and CD10 expression was analyzed in different OSCC cell lines after the cisplatin treatment. Genes related to chemotherapy resistance, cancer stem cells and the epithelial–mesenchymal transition were detected by quantitative real-time PCR (qPCR) in $CD10^{high}$ and $CD10^{low}$ OSCC cells. Mouse xenograft model and venous metastasis model were used to explore the potential regulatory mechanism of the resistance effect of CD10 on cisplatin.

Results: The higher expression of CD10 gene in different cell lines displayed enhanced cisplatin resistance ability. The expression of genes related to chemotherapy resistance, cell stemness, and the epithelial–mesenchymal transition was significantly higher in $CD10^{high}$ cells compared with $CD10^{low}$ cells. Moreover, the combination of cisplatin and Hh pathway inhibitors significantly reduced the resistance of CD10 to cisplatin in the xenograft model and venous metastasis models.

Conclusion: CD10-positive cells are implicated in developing cisplatin resistance of OSCC, which could be related to its cancer stem cell characteristics regulated by the Hedgehog pathway.

KEYWORDS

cancer stem cell, CD10, chemotherapy resistance, Hedgehog pathway, oral squamous cell carcinoma

Yifei Wang and Qingxiang Li contribute equally to this work.

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1 | INTRODUCTION

Squamous cell carcinoma (SCC) is the most common epithelial malignant tumor of the head and neck region (Bray et al., 2018; Sturgis et al., 2018). An advanced oral SCC can seriously affect the patient's chewing, speech, and appearance of patients with a reduction of life quality. Surgery and radiation therapy have shown to be ineffective for advanced oral cancer, while chemotherapy with cisplatin often shows resistance.

Cancer stem cell (CSC) was reported to be associated with the chemotherapy resistance of cancer (Donnenberg & Donnenberg, 2005). CSCs have the potential of self-renewal, hierarchical differentiation, and tumor formation (Clarke et al., 2006; Ishizawa et al., 2010), which are regulated by canonical embryonic stem cell transcription factors (SOX2, OCT4, and NANOG) and several key signaling pathways (NOTCH, WNT/CTNNB1, and SHH) (Birkeland et al., 2015; Lazarevic et al., 2018). CD10 is a newly discovered CSC surface marker, which has been associated with local recurrence, distant metastases, and a higher histologic tumor grade (Fukusumi et al., 2014; Piattelli et al., 2006).

The role of the Hedgehog (Hh) signaling pathway has been well studied in embryonic development. Many developmental anomalies and cancers have been associated with the deregulation of the Hh signaling pathway (Bora-Singhal et al., 2020). It has been reported that Hh ligands can maintain a stemness signature through pluripotency genes, including SOX2, NANOG, and BMI1 (Zhu et al., 2019). Despite the importance of Hh signaling in cancer development, its mechanism underlying the regulation of CD10 in cisplatin resistance of OSCC remains to be elucidated.

In this study, we investigated the role of CD10 in cisplatin resistance of oral squamous cell carcinoma (OSCC) and its association with the Hh signaling pathway and cancer stem cells. We aimed to provide a theoretical basis for using CD10 as a diagnostic or therapeutic target for OSCC.

2 | MATERIALS AND METHODS

2.1 | Cell culture

The human immortalized epidermal cell line HaCaT (RRID: CVCL_0038) and two human OSCC cell lines CAL27 (RRID: CVCL_1107) and WSU-HN6 (RRID: CVCL_5516) were used in this study. CAL27 was obtained from the American Type Culture Collection (ATCC, CRL-2095). HaCaT and WSU-HN6 were obtained from the Central Laboratory of Peking University School and Hospital of Stomatology. The cells were cultured in DMEM (Life Technology) containing 10% FBS (Gibco) and 1% penicillin/streptomycin (Gibco) in a humidified atmosphere containing 5% CO2/95% air at 37°C. The cells were authenticated by STR analysis and Mycoplasma detection before used. All cells were cryopreserved for more than 6 months, and the general length of time between thawing and use was not exceeding 3 months.

2.2 | Magnetic activated cell sorting

CD10 ^{high} and CD10 ^{low} cells were separated by the CD10 Magnetic activated cell sorting (MACS) kit (Miltenyi). Briefly, the cells were counted. Then, 10^7 cells were resuspended in a 40 μl buffer, which was mixed with 10 μl of Anti-CD10-Biotin at 4°C for 10 min. Consequently, cells were incubated with 30 μl buffer and 20 μl of Anti-Biotin-Microbeads at 4°C for 15 min. After being washed with the buffer, the cells flowed into the LS sorting column, which was placed in a magnetic field. The unlabeled cells were collected (CD10 ^{low} cells). The LS sorting column was then taken out from the magnetic field; the labeled cells were flushed out and collected (CD10 ^{high} cells).

2.3 | Chemotherapeutic drug sensitivity test

The cells were seeded at a density of 5,000 per well in 96-well culture plate. Cells were then exposed to gradually increased concentration (5, 10, 15, or 45 μM) of cisplatin (diluted with PBS; Jiangsu Haosen Pharmaceutical Co., Ltd.) for 24, 48, 72, or 96 hr. At each time point, 10 μl of sterile CCK-8 solution (Bimake) was added to each well and incubated for another 3 hr at 37°C. The absorbance values were measured at 450 nm using a microplate reader respectively. Cell viability under each condition was calculated as the percent of the control value. Each condition was performed in five wells, and data were obtained from at least three separate experiments.

2.4 | Flow cytometry

After MACS sorting, 10^5 CD10^{low} cells and 10^5 CD10^{high} cells were stained with PE-labeled mouse anti-human CD10 antibody (Invitrogen) at room temperature for 30 min in the dark. Then the cells were analyzed using Beckman Coulter XL instrument.

2.5 | Quantitative real-time PCR and gene expression analysis

Total RNA from the cells was extracted using TRIzol reagent (Ambion) according to the product's protocol. The RNA quantity and purity were measured with a spectrophotometer (Bio Tek). The cDNA was synthesized using a reverse transcription kit (Promega). Real-time PCR assays were performed using SYBR Green (Roche, Switzerland) in the ABI 7500 Real-Time PCR Detection System (Applied Biosystems). Ribosomal protein S18 (RPS18) was used as the endogenous standard. The PCR program consisted of pre-denaturation at 95°C for 10 min, followed by 40 cycles amplification of 95°C for 15 s and 60°C for 1 min. The primer was purchased from Shanghai Shenggong Co., Ltd., and its sequence is shown in Table S1. The relative expression level was normalized to the amount of RPS18 and calculated using the $2^{-\Delta\Delta Ct}$ method.

2.6 | Spheroid formation assay

CD10^{low} and CD10^{high} cells were seeded at a density of 1,000 per well in 12-well ultra-low attachment culture plates in serum-free DMEM-F12 medium (Invitrogen) containing 2% B27 (Sigma), EGF (20 ng/ml; R&D) and bFGF (10 ng/ml; R&D). After 15 days, the number of spheroids with a diameter of over 100 μm (Johnson et al., 2013) per well was counted under an inverted light microscope. The assay was performed three times.

2.7 | Immunofluorescence

After sorted with CD10 MACS kit or treated with siRNA, the cells were fixed in 4% paraformaldehyde for 15 min at RT, followed by incubation in 0.1% (v/v) Triton-100-PBS. Subsequently, cells were blocked with 10% goat serum (Zhongshan Biosciences Inc.) and then incubated with anti-Gli1 antibody (1:200, Novus) at 4°C overnight. The cells were then stained with FITC- or TRITC-labeled secondary antibody (Zhongshan Biosciences Inc.) for 1 hr at RT. Nuclear staining was performed by incubation with 4′,6-diamidino-2-phenylindole (DAPI; Zhongshan Biosciences Inc.). The images were then captured using an optimal fluorescent microscope (Olympus).

2.8 | Xenograft studies

Forty-two 4-week-old female BALB/c-nude mice (Vital River Laboratory Animal Technology) were housed in a specific pathogen-free environment with a temperature of 22 ± 1°C, the relative humidity of 50 ± 1%, and a light/dark cycle of 12/12 hr. All animal studies were performed in accordance with the National Institute of Health (NIH), USA guidelines on the care and use of animals for experimental procedures, and in accordance with local laws and regulations. The study was approved by the Peking University institutional animal care and conducted according to the AAALAC and the IACUC guidelines (2018/06/27, NO. LA2018249). The details of xenograft studies were described in Supplementary Materials And Methods.

2.9 | Mouse model of distant tumor metastasis

Twenty mice were randomly divided into 4 groups (5 mice per group). One group received a vain tail injection of 2×10^5 of CD10^{low} WSU-HN6 cells, while 2×10^5 CD10^{high} WSU-HN6 cells were injected into the other 3 groups. Cisplatin was diluted with PBS, while the vector of GDC0449 was composed of 2% DMSO, 30% PEG300, and 5% Tween-80 diluted in sterilized PBS. The group with CD10^{low} WSU-HN6 cells accepted vector injection, and the groups with CD10^{high} WSU-HN6 cells were injected with vector, cisplatin, and cisplatin plus with GDC0449 respectively. Drug administration followed the plan described above. After 2 weeks, the PET/CT Imaging System was used to detect distant metastasis (Li et al., 2020).

2.10 | Statistical analysis

An independent *t* test was used to compare the difference between the two groups, including the results of CCK-8 assay, Quantitative real-time PCR (qPCR), and spheroid formation assay. One-way ANOVA was used to compare the difference among more than two groups, such as the expression of CD10 among HaCaT, CAL27, and WSU-HN6. Differences were considered significant when the *p* value was <.05. All statistical analyses were performed using SPSS 22.0 for Windows.

3 | RESULTS

3.1 | Expression of CD10 associated with cisplatin resistance

To explore whether CD10 is implicated with cisplatin resistance, we firstly detected the expression of CD10 in a human-immortalized epidermal cell line (HaCaT) and two human OSCC cell lines (CAL27 and WSU-HN6). By real-time PCR, we found that CD10 expression was significantly higher in OSCC cell lines compared to HaCaT, and WSU-HN6 cells showed higher CD10 expression compared to CAL27 cells (Figure 1a). Furthermore, flow cytometry results indicated that the proportion of CD10-positive cells in WSU-HN6 cells was also higher than that in CAL27 (Figure 1b). Moreover, cisplatin resistance assay indicated that the resistance to cisplatin increased in time- and dose-dependent manners. Besides, WSU-HN6 showed better viability than CAL27 (Figure 1c).

3.2 | CD10^{high} cells revealed enhanced cisplatin resistance in OSCC cell line

To further test the association of CD10 with cisplatin resistance, CD10-positive and -negative subpopulation were isolated from the WSU-HN6 cell line. The efficiency analysis of MACS separation for CD10 was carried by flow cytometry. The percentage of CD10-positive cells among the CD10^{low} cell subgroup was 22.1%, and the percentage among the CD10^{high} subgroup was 80.2% (Figure S1a). Then, we treated the two subpopulations with different concentrations of cisplatin. As expected, after treating cells with different concentrations of cisplatin (5, 15, 45 μM) for a different time (48 or 72 hr), the CD10^{high} cells were more refractory to cisplatin than CD10^{low} cells (Figure 2a).

ABCB1 and ABCG2 genes that participate in chemotherapy resistance (Begicevic & Falasca, 2017) were highly expressed in CD10^{high} cells compared to CD10^{low} cells (Figure 2b).

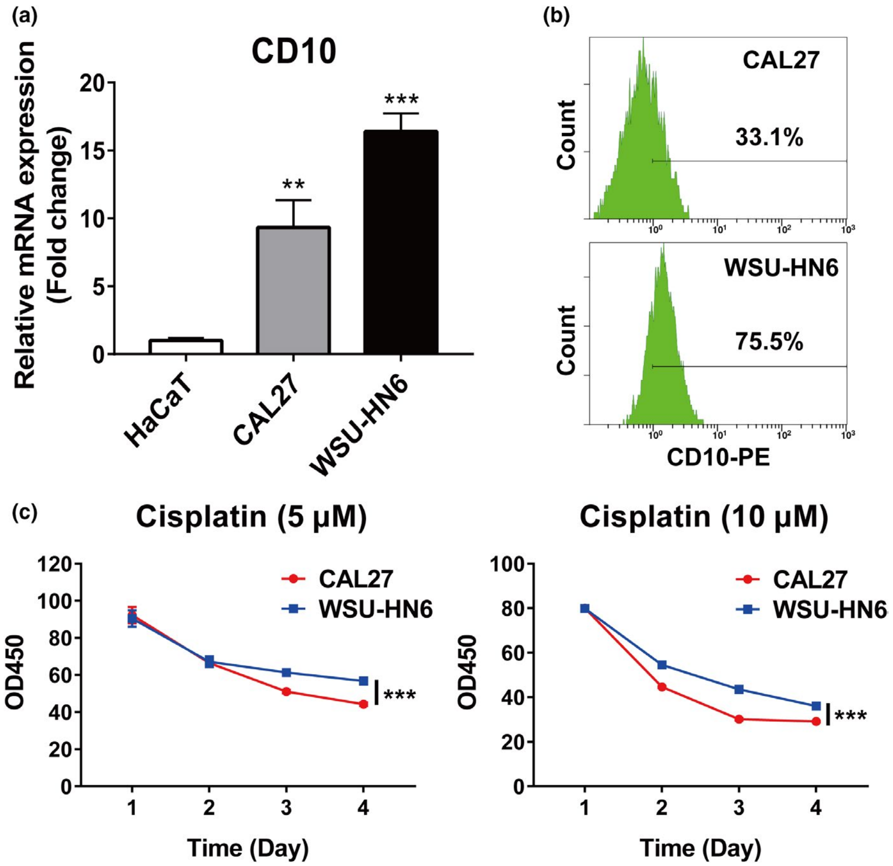


FIGURE 1 The expression of CD10 was associated with cisplatin resistance. (a) The expression of CD10 among HaCaT, CAL27, and WSU-HN6 was detected by qPCR (***, $p < .005$; **, $p < .01$). (b) CD10-positive cell percentage was analyzed by flow cytometry analysis in CAL27 and WSU-HN6 cells. (c) Cell viability after cisplatin treatment between CAL27 and WSU-HN6 cell lines was detected by CCK-8 assay (***, $p < .005$) [Colour figure can be viewed at wileyonlinelibrary.com]

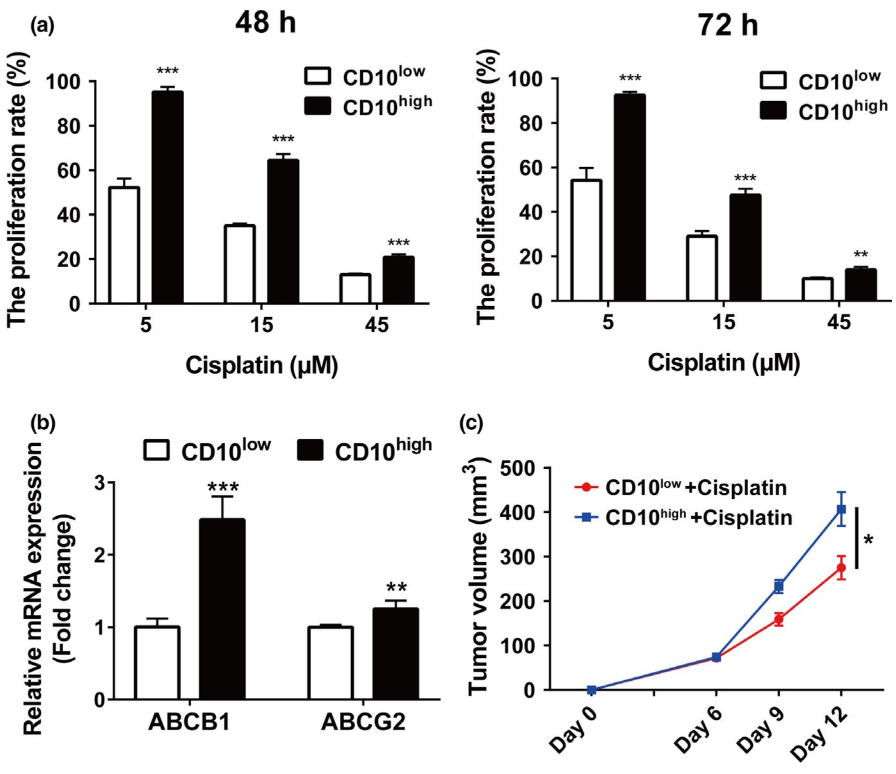


FIGURE 2 $CD10^{high}$ cells revealed enhanced cisplatin resistance in the OSCC cell line. (a) Cell viability of $CD10^{high}$ and $CD10^{low}$ cells after cisplatin treatment was detected by CCK-8 assay (***, $p < .005$; **, $p < .01$). (b) The expression of chemoresistance-related genes ABCB1 and ABCG2 in $CD10^{high}$ and $CD10^{low}$ cells was analyzed by qPCR (***, $p < .005$; **, $p < .01$). (c) Tumor volume variation of xenograft tumor with $CD10^{high}$ or $CD10^{low}$ cells under the treatment of cisplatin (*, $p < .05$) [Colour figure can be viewed at wileyonlinelibrary.com]

By using a nude mice xenograft model, we found that although the tumors generated from $CD10^{high}$ and $CD10^{low}$ cells revealed similar growth patterns without cisplatin treatment (Figure S1b), the $CD10^{high}$ cells showed a faster tumor growth than the $CD10^{low}$ cells after cisplatin treatment (Figure 2c). These results further demonstrated that CD10-positive cells participated in the cisplatin resistance of OSCC.

3.3 | CD10-positive cells showed enhanced CSC-associated characteristics

Cancer stem cells has an important role in the chemotherapy resistance of cancers (Chen et al., 2017). Thus, we then explored the association between CD10 and CSC in OSCC. At the cellular level, CSC showed enhanced self-renewal and slow-growing phase of the cell cycle (Xiao et al., 2018). By spheroid formation assay, we found that CD10^{high} cells presented a stronger ability of spheroid formation than CD10^{low} cells. In addition, both the number and size of the spheroid colonies in CD10^{high} cells were larger than those in CD10^{low} cells (Figure 3a). This indicated that CD10^{high} cells possessed higher self-renewal ability. Besides that, by cell cycle detection, we found that compared with CD10^{low} cells, CD10^{high} cells revealed more G0/G1 phase cells and fewer G2/M phase cells, which represented the arrested cell cycle (Figure S2a).

Next, we analyzed the molecular level of CD10 and CSC in OSCC. We found that the expression of several well-known CSC genes, including CD44, ALDH1, BMI1, NANOG, OCT4, and SOX2, were higher in CD10^{high} cells than CD10^{low} cells (Figure 3b). We also explored the genes related to epithelial–mesenchymal transition (EMT), another process occurring in CSC (Kajiyama et al., 2007; Mani et al., 2008). Compared to CD10^{low} cells, CD10^{high} cells had a lower expression of E-Cadherin (the epithelial-associated gene), and a higher expression of N-Cadherin, Vimentin, and Slug (the mesenchymal-related genes) (Figure 3c). This indicated that CD10-positive cells of OSCC revealed enhanced mesenchymal characteristics.

The function assays associated with the EMT process were also performed. By preforming the transwell assay, we found that CD10^{high} cells exhibited stronger migration and invasion capacity than CD10^{low} cells (Figure S2b,c).

To further confirm these findings in vivo, we performed tumorigenicity assay in BALB/c-nude mice with different CD10^{high} or CD10^{low} cells numbers separated from WSU-HN6. Tumor formation was observed in mice injected with a different number of CD10^{high} cells (5×10^3, 5×10^4, 5×10^5). In contrast, the tumor was injected only in mice treated with 5×10^5 CD10^{low} cells, but not in 5×10^3 and 5×10^4 CD10^{low} cell groups. These data confirmed the enhanced CSC-associated character of CD10^{high} cells in OSCC (Figure 3d).

3.4 | Hedgehog pathway has a regulatory role in CD10^{high} cells-associated cisplatin resistance of OSCC

Hedgehog pathway is crucial for cell self-renewal, tissue maintenance, and cell regeneration. In this study, we investigated its role in the cisplatin resistance of CD10^{high} cells in OSCC. We first detected the expression of representative genes of the Hedgehog pathway. Higher expression of Gli1 was found in CD10^{high} cells compared to CD10^{low} cells (about 5 times, Figure 4a). Besides, knockdown of

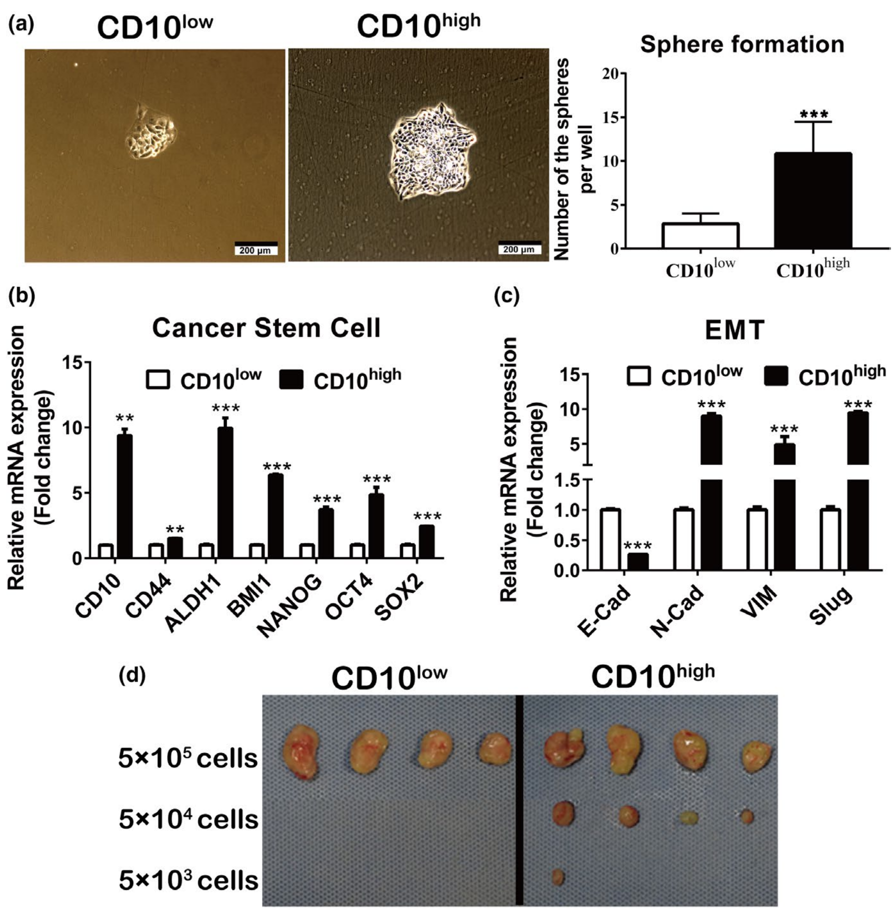


FIGURE 3 CD10-positive cells showed enhanced cancer stem cell (CSC)-associated characters. (a) Tumor formation capacity of CD10^{high} and CD10^{low} cells was detected by in vitro tumor spheroid formation assay. Photographs were taken under 40 × microscope, and the scale bar was 200μm (***, $p < .005$). (b) The expression of genes related to CSCs between CD10^{high} and CD10^{low} cells was detected by qPCR (***, $p < .005$; **, $p < .01$). (c) The expression of genes related to EMT between CD10^{high} and CD10^{low} cells was detected by qPCR (***, $p < .005$; **, $p < .01$). (d) In vivo tumorigenicity of CD10^{high} and CD10^{low} cells was detected by injecting cells into the back of BALB/c-nude mice [Colour figure can be viewed at wileyonlinelibrary.com]

CD10 by siRNA downregulated Gli1 and SMO signaling in WSU-HN6 cells (Figure 4b). At the protein level, we performed immunofluorescent staining to observe the nuclear translocation of Gli1. The results showed that the expression of Gli1 in nuclear was more obvious in CD10^{high} cells compared to CD10^{low} cells (Figure 4c). Once again, after the knockdown of CD10 by siRNA, the expression of Gli1 in nuclear was decreased (Figure 4d).

After that, the xenograft study was performed. In vivo study revealed that the Hh inhibitor could significantly eliminate the cisplatin resistance in CD10^{high} cells, while the addition of Hh agonist partially enhanced the cisplatin resistance of CD10^{low} cells (Figure 4e). Moreover, CD10^{high} cells led to more obvious vertebra metastasis than CD10^{low} cells. Cisplatin could reduce vertebra metastasis of CD10^{high} cells, while by the combination of cisplatin and GDC0449, the vertebra metastasis of CD10^{high} cells was almost as same as CD10^{low} cells (Figure 4f). This result indicated that the Hh pathway promotes the cisplatin resistance of CD10-positive cells in OSCC.

4 | DISCUSSION

Chemotherapy is a necessary treatment approach for controlling advanced oral cancers. Yet, chemotherapy resistance, either intrinsic before or acquired after chemotherapy treatment, has a crucial role in the recurrence of cancer, which is also one of the leading causes of cancer-related death worldwide (Housman et al., 2014). A better understanding of the mechanism of chemotherapy resistance may be helpful in guiding cancer chemotherapy and improving patients' survival. Chemotherapy resistance is regulated by many factors, including drug efflux, drug target alterations, enhanced DNA damage repair and senescence escape, epigenetic alterations, and tumor heterogeneity. CSCs, a pre-existing subpopulation of insensitive cells within heterogeneous tumor cell populations, are activated upon drug treatment. After activation, these cells may lead to resistance to chemotherapeutic treatment (Vasan et al., 2019).

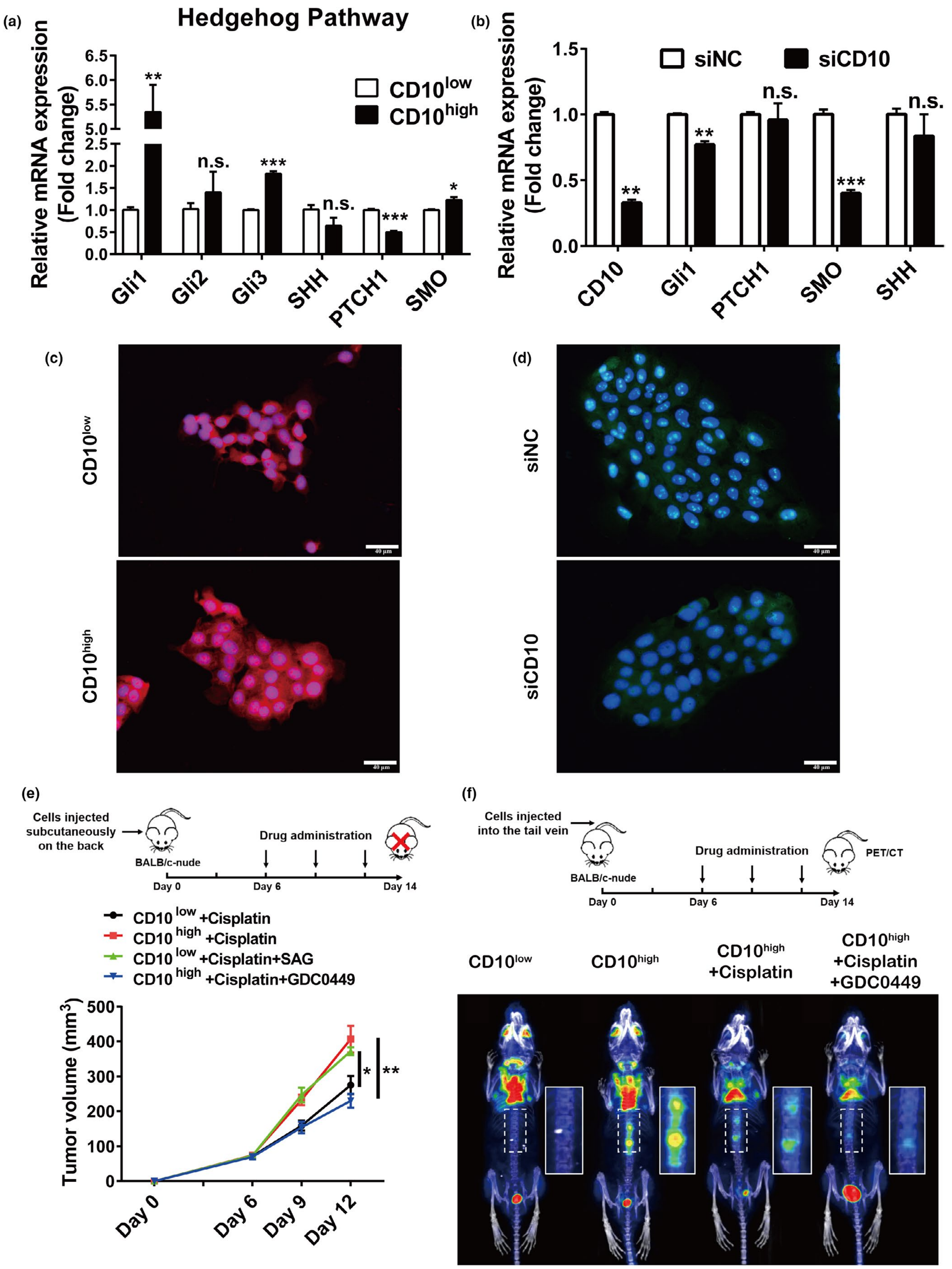
The CD10 protein is a new cell surface glycoprotein metal-binding enzyme used as an immunohistochemical marker to distinguish between normal endometrial stroma and endometrial stromal tumors (McCluggage et al., 2001). Fukusumi, et al. suggested that CD10 is associated with cisplatin resistance and CSC-like properties of head and neck squamous cell carcinoma cell lines (Fukusumi et al., 2014). Yet, the specific regulatory mechanism of CD10-positive cells during the cisplatin resistance still remains unclear.

In this study, we observed stronger cisplatin resistance in OSCC cell line with enhanced expression of CD10 gene. Furthermore, the cell survival rate of CD10^{high} cells was higher than CD10^{low} cells after cisplatin treatment. Molecules closely related to chemotherapy resistance include ATP-binding cassette (ABC) drug transporters such as ABCB1 and ABCG2, which protect cancer stem cells from chemotherapeutic drugs (Dean et al., 2005). In this study, we found an increased expression of the drug-resistant genes ABCB1 and ABCG2 in CD10^{high} cells, which further suggested that CD10 is implicated in developing cisplatin resistance of oral squamous cell carcinoma.

At the same time, through in vitro spheroid formation experiments and cell cycle assays, it was confirmed that CD10^{high} cells have stronger stem cell characteristics. At the genetic level, CD10^{high} cells significantly expressed CSC-associated genes CD44, ALDH1, BMI1, NANOG, OCT4, and SOX2 (Lazarevic et al., 2018; Xia, 2014). In addition, EMT is another factor that can induce stem cell characteristics and has a vital role in tumorigenesis and chemotherapy resistance. Many studies have shown that the acquisition of CSCs is related to EMT (Lazarevic et al., 2020; Singh & Settleman, 2010; Thiery et al., 2009; Zhou et al., 2017). We found that CD10^{high} cells have low expression of epithelial-associated genes and high expression of mesenchymal cell-related genes, which further suggested that CD10^{high} cells have more robust CSC characteristics. In addition, the tumorigenicity assay in BALB/c-nude mice with different numbers of CD10^{high} or CD10^{low} cells affirmed its CSC characteristics. Furthermore, we also explored the possibility of CSC being regulated by SHH and found that the CD10^{high}-cells-based cisplatin resistance of OSCC was significantly weakened after the combination of Hh inhibitors within in vivo models.

The Hh pathway has an essential role in embryonic development and organ formation in animals (Guo et al., 2018). Mutation or incorrect expression of its molecules could activate the pathway, which ultimately leads to the occurrence and development of cancer (Bora-Singhal et al., 2020; Wu et al., 2017). In our study, we found an enhanced Hh pathway activation in CD10^{high} cells compared to CD10^{low} cells, which was revealed by the higher expression and nuclear translocation of Gli1. The association between CD10 and Hh pathway in OSCC was further confirmed by knockdown CD10, which inhibited the activation of the Hh pathway, and by adding the agonist of Hh to CD10^{low} cells, which promoted their growth. These data were further confirmed in vivo; Hh inhibitor could eliminate the cisplatin resistance in CD10^{high} cells, while the addition of Hh agonist partially enhanced the cisplatin resistance of CD10^{low} cells CD10^{low} cells. These results demonstrated that

FIGURE 4 Hedgehog pathway has a regulatory role in CD10^{high} cells associated with cisplatin resistance of OSCC. (a) The expression of Hh pathway associated genes in CD10^{high} and CD10^{low} cells was analyzed by qPCR (***, $p < .005$; **, $p < .01$; *, $p < .05$; n.s., no statistic difference). (b) The expression of Hh pathway-associated genes of WSU-HN6 cells treated by siNC or siCD10 detected by qPCR (***, $p < .005$; **, $p < .01$, n.s., no statistic difference). (c) The expression of Gli1 in CD10^{high} and CD10^{low} cells was observed by immunofluorescence. Photographs were taken under 200 × microscope, and the scale bar was 40 μm. (d) The expression of Gli1 in WSU-HN6 cells treated with siNC or siCD10 was observed by immunofluorescence. Photographs were taken under 200 × microscope, and the scale bar was 40μm. (e) Tumor volume variation of xenograft tumor with CD10^{high} or CD10^{low} cells under the treatment of cisplatin with or without Hh inhibitor GDC0449 or agonist SAG (**, $p < .01$; *, $p < .05$). (f) Representative PET/CT images of the mice injected with CD10^{high} or CD10^{low} cells and treated with vector, cisplatin, or with Hh inhibitor GDC0449. The insets show the magnified boxed region [Colour figure can be viewed at wileyonlinelibrary.com]


(a)
Hedgehog Pathway
Relative mRNA expression (Fold change)
CD10low
CD10high
Gli1
Gli2
Gli3
SHH
PTCH1
SMO
n.s.
(b)
Relative mRNA expression (Fold change)
siNC
siCD10
CD10
Gli1
PTCH1
SMO
SHH
(c)
CD10low
CD10high
(d)
siNC
siCD10
(e)
Cells injected subcutaneously on the back
BALB/c-nude
Drug administration
Day 0
Day 6
Day 14
CD10 low +Cisplatin
CD10 high +Cisplatin
CD10 low +Cisplatin+SAG
CD10 high +Cisplatin+GDC0449
Tumor volume (mm3)
Day 0
Day 6
Day 9
Day 12
(f)
Cells injected into the tail vein
BALB/c-nude
Drug administration
PET/CT
Day 0
Day 6
Day 14
CD10low
CD10high
CD10high +Cisplatin
CD10high +Cisplatin +GDC0449

the Hh pathway has a role in CD10^{high} cells associated with chemotherapy resistance in OSCC.

Our data provide a theoretical basis for solving the problem of poor sensitivity of OSCC chemotherapy. Moreover, further studies should take into consideration of tumor microenvironment. Su et al proposed that CD10^{+}/GPR77^{+} cancer-associated fibroblasts mediate IL-6/8 secretion through the NF-κB pathway, support the cancer stemness, and promote tumor formation and chemotherapy resistance (Su et al., 2018). Therefore, the establishment of a cisplatin resistance microenvironment research model that focused on tumor cells and stromal cells and their interactions may further elucidate the actual state of the tumor.

To sum up, CD10-positive cells are implicated in developing cisplatin resistance of OSCC, which could be related to its cancer stem cell characteristics regulated by the Hedgehog pathway.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

AUTHOR CONTRIBUTIONS

Yifei Wang: Conceptualization; Project administration; Writing-original draft. **Qingxiang Li:** Investigation; Project administration; Writing-review & editing. **Le Xu:** Methodology; Project administration. **Junpeng Chen:** Project administration. **Yinfei Pu:** Project administration. **Lin Wang:** Methodology; Supervision; Writing-review & editing. **Hongfang Sun:** Methodology; Project administration; Software. **Yuxing Guo:** Conceptualization; Funding acquisition; Supervision; Validation; Writing-review & editing. **Chuanbin Guo:** Conceptualization; Funding acquisition; Supervision; Writing-review & editing.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The animal study was performed in accordance with the National Institute of Health (NIH), USA guidelines on the care and use of animals for experimental procedures, and in accordance with local laws and regulations. The study was approved by the Peking University institutional animal care and conducted according to the AAALAC and the IACUC guidelines (2018/06/27, NO. LA2018249).

CONSENT FOR PUBLICATION

None of individual person's data were included in this study. Consent to publish has been obtained from all authors.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/odi.13673.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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