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# Cancer-derived IgG involved in cisplatin resistance through PTP-BAS/Src/PDK1/AKT signaling pathway

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## Abstract

**Objectives:** This study aimed to explore whether knockdown of cancer-derived IgG (CIgG) could enhance cisplatin-induced anti-cancer effects.

**Materials and methods:** Cancer-derived IgG was knocked down by siRNA or Tet-on shRNA in the absence or presence of cisplatin in WSU-HN6 or CAL27 cells. Cell proliferation, apoptosis, and mobility were evaluated using CCK-8, flow cytometry, and transwell assays, respectively. Molecular events were investigated using real-time PCR and Western blot assays.

**Results:** Knockdown of CIgG significantly promoted cisplatin-induced apoptosis and inhibition of cell proliferation, migration, and invasion. Cisplatin upregulated CIgG expression and phosphorylation of AKT and PDK1, while knockdown of CIgG downregulated phosphorylation of AKT and PDK1, and blocked cisplatin-induced upregulation of AKT and PDK1 phosphorylation. Moreover, knockdown of CIgG blocked cisplatin-induced upregulation of Src phosphorylation, and knockdown of Src blocked cisplatin-induced upregulation of AKT and PDK1 phosphorylation. Overexpression of Src upregulated AKT and PDK1 phosphorylation. Furthermore, knockdown of CIgG upregulated PTP-BAS mRNA and protein expression, whereas cisplatin downregulated PTP-BAS protein, but not mRNA expression; knockdown of PTP-BAS upregulated phosphorylation of Src, PDK1, AKT, and blocked CIgG knockdown-mediated enhancement of cisplatin-induced inhibition of cell proliferation.

**Conclusion:** Knockdown of CIgG enhanced the anti-cancer effects of cisplatin through PTP-BAS/Src/PDK1/AKT signaling pathway in oral squamous cell carcinoma.

KEYWORDS

cancer-derived IgG, cisplatin resistance, head and neck squamous cell carcinoma, PTP-BAS, Src

## 1 | INTRODUCTION

Cancer-derived IgG (CIgG), also known as CA215, is a new-found IgG molecular that is generated only from cancer cells, including carcinomas of breast, colon, liver, lung, head, and neck (Chen et al., 2010; Zhu et al., 2008). RP215, an antibody which is produced by using the cell extract of OC-3-VGH ovarian cancer cells as antigen, was originally considered as an antibody to an unknown pan cancer marker and later confirmed as CIgG monoclonal antibody (Lee, Laflamme, Chien, & Ting, 2008). CIgG significantly correlates to cancer initiation, proliferation, and metastasis, and participates in tumor immune escape (Liao et al., 2015; Qiu et al., 2003; Wang et al., 2019; Yang et al., 2013). Moreover, patients with high CIgG expression have poor prognosis (Liu

et al., 2015; Tang et al., 2018). Previous study showed integrin–FAK signaling pathway as a main target of CIgG (Tang et al., 2018). As an IgG molecule produced only by cancer cells and almost has no cross-reactivity with normal IgG, CIgG suggests its great potential in tumor treatment. Yet, its function and molecular mechanisms remain largely unknown.

Head and neck squamous cell carcinoma (HNSCC) ranks seventh by incidence among the most common cancer in 2018, with 3.5 million cases (2% of the total cancers) from lip and oral cavity (Ferlay et al., 2019). Late diagnosis and high recurrent rate lead to a low 5-year survival rate (Amit et al., 2013; Cheraghlou, Schettino, Zogg, & Judson, 2018). For late-stage lesions, unresectable tumors, and recurrent or metastatic HNSCC patients, cisplatin remains the first-line drugs for chemotherapy (Ettinger, Ganry, & Fernandes, 2019; Huang & O'Sullivan, 2013). However, acquired cisplatin resistance has limited its clinical application with molecular mechanism remains complex and largely unknown. Changes of DNA repair abilities, altered proteins that relate with cisplatin accumulation, and cytosolic inactivation of cisplatin are believed to contribute to cisplatin resistance (Amable, 2016; Galluzzi et al., 2012; Ghosh, 2019). Recently, tumor microenvironment and long non-coding RNAs and downstream pathways are also shown to involve in cisplatin resistance (Abu, Hon, Jeyaraman, & Jamal, 2018; Chen & Chang, 2019). Nevertheless, it is still of clinical and theoretical significance to explore the signaling pathway that could reverse cisplatin resistance.

Src (c-Src) might also be involved in cisplatin resistance, since increased Src expression or Src phosphorylation was detected in cisplatin-resistant cell lines (Huang, Kuo, Wang, Yeh, & Wang, 2019; Yang et al., 2019). Src is the first described proto-oncogene directly linked to cancer development. High expression or over-activation of Src has been observed in a variety of cancer cells and plays a key role in cancer genesis, metastasis, and prognosis (Irby & Yeatman, 2000). Two main phosphorylation sites that regulate Src activity include autophosphorylation site (Y419, human; Y416, chicken) and negative regulatory residue (Y530, human; Y527, chicken). C-terminal SRC kinase (CSK) phosphorylates Y530, lead to a suppression of Src activity. For autophosphorylation site Y419, tyrosine phosphatase PTP-BAS (also called PTPN13, PTPL1, or FAP-1) directly dephosphorylates it and inhibits the function of Src (Glondu-Lassis et al., 2010). AKT could be involved in Src-mediated cisplatin resistance, as Src inhibitor dasatinib downregulates AKT phosphorylation (Chen et al., 2015). It is worth noticing that cisplatin-resistant cells also develop a certain degree of resistance to dasatinib (Yang et al., 2019). Recently, we also showed that T308 phosphorylation of AKT is important in cisplatin resistance (Zhao, Li, & Gan, 2018). Importantly, Src also regulates 3-phosphoinositide-dependent protein kinase 1 (PDK1) and AKT phosphorylation (Grillo et al., 2000; Thapa, Choi, Tan, Wise, & Anderson, 2015; Yang et al., 2008). All these results imply that Src might be involved in cisplatin resistance through regulating AKT phosphorylation.

PTP-BAS is a negative regulator of Src activation. PTP-BAS is a 277 kDa non-receptor type protein tyrosine phosphatase, which was identified as a tumor suppressor (Yeh et al., 2006). Its function involves in cell proliferation, apoptosis, and aggressiveness (Bompard, Puech, Prebois, Vignon, & Freiss, 2002; Freiss, Puech, & Vignon, 1998; Glondu-Lassis et al., 2010). Some cancer cells express low level of PTP-BAS because of gene deletion including ovarian and liver cancers (Inazawa et al., 1996), promoter hypermethylation including hepatocellular carcinoma and multiple lymphomas (Hoover et al., 2009; Zhan et al., 2016), and mutations including colorectal cancer (Wang et al., 2004). Clinical studies showed correlation between PTP-BAS and prognosis (D'Hondt et al., 2017; Revillion et al., 2009). Downstream molecules of PTP-BAS include Fas, Src, PTEN, and insulin receptor substrate-1 (IRS-1)-mediated PI3K/AKT signaling pathway (Bompard et al., 2002; Freiss et al., 1998; Glondu-Lassis et al., 2010). However, PTP-BAS was never studied in HNSCC. Whether PTP-BAS is involved in cisplatin resistance in HNSCC remains to be explored.

In this study, we showed for the first time that knockdown of CIgG enhanced anti-cancer effects of cisplatin through PTP-BAS/Src/PDK1/AKT signaling pathway in oral squamous cell carcinoma.

## 2 | MATERIAL AND METHODS

### 2.1 | Cell lines

WSU-HN6 and CAL27 cell lines were derived from human oral tongue squamous cell carcinoma. 293T was derived from human embryonic kidney. WSU-HN6 was obtained from Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine (Shanghai, PR China), and was applied in previous studies (Jeon et al., 2004; Lv et al., 2014). CAL27 and 293T were purchased from American Type Culture Collection (Manassas, VA, USA). All cells were cultured in Dulbecco's Modified Eagle's Medium (Gibco) with 10% fetal bovine serum (FBS) and 1% Pen Strep (Gibco) at 37°C with 5% CO2.

### 2.2 | Antibodies and reagents

Anti-CIgG antibodies RP215 (sc-69849) were purchased from Santa Cruz Biotechnology. Anti-phospho-Src (Y416, but can also recognize Y419 of human Src) antibodies (#2101), anti-Src antibodies (#2108), anti-phospho-PDK1 (S241) antibodies (#3061), anti-PDK1 antibodies (#3062), anti-GFP antibodies (#2555), anti-phospho-AKT (T308) antibodies (#13038), anti-AKT (pan) antibodies (#4685) were purchased from Cell Signaling Technology. Anti-PTP-BAS (25944-1-AP) and anti-β-actin (I-19) antibodies were purchased from Proteintech Group. Cisplatin and BX-795 were purchased from Selleck Chemicals. Cisplatin was dissolved in N, N-dimethylformamide, and a concentration of 30 μM was prepared and stored at −20°C. BX-795 was dissolved in dimethyl sulfoxide and stored at −20°C. The doses of

cisplatin and BX-795 were applied according to the $IC_{50}$ obtained in preliminary experiments and empirically in combination of CIgG knockdown.

## 2.3 | Small interfering RNAs, Plasmids, and Primers

Small interfering RNAs (siRNA) used for transient transfection were synthesized according to the following sequence: human CIgG siRNA 1:5′-GGUGGACAAGACAGUUGAG-3′; siRNA 2:5′-AGUGCAAGGUCUCCAACAA-3 (Liao et al., 2015); Src: 5′–AUUUUGGCAAGAUCACCAGA-3′ (designed according to Src mRNA transcript variant X4, NCBI Reference Sequence: XM_017028027.2, from 493 to 512); PTP-BAS: 5′–GGAAAGAAGAGUUCGUUUA-3′ (Glondu-Lassis et al., 2010). Short hairpin RNA (shRNA) used for stable knockdown of human CIgG was synthesized according to the following sequence: 5′-CCGGGGGGTGGACAAGACAGTTGAGTCAGAGCTCAACTGTCTTGTCCACCCCTTTTT-3′ (sense); 5′-AATTAAAAAGGGGTGGACAAGACAGTTGAGCTCTGACTCAACTGTCTTGTCCACCCC-3′ (antisense), and then inserted into Tet-pLKO-puro plasmids (Addgene Cambridge, MA, USA) at AgeI and EcoRI sites to construct Tet-on shCIgG plasmid. T308 of AKT was mutated into aspartic acid to construct constitutively active AKT (ca-AKT) with the following sequence: 5′-GCCACCATGAAGGACTTTTGCGGCACA-3′ (sense); 5′-TGTGCCGCAAAAGTCCTTCATGGTGGC-3′ (antisense) (Alessi et al., 1996) and inserted into pLVX-AcGFP-N1 vectors (Clontech) at EcoRI and BamHI sites to construct ca-AKT-GFP plasmid. Y419 of human Src was mutated into phenylalanine with the following sequence: GGCTCGGCTCATTGAAGACAATGAGTTCACGGCGCGGCAAGGTGCCAAATTCC-3′ (sense); 5′-GGAATTTGGCACCTTGCCGCGCCGTGAACTCATTGTCTTCAATGAGCCGAGCC-3′ (antisense) and then inserted into pEGFP-C1 vectors (Clontech) at EcoRI and XhoI sites to construct mutant Src Y419F plasmid. Primers for real-time PCR were as follows: human PTP-BAS: 5′-TTGGAATGACACTGTATTGGGG-3′ (sense); 5′-CCAAGCAGTATGCTGTTGAGAT-3′ (antisense); human β-actin: 5′-CGGGAAATCGTGCGTGAC-3′ (sense); 5′-CAGGCAGCTCGTAGCTCTT-3′ (antisense). Src (NG_023033.1) coding sequence amplified from cDNA of WSU-HN6 was inserted into pEGFP-C1 vectors at EcoRI and XhoI sites to construct Src-GFP plasmid.

## 2.4 | Stable transfection with lentivirus

The plasmids Tet-on shCIgG, ca-AKT-GFP, and pLVX-AcGFP-N1 were respectively co-packed by Gag, Rev, and VSVG and transfected into 293T cells; lentiviral supernatants were collected and added into WSU-HN6 as described previously (Zhao et al., 2018). The infected WSU-HN6 cells were screened after adding lentiviral supernatants for 48 hr, using 1 μg/ml puromycin. Knockdown of CIgG, overexpression of Src, and constitutively active AKT in WSU-HN6 cells were confirmed by Western blot assays.

## 2.5 | Cell proliferation assay

Cell Counting Kit-8 (CCK-8; Dojindo) was used to perform cell proliferation assay (Tominaga et al., 1999). Cells transfected with CIgG or scrambled siRNA were seeded into 96-well plates ($3 \times 10^3$ per well) and treated with cisplatin (10 μM) or DMF (vehicle). After 48 hr treatment, growth medium containing 10% CCK-8 was added to each well and incubated at 37°C for 2 hr. Absorbance at 450 nm was determined to analyze cell proliferation.

## 2.6 | Assessment of cell apoptosis

Cell apoptosis was evaluated by flow cytometry assay. After 20 hr treatment of cisplatin, cells were washed with PBS. Cells were digested and incubated using FITC-Annexin V and PI for 15 min at room temperature in the dark. Flow cytometry analysis was performed using a Beckman Coulter EPICS Altra HSS flow cytometer (Beckman Coulter). Data were analyzed using PolyFlo software (PolyFlo Inc.), and Annexin V+/PI- cells (R4 zone) were calculated as early apoptotic cells.

## 2.7 | Transwell migration and invasion assay

Transwell chambers (Corning Costar) were used for migration and invasion assays. After CIgG siRNA transfection and starvation for 12 hr, cells ($1 \times 10^5$ per well) were seeded in the upper chambers with serum-free medium. Lower chambers contained DMEM with 10% FBS in the presence or absence of cisplatin. For migration assays, cells were incubated at 37°C for 16 hr. For invasion assay, cells were seeded into the upper chamber that had been precoated with extracellular matrix gel (BD, Biosciences) and incubated at 37°C for 12 hr. Unattached cells on the top were wiped off, whereas migrated or invaded cells were counted by crystal violet (0.1%) staining. Six randomly selected fields of cell amount were counted, and results were presented in ratio as means ± *SD*.

## 2.8 | Protein extraction and Western blot analysis

Cells were washed with phosphate-buffered saline (PBS) and lysed with RIPA lysis buffer (Applygen) on ice for 30 min. Protein concentrations were determined by BCA protein assay (Thermo Fisher Scientific Inc.). Twenty micrograms of protein samples was separated by 6% or 10% SDS–PAGE and transferred electrophoretically onto to polyvinylidene fluoride membrane (Millipore), then blocked the membrane with 5% non-fat milk in TBS-T (50 mmol/L Tris, pH 7.5; 150 mmol/L NaCl; 0.05% Tween 20) for 1 hr and probed with antibodies diluted at 1:1,000 (anti-PTP-BAS antibody diluted at 1:300) for 14 hr at 4°C. Secondary antibodies were diluted at 1:10,000 and incubated for 1 hr at room temperature. β-actin was used for internal controls of equal loading. Densitometric analyses of Western blots (Gassmann, Grenacher,

Rohde, & Vogel, 2009) were performed using ImageJ software. First, the relative density of target proteins was calculated for each group from the equation that the density values of target protein was divided by the density values of its corresponding internal control protein (β-actin or total target protein in case of phosphorylation). Second, the relative density of target protein in non-control groups was divided by the relative density of target protein in the control group. The data were presented as the fold change to that of the control in the Figures.

### 2.9 | Coefficient of drug interaction

Coefficient of drug interaction (CDI) was performed based on previous study (Cao & Zhen, 1989; Xu et al., 2007) and used to assess synergy between cisplatin treatment and CIgG or Src knockdown. Based on the absorbance or cell amount, A or B is the ratio of the cisplatin group or CIgG knockdown group to that of the control group; AB is the ratio of the combination treatment group to that of the control group. CDI calculated as AB/ (A × B). Thus, CDI value <1 indicates that the treatments are synergistic. CDI <0.7 indicates that the treatments are significantly synergistic, and CDI > 1 indicates that the treatments are antagonistic.

### 2.10 | Patient samples

All samples were obtained from Biobank of Peking University School and hospital of Stomatology. Twenty clinical specimens of tongue squamous cell carcinoma and adjacent normal tissues, which were confirmed by pathological examination, were tested for CIgG protein expression using Western blot assays. The experiment was approved by the Ethics Committee of Peking University School and hospital of Stomatology.

### 2.11 | Statistical analysis

Statistical analysis was analyzed using SPSS 22. All experiments were repeated at least three times, and all data were presented as the mean ± *SD*. Two-tailed *t* test was used to analyse the difference of CIgG protein expression between the tumor tissues and the adjacent normal tissues. One-way ANOVA was used to analyse differences among multiple groups. Probability value was calculated and less than .05 was considered to be statistically significant.

## 3 | RESULTS

### 3.1 | Knockdown of CIgG synergistically enhanced cisplatin-induced apoptosis and inhibition of cell proliferation, migration, and invasion

As shown in Figure 1a, knockdown of CIgG or cisplatin treatment resulted in a slight inhibition of proliferation compared to control group, whereas combination of cisplatin with CIgG knockdown induced a synergistic inhibition of cell proliferation in both WSU-HN6 and CAL27 cell lines, compared to the group of cisplatin treatment alone or knockdown of CIgG alone, and the coefficient of drug interaction (CDI) is 0.33 and 0.12, respectively, less than 0.7, suggesting that the combination of CIgG knockdown and cisplatin produced significantly synergistic inhibitory effect. Cell apoptosis was evaluated using flow cytometry, and the number of WSU-HN6 apoptotic cells in the combination group (45.12%) was three times that of the group of cisplatin treatment alone (10.74%) or knockdown of CIgG alone (9.47%), similar results were also confirmed in CAL27 cells (Figure 1b). Reduction in 18% in migratory cells was observed in the group of cisplatin treatment alone, still when combined with CIgG knockdown, knockdown of CIgG significantly promoted cisplatin-induced inhibition of WSU-HN6 cell migration and invasion, with CDI for 0.27 and 0.34, respectively (Figure 1c,d). Knockdown of CIgG also significantly promoted cisplatin-induced inhibition of CAL27 cell migration (Figure S1).

Moreover, we examined the protein expression of CIgG in cancer and adjacent tissues in 20 samples of tongue squamous cell carcinoma. The expression of CIgG in cancer tissues was higher than that in adjacent tissues in 17 samples with 13 samples more than twofold higher (65%, 13/20), and lower than that in adjacent tissues in 3 samples (Figure 1e,f). We also noticed that CIgG expression was detectable in adjacent tissues in 13 samples.

### 3.2 | Effects of CIgG knockdown on cisplatin treatment via blocking cisplatin-induced upregulation of PDK1/AKT signaling

T308 phosphorylation of AKT plays an important role in cisplatin resistance (Zhao et al., 2018) and is regulated by phosphatase PP2A (Kuo et al., 2008) and phosphokinase PDK1 (Dangelmaier et al., 2014). To explore the mechanism underlying the synergistic effect of CIgG knockdown on anti-cancer effects of cisplatin, we first examined CIgG expression and phosphorylation of AKT and PDK1 after treatment with cisplatin in WSU-HN6 cells. As shown in Figure 2a,b, cisplatin upregulated CIgG expression and phosphorylation of AKT (both T308 and S473) and PDK1 (S241) in a dose- and time-dependent manner. Knockdown of CIgG significantly downregulated T308, but not S473, phosphorylation of AKT, and S241 phosphorylation of PDK1, and completely blocked cisplatin-induced upregulation of phosphorylation of AKT and PDK1 in both two cell lines (Figure 2c). Although knockdown of CIgG did not affect S473 phosphorylation of AKT, it also blocked cisplatin-induced upregulation S473 phosphorylation of AKT (Figure 2c).

Similarly, PDK1 inhibitor BX795 (inhibiting T308 phosphorylation of AKT without affecting S473 of AKT) also significantly promoted cisplatin-induced inhibition of cell proliferation (Figure S2) and blocked cisplatin-induced upregulation of AKT T308 phosphorylation. Although BX795 did not affect S473 phosphorylation of AKT, it also blocked cisplatin-induced upregulation of S473 phosphorylation

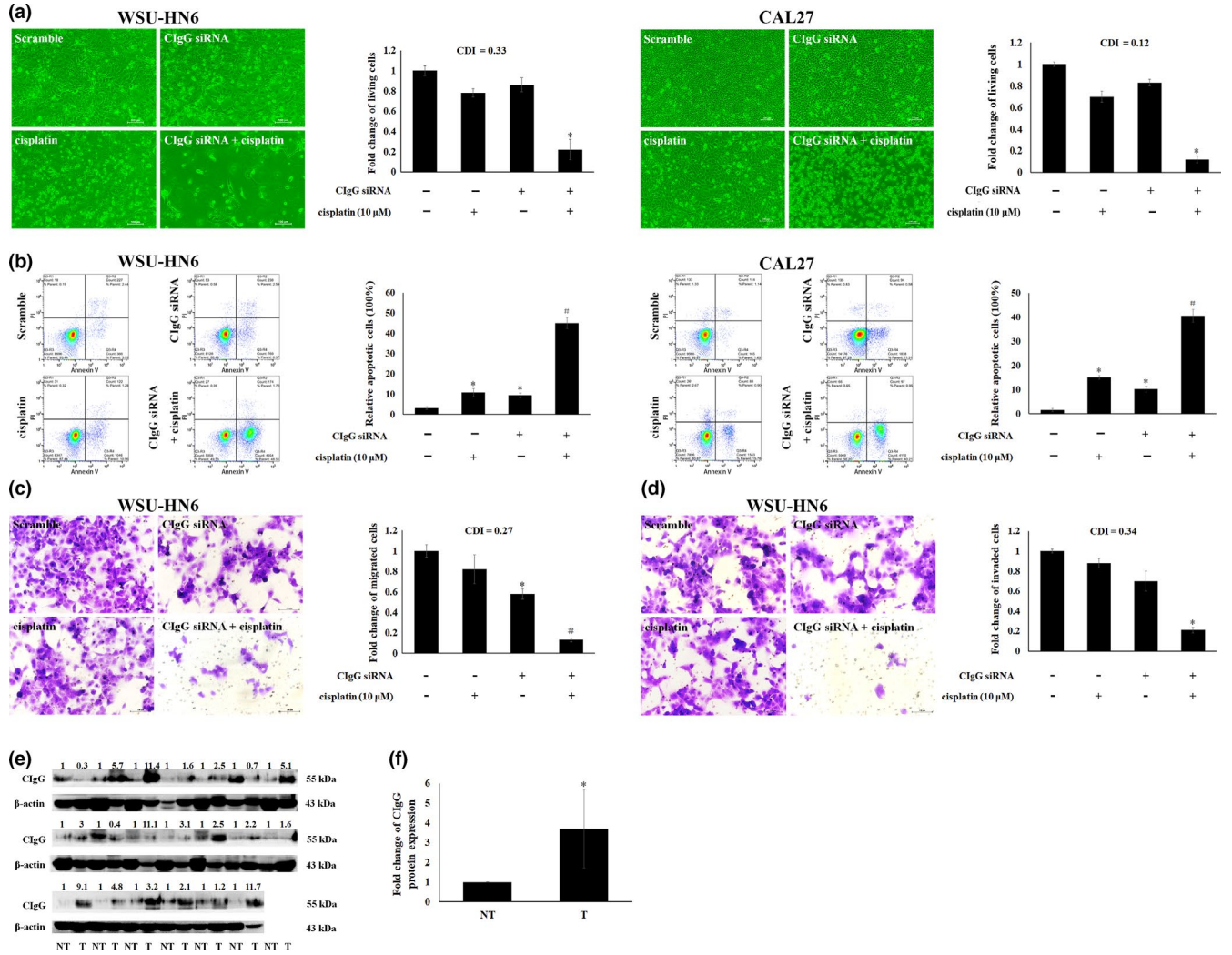


**FIGURE 1** Knockdown of cancer-derived IgG (CIgG) synergistically enhanced cisplatin-induced anti-cancer effects in WSU-HN6 and CAL27 cells, and CIgG protein highly expressed in tongue cancer. (a) Microphotographs of cells and quantified data of cell proliferation after different treatments. WSU-HN6 and CAL27 cells transfected with CIgG siRNA or scrambled siRNA were treated with or without cisplatin (10 μM) for 48 hr and subjected to CCK-8 assays. $^{*}p < .05$ versus rest three groups ($n = 6$). (b) Flow cytometry analysis and quantified data of cell apoptosis after different treatments. WSU-HN6 and CAL27 cells with transfection of CIgG siRNA or scrambled siRNA were treated with or without cisplatin (10 μM) for 20 hr and subjected to flow cytometry assay with Annexin V and PI staining. $^{*}p < .05$ versus control group; $^{\#}p < .05$ versus cisplatin group or CIgG siRNA group ($n = 3$). (c) Microphotographs of cells and quantified data of cell migration after different treatments. WSU-HN6 cells with transfection of CIgG siRNA or scrambled siRNA were seeded into the upper chamber after starvation for 12 hr and then treated with or without cisplatin (10 μM) for 16 hr. $^{*}p < .05$ versus control group; $^{\#}p < .05$ versus CIgG knockdown group or cisplatin group ($n = 4$); scale bar: 100 μm. (d) Microphotographs of cells and quantified data of cell invasion after different treatments. WSU-HN6 cells with transfection of CIgG siRNA or scrambled siRNA were seeded into the upper chamber coated with extracellular matrix gel after starvation for 12 hr and then treated with or without cisplatin (10 μM) for 12 hr. $^{*}p < .05$ versus the rest three groups ($n = 4$); scale bar: 100 μm. (e, f) CIgG protein expression in tongue cancer tissues and adjacent tissues. The protein expressions of CIgG in the tumor tissue and adjacent normal tissue of 20 cases were assessed by Western blot. $^{*}p < .05$ ($n = 20$); NT, adjacent normal tissue; T, tumor [Colour figure can be viewed at wileyonlinelibrary.com]

(Figure 2d). To further understand the role of AKT phosphorylation in the effect of CIgG knockdown on cisplatin treatment, we constructed WSU-HN6 and CAL27 cell lines with constitutively activated AKT through T308 phosphorylation (Figure S3) (Alessi et al., 1996). In these cells, knockdown of CIgG failed to promote cisplatin-induced inhibition of cell proliferation, compared to the control cells (Figure 2e). All these results implied that PDK1/AKT signaling was involved in the effect of CIgG knockdown on cisplatin treatment.

### 3.3 | Effects of CIgG knockdown on cisplatin treatment via blocking cisplatin-induced upregulation of Src/PDK1/AKT signaling

Since Src could be upstream regulator of AKT in cisplatin resistance (Chen et al., 2015), we thus examined whether Src was also involved in the effects of CIgG knockdown on cisplatin treatment. As shown in Figure 3a,b, knockdown of CIgG downregulated Y419 phosphorylation

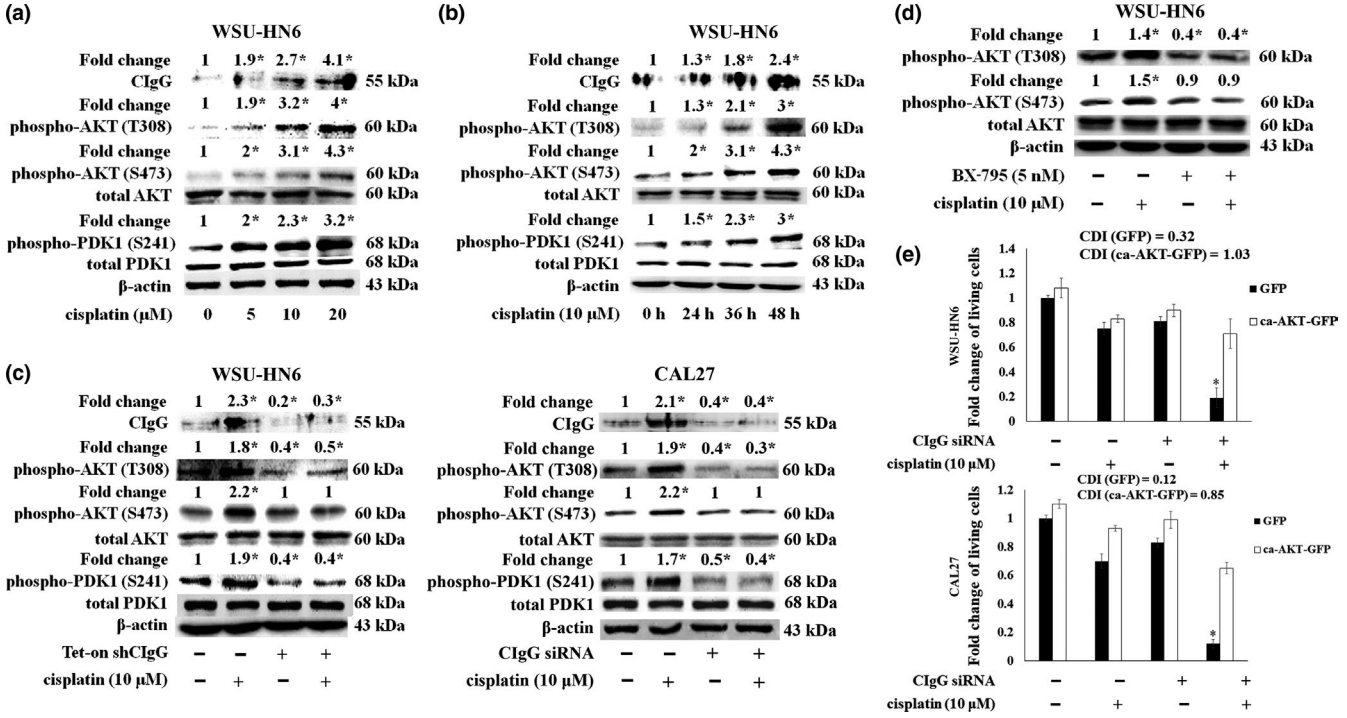


**FIGURE 2** Effects of cancer-derived IgG (CIgG) knockdown on cisplatin treatment via blocking cisplatin-induced upregulation of PDK1/AKT signaling. (a) Cisplatin upregulated CIgG expression and phosphorylation of AKT (T308 and S473), PDK1 (S241) in a dose-dependent manner. WSU-HN6 cells were exposed to different dosage of cisplatin for 48 hr. Total protein was extracted and subjected to Western blot for analysis. $^*p < .05$ versus control group ($n = 3$). (b) Cisplatin upregulated CIgG expression and phosphorylation of AKT (T308 and S473), PDK1 (S241) in a time-dependent manner. WSU-HN6 cells were exposed to cisplatin (10 μM) and protein was collected at indicated time points and then subjected to Western blot analysis. $^*p < .05$ versus the control group ($n = 3$). (c) Knockdown of CIgG blocked cisplatin-induced phosphorylation of AKT and PDK1. WSU-HN6 cells with stable transfection of Tet-on shCIgG or scrambled Tet-on shRNA in the presence or absence of doxycycline (100 nM) and CAL27 cells with transfection of CIgG siRNA or scrambled siRNA were treated with or without cisplatin (10 μM) for 36 hr. Total protein was extracted and subjected to Western blot for analysis. $^*p < .05$ versus the control group ($n = 3$). (d) PDK1 inhibitor BX795 blocked cisplatin-induced AKT phosphorylation. WSU-HN6 cells were treated with BX-795 (5 nM) or cisplatin (10 μM) or both for 30 hr. Total protein was extracted and subjected to Western blot for analysis. $^*p < .05$ versus the control group ($n = 3$). (e) Constitutively activated AKT T308 phosphorylation abolished promoting effect of CIgG knockdown on cisplatin-induced inhibition of cell proliferation. WSU-HN6 and CAL27 cells with infection of pLVX-AcGFP-N1 vectors or ca-AKT-GFP vectors were transfected with CIgG siRNA or scrambled siRNA and treated with or without cisplatin (10 μM) for 48 hr and subjected to CCK-8 assay. $^*p < .05$ versus the rest three groups ($n = 5$)

of Src and blocked cisplatin-induced upregulation of Y419 phosphorylation of Src in WSU-HN6 and CAL27 cells. Treatment with cisplatin upregulated Y419 phosphorylation of Src in a dose- and time-dependent manner (Figure S4 and Figure S5. Knockdown of Src downregulated phosphorylation of PDK1 and AKT, blocked cisplatin-induced upregulation of PDK1 and AKT phosphorylation, and synergistically enhanced cisplatin-induced inhibition of cell proliferation (Figure 3c and Figure S6). Overexpression of Src upregulated phosphorylation of both exogenous and endogenous Src, and S241 phosphorylation of PDK1 and T308 phosphorylation of AKT, but failed to rescue CIgG knockdown-induced downregulation of phosphorylation of Src, PDK1, and AKT (Figure 3d), and also failed to reverse the corresponding enhancement of CIgG knockdown on cisplatin-induced inhibition of cell proliferation (Figure 3e). These results implied that CIgG could target an upstream molecular of Src.

Y419 of Src is a key site for Src autophosphorylation or activation. To examine whether Y419 phosphorylation of Src was required for phosphorylation (activation) of endogenous Src, PDK1, and AKT, we also constructed a mutant Src Y419F plasmid. Overexpression of mutant Src Y419F failed to induce phosphorylation of endogenous Src, PDK1, and AKT (Figure 3f).

### 3.4 | Effects of CIgG knockdown on cisplatin treatment via blocking cisplatin-induced upregulation of PTP-BAS/Src/PDK1/AKT signaling

Since Src Y419 phosphorylation is regulated by tyrosine phosphatase PTP-BAS, we further examined whether PTP-BAS was also involved in cisplatin resistance. As shown in Figure 4a, knockdown of PTP-BAS upregulated phosphorylation of Src, PDK1 and AKT, whereas knockdown of CIgG upregulated PTP-BAS protein expression and correspondingly downregulated phosphorylation of Src, PDK1, and AKT. Moreover, knockdown of PTP-BAS still upregulated

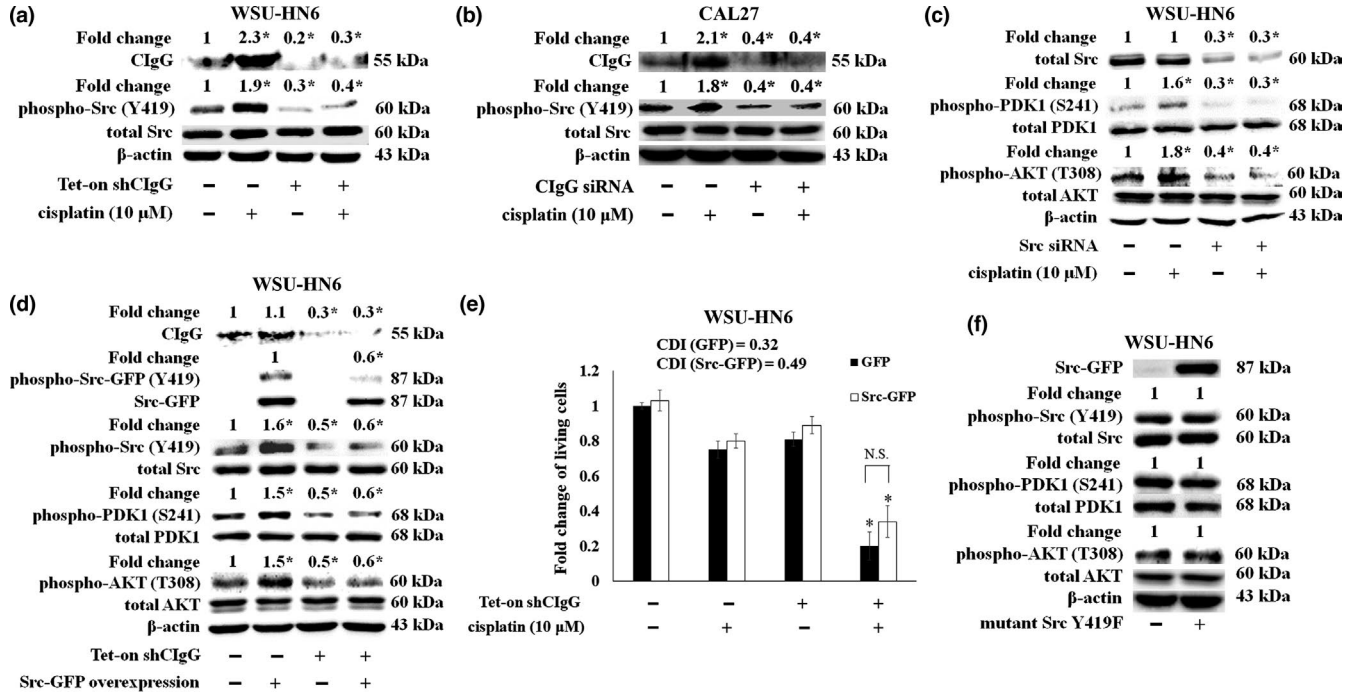


**FIGURE 3** Effects of cancer-derived IgG (CIgG) knockdown on cisplatin treatment via blocking cisplatin-induced upregulation of Src/PDK1/AKT signaling. (a) Knockdown of CIgG blocked cisplatin-induced Src phosphorylation in WSU-HN6 cells. WSU-HN6 cells with stable transfection of Tet-on shCIgG or scrambled Tet-on shRNA were treated with or without cisplatin (10 μM) in the presence or absence of doxycycline (100 nM) for 36 hr. Total protein was extracted and subjected to Western blot for analysis. *$p < .05$ versus the control group ($n = 3$). (b) Knockdown of CIgG blocked cisplatin-induced upregulation of Src phosphorylation in CAL27 cells. CAL27 cells transfected with CIgG siRNA or scrambled siRNA were treated with or without cisplatin (10 μM) for 36 hr. Total protein was extracted and subjected to Western blot for analysis. *$p < .05$ versus the control group ($n = 3$). (c) Knockdown of Src blocked cisplatin-induced phosphorylation of PDK1 and AKT. WSU-HN6 cells with transfection of Src siRNA or scrambled siRNA were treated with without cisplatin (10 μM) for 36 hr. Total protein was extracted and subjected to Western blot for analysis. *$p < .05$ versus the control group ($n = 3$). (d) Overexpression of Src upregulated phosphorylation of both exogenous and endogenous Src, PDK1, and AKT, and failed to reverse CIgG knockdown-induced downregulation of phosphorylation of Src, PDK1, and AKT. WSU-HN6 cells with stable transfection of Tet-on shCIgG or scrambled Tet-on shRNA were transfected with Src-GFP vector or GPF vector in the presence or absence of doxycycline (100 nM) for 48 hr. Total protein was extracted and subjected to Western blot for analysis. *$p < .05$ versus the control group ($n = 3$). (e) Overexpression of Src failed to reverse promoting effect of CIgG knockdown on cisplatin-induced inhibition of cell proliferation. WSU-HN6 cells with stable transfection of Tet-on shCIgG or scrambled Tet-on shRNA were transfected with Src-GFP vector or GPF vector in the presence or absence of doxycycline (100 nM) and treated with or without cisplatin (10 μM) for 48 hr and subjected to CCK-8 assay. *$p < .05$ versus the rest three groups ($n = 4$); N.S.: no significant difference. (f) Overexpression of mutant Src Y419F showed no effects on phosphorylation of Src, PDK1, and AKT. WSU-HN6 cells were transfected with GFP vectors or mutant Src Y419F vectors for 48 hr. Total protein was extracted and subjected to Western blot for analysis

phosphorylation of Src, PDK1, and AKT under the condition of CIgG knockdown. Correspondingly, knockdown of PTP-BAS totally blocked the synergistic effect of CIgG knockdown on cisplatin-induced inhibition of cell proliferation (Figure 4b). Moreover, cisplatin upregulated CIgG expression but downregulated PTP-BAS protein expression, and knockdown of CIgG still upregulated PTP-BAS protein expression in the presence of cisplatin, implying that CIgG negatively regulated PTP-BAS (Figure 4c).

### 3.5 | Knockdown of CIgG upregulated PTP-BAS mRNA expression and cisplatin only downregulated PTP-BAS protein, but not mRNA, expression

As shown in Figure 5a,b, knockdown of CIgG upregulated PTP-BAS mRNA by about 2.25-fold, whereas cisplatin did not affect PTP-BAS mRNA expression. Moreover, cisplatin could downregulate PTP-BAS protein expression in a dose- and time-dependent manner (Figure 5c and Figure S7).

## 4 | DISCUSSION

In the present study, we showed that knockdown of CIgG enhanced anti-cancer effects of cisplatin through PTP-BAS/Src/PDK1/AKT signaling pathway in two oral squamous cell carcinoma cell lines WSU-HN6 and CAL27. Our results imply that CIgG might be involved in cisplatin resistance in oral squamous cell carcinoma.

Although CIgG was identified in cancer cells several decades ago, its role in tumorigenesis remains obscure. In this study, we showed that knockdown of CIgG synergistically enhanced cisplatin-induced apoptosis and inhibition of cell proliferation, migration, and invasion in

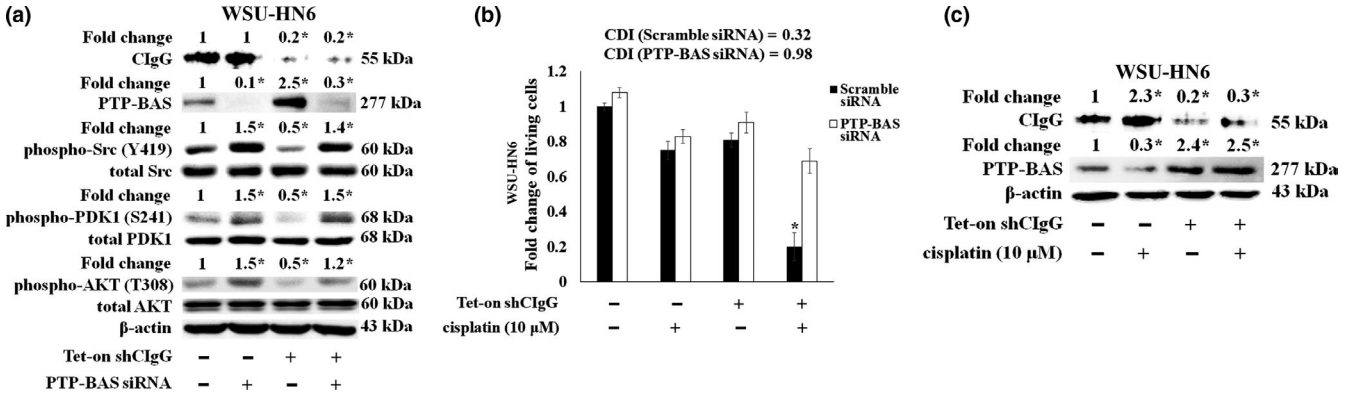


**FIGURE 4** Effects of cancer-derived IgG (CIgG) knockdown on cisplatin treatment via blocking cisplatin-induced upregulation of PTP-BAS/Src/PDK1/AKT signaling. (a) Knockdown of PTP-BAS upregulated phosphorylation of Src, PDK1, and AKT, and reversed CIgG knockdown-induced downregulation of phosphorylation of Src, PDK1, and AKT. WSU-HN6 cells with stable transfection of Tet-on shCIgG or scrambled Tet-on shRNA were transiently transfected with PTP-BAS siRNA or scrambled siRNA in the presence or absence of doxycycline (100 nM) for 48 hr. Total protein was extracted and subjected to Western blot for analysis. $^*p < .05$ versus the control group ($n = 3$). (b) Knockdown of PTP-BAS abolished promoting effect of CIgG knockdown on cisplatin-induced inhibition of cell proliferation. WSU-HN6 cells with stable transfection of Tet-on shCIgG or scrambled Tet-on shRNA were transiently transfected with PTP-BAS siRNA or scrambled siRNA in the presence or absence of doxycycline (100 nM) and treated with or without cisplatin (10 μM) for 48 hr and subjected to CCK-8 assay. $^*p < .05$ versus the control group ($n = 3$). (c) Cisplatin downregulated PTP-BAS protein expression and knockdown of CIgG still upregulated PTP-BAS protein expression in the presence of cisplatin. WSU-HN6 cells with stable transfection of Tet-on shCIgG or scrambled Tet-on shRNA were treated with or without cisplatin (10 μM) in the presence or absence of doxycycline (100 nM) for 36 hr. Total protein was extracted and subjected to Western blot for analysis. $^*p < .05$ versus the control group ($n = 3$)

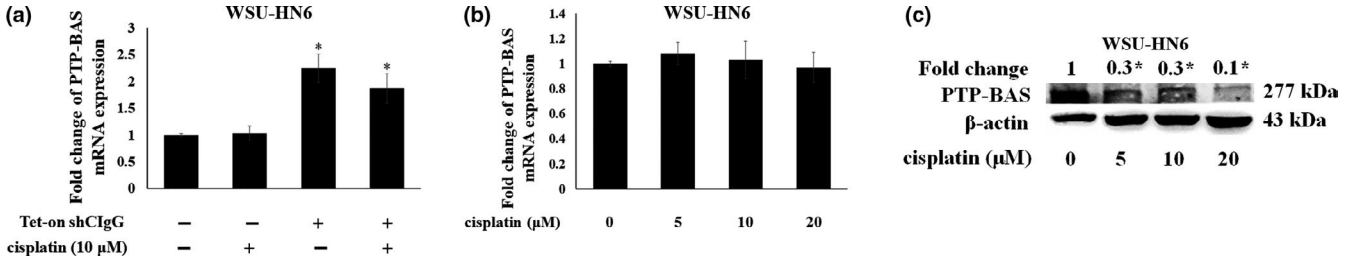


**FIGURE 5** Knockdown of CIgG upregulated PTP-BAS mRNA expression and cisplatin only downregulated PTP-BAS protein, but not mRNA, expression. (a) Knockdown of CIgG upregulated PTP-BAS mRNA expression and cisplatin showed little effects on PTP-BAS mRNA expression. WSU-HN6 cells with stable transfection Tet-on shCIgG or scrambled Tet-on shRNA were treated with or without cisplatin (10 μM) in the presence or absence doxycycline (100 nM) for 36 hr. mRNA were quantitated by real-time PCR. $^*p < .05$ versus the control group or cisplatin group ($n = 3$). (b) Cisplatin did not affect PTP-BAS mRNA expression. WSU-HN6 cells were exposed to different dosages of cisplatin for 48 hr. mRNA were quantitated by real-time PCR. (c) Cisplatin downregulated PTP-BAS protein expression in a dose-dependent manner. WSU-HN6 cells were exposed to different dosages of cisplatin for 48 hr. Total protein was extracted and subjected to Western blot for analysis. $^*p < .05$ versus the control group ($n = 3$)

WSU-HN6 cells and CAL27 cells. These results arouse our interest to ask whether clinical application of CIgG monoclonal antibody (RP215) could enhance the effects of cisplatin on reducing cancer volume or metastasis before operation of some oral cancer cases. Studies in oral cancer cell-line-derived xenograft or patient-derived xenograft models are first needed in future. We also observed that CIgG expression in cancer tissues was more than twofold higher than that in adjacent tissues in 65% (13/20) of tongue cancer samples. These results, to some extent, help explain why tongue cancer is generally not sensitive to cisplatin chemotherapy (Szturz et al., 2019). We also noticed that CIgG expression was detectable in the adjacent tissues in 11 samples and even higher in the adjacent tissues in 3 cases among the total 20 samples. This result also implies that some genes have already changed in the adjacent tissues, although microscopically normal.

Involvement of CIgG in cisplatin resistance was dependent on PDK1/AKT signaling pathway. We showed that cisplatin upregulated both CIgG expression and phosphorylation of PDK1 and AKT (both T308 and S473). Knockdown of CIgG downregulated phosphorylation of PDK1 and AKT T308, and blocked cisplatin-induced upregulation of PDK1 and AKT T308 phosphorylation. Moreover, PDK1 as the regulator of AKT T308 phosphorylation, its inhibitor BX795 also significantly promoted cisplatin-induced inhibition of cell proliferation, and blocked cisplatin-induced upregulation of AKT T308 phosphorylation. Furthermore, in the constitutively activated AKT T308 cells, knockdown of CIgG failed to enhance cisplatin-induced inhibition of cell proliferation. These results suggested that CIgG involved in cisplatin resistance via PDK1/AKT signaling pathway. However, the mechanism underlying cisplatin-induced CIgG expression remains to be elucidated in the future.

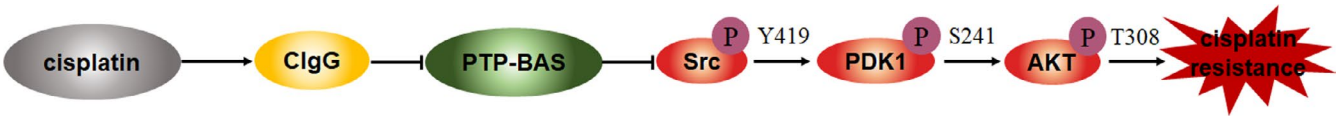


**FIGURE 6** A schematic model of cisplatin, CIgG and PTP-BAS/Src/PDK1/AKT signaling pathway in cisplatin resistance in oral squamous cell carcinoma [Colour figure can be viewed at wileyonlinelibrary.com]

Involvement of CIgG in cisplatin resistance was dependent on Src/PDK1/AKT signaling pathway. Src is also an upstream regulator of AKT and can be auto phosphorylated at Y419. We observed that cisplatin could upregulate Y419 phosphorylation of Src and that knockdown of CIgG simultaneously downregulated phosphorylation of Src, PDK1, and AKT in both WSU-HN6 cells and CAL27 cells. Moreover, we detected in WSU-HN6 cells that knockdown of Src downregulated phosphorylation of PDK1 and AKT T308 and enhanced cisplatin-induced inhibition of cell proliferation, and overexpression of wild-type Src, but not the mutant Src Y419F, could induce phosphorylation of PDK1 and AKT. The results of overexpression of wild-type Src and mutant Src Y419F also confirmed that Y419 of Src was important for self-activation and its induction of PDK1 and AKT phosphorylation, and that Src regulation of AKT was dependent on PDK1. All these results indicated that Src was a positive regulator of PDK1/AKT signaling in the cisplatin treatment. Our results of Src overexpression inducing endogenous Src Y419 phosphorylation in WSU-HN6 cells were consistent with a previous study in 293T cells (Wang et al., 2015). Although overexpression of Src could induce phosphorylation of both exogenous and endogenous Src, knockdown of CIgG could still downregulate phosphorylation of both exogenous and endogenous Src, and phosphorylation of PDK1 and AKT even under the condition of Src overexpression. Correspondingly, overexpression of Src failed to reverse CIgG knockdown-induced inhibition of cell proliferation. These results suggested that CIgG might also affect a phosphatase of Src, leading us to proving it later. Nevertheless, Src/PDK1/AKT signaling pathway was involved in CIgG-mediated cisplatin resistance.

Involvement of CIgG in cisplatin resistance was dependent on PTP-BAS/Src/PDK1/AKT signaling pathway. Phosphatase PTP-BAS is an important regulator of Src Y419 phosphorylation. Whether PTP-BAS was involved in cisplatin resistance remains unknown. In this study, we proved for the first time that PTP-BAS was involved in CIgG-mediated cisplatin resistance and that CIgG was a negative regulator of PTP-BAS in WSU-HN6 cells. Considering that knockdown of CIgG upregulated PTP-BAS both mRNA and protein expression, CIgG regulated PTP-BAS protein expression possibly through affecting its mRNA expression. Knockdown of PTP-BAS still upregulated phosphorylation of Src, PDK1, and AKT under the condition of CIgG knockdown, and correspondingly totally blocked the synergistic effect of CIgG knockdown on cisplatin-induced inhibition of cell proliferation. These results also indicated that PTP-BAS was a negative regulator of Src/PDK1/AKT signaling in the cisplatin treatment. Taken together, our results suggest a new mechanism that cisplatin induces CIgG resulting in downregulation of PTP-BAS expression and then sequentially inducing phosphorylation of Src, PDK1, and AKT leading to cisplatin resistance (Figure 6).

In conclusion, knockdown of CIgG enhanced anti-cancer effects of cisplatin through PTP-BAS/Src/PDK1/AKT signaling pathway in oral squamous cell carcinoma.

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## CONFLICT OF INTEREST

None to declare.

## AUTHOR CONTRIBUTIONS

**Luming Wang:** Conceptualization; Data curation; Formal analysis; Methodology; Software; Validation; Visualization; Writing–original draft; Writing–review & editing. **Ye-Hua Gan:** Conceptualization; Formal analysis; Funding acquisition; Methodology; Project administration; Resources; Supervision; Validation; Writing–original draft; Writing–review & editing.

## PEER REVIEW

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## SUPPORTING INFORMATION

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

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