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ORIGINAL ARTICLE

Mechanism of sensitivity to cisplatin, docetaxel, and 5-fluorouracil chemoagents and potential erbB2 alternatives in oral cancer with growth differentiation factor 15 overexpression

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Abstract

The aim of this study was to: (a) explore the potential mechanism of cancer cell sensitivity to cisplatin, docetaxel, and 5-fluorouracil (TPF) in oral squamous cell carcinoma (OSCC) patients overexpressing growth differentiation factor 15 (GDF15); and (b) identify potential alternative agents for patients who might not benefit from inductive TPF chemotherapy. The results indicated that OSCC cells overexpressing GDF15 were sensitive to TPF through a caspase-9-dependent pathway both in vitro and in vivo. Immunoprecipitation combined with mass spectrometry revealed that the erbB2 protein was a potential GDF15-binding protein, which was verified by coimmunoprecipitation. Growth differentiation factor 15 overexpression promoted OSCC cell proliferation through erbB2 phosphorylation, as well as downstream AKT and Erk signaling pathways. When GDF15 expression was blocked, the phosphorylation of both the erbB2 and AKT/Erk pathways was downregulated. When OSCC cells with GDF15 overexpression were treated with the erbB2 phosphorylation inhibitor, CI-1033, cell proliferation and xenograft growth colony formation were significantly blocked ($P < .05$). Thus, GDF15-overexpressing OSCC tumors are sensitive to TPF chemoagents through caspase-9-dependent pathways. Growth differentiation factor 15 overexpression promotes OSCC proliferation through erbB2 phosphorylation. Thus, ErbB2 inhibitors could represent potential targeted drugs or an alternative therapy for OSCC patients with GDF15 overexpression.

KEYWORDS
GDF15, oral squamous cell carcinoma, ErbB2, TPF induction chemotherapy

Abbreviations: ErbB2, receptor tyrosine-protein kinase erbB-2; GDF15, growth differentiation factor 15; GFRAL, glial-derived neurotrophic factor receptor alpha-like; HIOEC, human immortalized oral epithelial cell; IP, immunoprecipitation; LC-MS/MS, liquid chromatography-tandem mass spectrometry; OSCC, oral squamous cell carcinoma; PARP, poly(ADP-ribose) polymerase; TPF, docetaxel, cisplatin, and 5-fluorouracil.

TongChao Zhao, Zhihang Zhou, and Wutong Ju contributed equally to this study.

1 | INTRODUCTION

Of the tumors that affect the oral and maxillofacial region, OSCC represents one of the most commonly occurring malignancies. Although advances in comprehensive sequence therapy and targeted therapy have recently been made regarding cancers of the head and neck, the overall survival rate of OSCC patients remains unsatisfactory. The standard treatment for OSCC continues to be characterized by radical surgery and subsequent adjuvant radiotherapy and/or chemoradiotherapy.[1,2] Moreover, induction chemotherapy is helpful for reducing or downstaging locally advanced cancers, improving the opportunity of lesion eradication and organ conservation.[3] In head and neck cancer, the TPF protocol has shown greater efficacy compared to the 5-fluorouracil and cisplatin protocol.[4] Unfortunately, our previous trial of adding inductive TPF chemotherapy failed to provide a significant survival benefit, compared to standard treatment in patients with clinical stage III or IVA OSCC based on UICC (2002); however, patients who showed a promising pathological reaction to inductive TPF chemotherapy have experienced a significant long-term survival benefit.[3,5] Therefore, the selection of biomarkers that can be used to accurately predict the efficacy of induction chemotherapy that will aid in the administration of personalized treatment for OSCC patients.

Growth differentiation factor 15 has been shown to play a role in cancer, anorexia/cachexia, and cardiovascular diseases.[6] Our previous study has confirmed that the expression of GDF15 is higher within OSCC tumors compared to that in the surrounding tissues.[7] The upregulation of GDF15 expression can significantly promote cellular proliferation and tumorigenesis in nude mice while reducing apoptosis in OSCC.[8] Based on our previous TPF trial in OSCC, patients who show low levels of GDF15 expression in biopsies are associated with better prognoses, compared to those with high levels of GDF15 expression.[8] However, they have similar clinical outcomes regardless of whether the patients received TPF induction chemotherapy. These results indicate that low GDF15 expression in OSCC patients could represent a favorable prognostic biomarker, and standard therapy is recommended for these patients. In contrast, as the clinical outcome is poor for patients who show high levels of GDF15 expression, the improvement of their clinical outcomes is extremely urgent. Fortunately, based on the findings of the TPF trial, among the patients with high GDF15 expression, only patients in the *c*T3/4N0M0 clinical stage could benefit from TPF induction chemotherapy before initiating standard treatment, compared with standard treatment alone.[9] Therefore, the method by which we can improve the prognosis of patients with *c*T3/4N0M0 clinical stage is the focus of our study.

In the present study, we undertook experiments both in vitro and in vivo to confirm the benefits of TPF chemoagents in OSCC cell lines with GDF15 overexpression. We subsequently screened GDF15-binding proteins in OSCC cells and revealed the potential molecular mechanism of the erbB2 inhibitor as a candidate for improving the effect of treatment in OSCC with GDF15 overexpression using both in vivo and in vitro approaches. Thus, we aimed to provide a theoretical basis for individualized treatment of OSCC patients with locally advanced lesions based on the use of GDF15 expression as a prognostic biomarker.

2 | MATERIALS AND METHODS

2.1 | In vitro and in vivo experiments

Oral squamous cell carcinoma cells, chemicals, and Abs, GDF15 siRNA and gene transfection, RT-PCR, western blot analysis, cellular growth, cellular colony formation, tumor models in nude mice, and immunohistochemistry are described in Appendix S1. This study was approved by the ethical committee of Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine (approval no. SH9H-2019-TK230-1 and SH9H-2019-A231-1).

2.2 | Liquid chromatography-MS/MS analysis

We used SDS-PAGE to separate the denatured proteins and the GDF15-overexpressing HB96 cell protein samples were prepared and subjected to in-gel digestion with sequencing-grade trypsin (Promega), as described previously.[10] After resuspending the samples in 5% acetonitrile and 0.1% formic acid, LC-MS/MS was carried out.

2.3 | Statistical analyses

All data were analyzed with SPSS 13.0 for Windows (SPSS Inc.). The data are presented as the mean ± SD. The means of the samples were compared using either an unpaired, two-tailed Student's *t* test or one-way ANOVA. A threshold value $P < .05$ indicated statistical significance.

3 | RESULTS

3.1 | Growth differentiation factor 15 expression and intervention in OSCC cell lines

The western blot and RT-PCR results revealed that four OSCC cell lines (CAL27, HB96, HN6, and HN30) displayed a higher expression of GDF15 compared to the HIOEC immortalized cell line (Figure S1A,B). After GDF15 was overexpressed or silenced in OSCC cells, the level of GDF15 protein and mRNA expression was verified, as shown in Figure S1C-G.

3.2 | Growth differentiation factor 15 overexpression increases the cytotoxicity of chemoagents both in vitro and in vivo through caspase-dependent apoptosis

To investigate the influence of GDF15 expression on the response to TPF, GDF15 expression intervention and treatment with a chemoagent were carried out in OSCC cells in vitro. Using a CCK-8 assay, GDF15

overexpression in both HB96 and HN6 cells was associated with increased sensitivity to TPF compared with the control (Figure 1A-F). Growth differentiation factor 15-silenced CAL27 and HB96 cells were less sensitive to TPF compared with the control (Figure 1G-L). The apoptotic effect of GDF15 expression and chemoagents on the cleavage of PARP, caspase-9, and caspase-3 was evaluated. Compared to the control, when GDF15-overexpressing HB96 and HN6 cells were treated with 12.5 nmol/L docetaxel for 72 hours, 10 μmol/L cisplatin for 72 hours, or 50 μmol/L 5-fluorouracil for 48 hours, a greater amount of PARP, caspase-9, and caspase-3 cleavage was observed. An accumulation of GDF15 protein induced by the chemoagents in the HB96 and HN6 cells was also found (Figures 2A and S2A,B). In contrast, the GDF15-silenced CAL27 and HB96 cells were less responsive to the chemoagents than the control, with decreased activation of caspase-9, caspase-3, and PARP (Figures 2B and S2C,D).

Compared with the PGMLV-GDF15 control group, following treatment with TPF chemoagents, the growth rate of xenografts in nude mice was significantly reduced in the GDF15-overexpressing PGMLV-GDF15 + TPF group (Figure 3A,B), which inversely increased in the PGMLV groups (PGMLV-NC and PGMLV + TPF) (Figure 3A,C). At the end of the treatment cycles, three xenografts in each group were randomly selected for H&E, TUNEL assay, and immunohistochemical staining. Compared with the control group, the GDF15-overexpressing group displayed an inhibition of the Ki-67 proliferation index and higher level of TUNEL-positive cells (Figure 3D,E), whereas the PGMLV group showed the opposite result. A western blot analysis of the other xenograft samples showed increased GDF15 expression when the cells were treated with TPF chemoagents, compared to those that were not treated with TPF chemoagents. Activation of the caspase activation-dependent apoptosis pathway was also found to clearly increase cleaved caspase-9, as well as the downstream effectors cleaved caspase-3 and cleaved PARP (Figure 3F).

3.3 | Growth differentiation factor 15 activates phosphorylation of erbB2 and then PI3K/AKT and MAPK signaling pathways

To screen for potential GDF15-binding proteins in OSCC, an anti-GDF15 Ab was used for an IP assay in the GDF15-overexpressing HB96 cells. After SDS-PAGE and staining with Coomassie blue, we found significant differences in the bands (Figure 4A). The

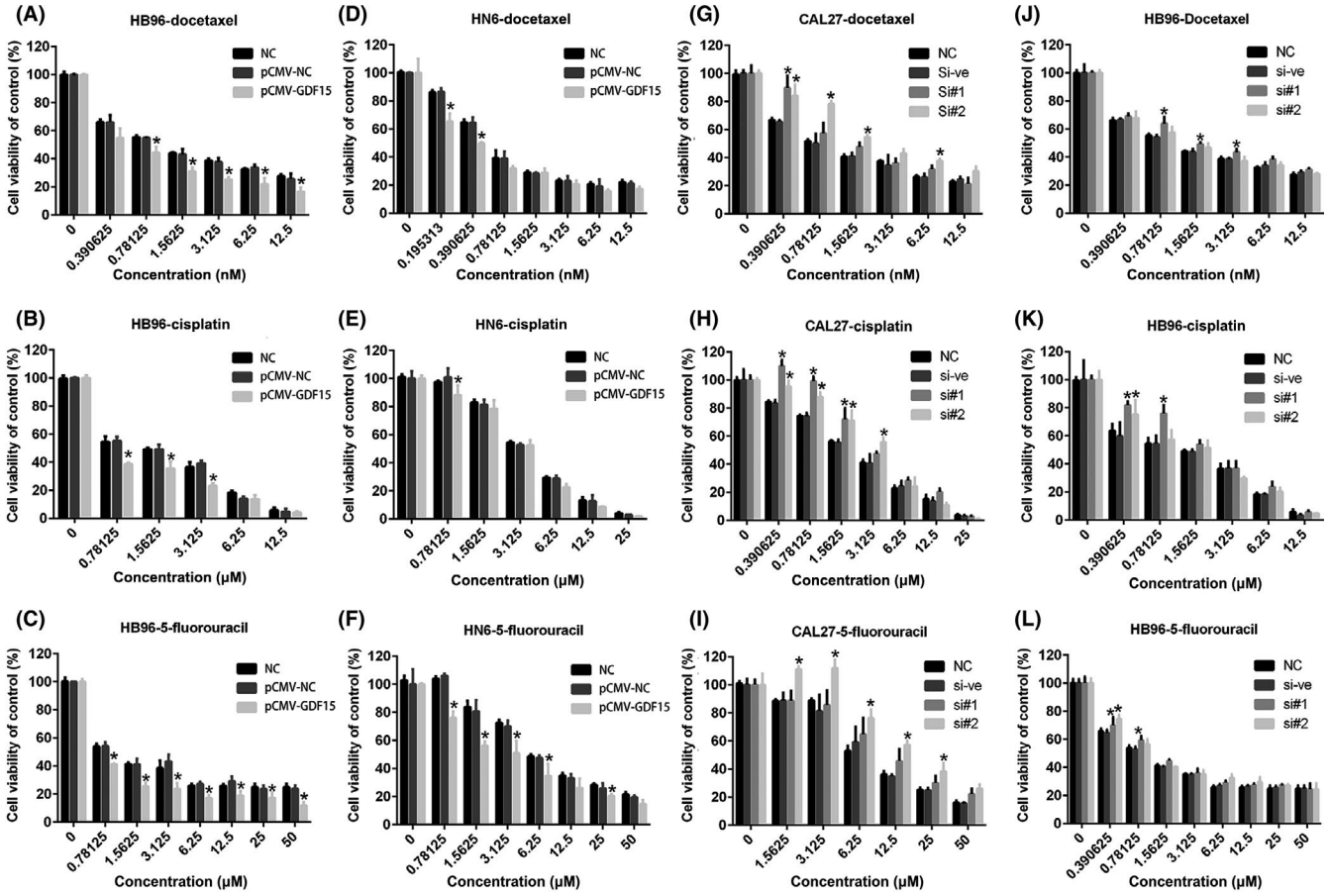


FIGURE 1 Influence of growth differentiation factor 15 (GDF15) expression on oral squamous cell carcinoma cell sensitivity to docetaxel, cisplatin, and 5-fluorouracil treatment in vitro. Increased sensitivity (decreased cell viability) was observed in GDF15-overexpressing HB96 and HN6 cells when treated with a series of concentrations of docetaxel (A, D), cisplatin (B, E) or 5-fluorouracil (C, F). Decreased sensitivity (increased cell viability) was observed in the GDF15-silenced CAL27 and HB96 cells when treated with a series of concentrations of docetaxel (G, J), cisplatin (H, K), or 5-fluorouracil (I, L). *$P < .05$

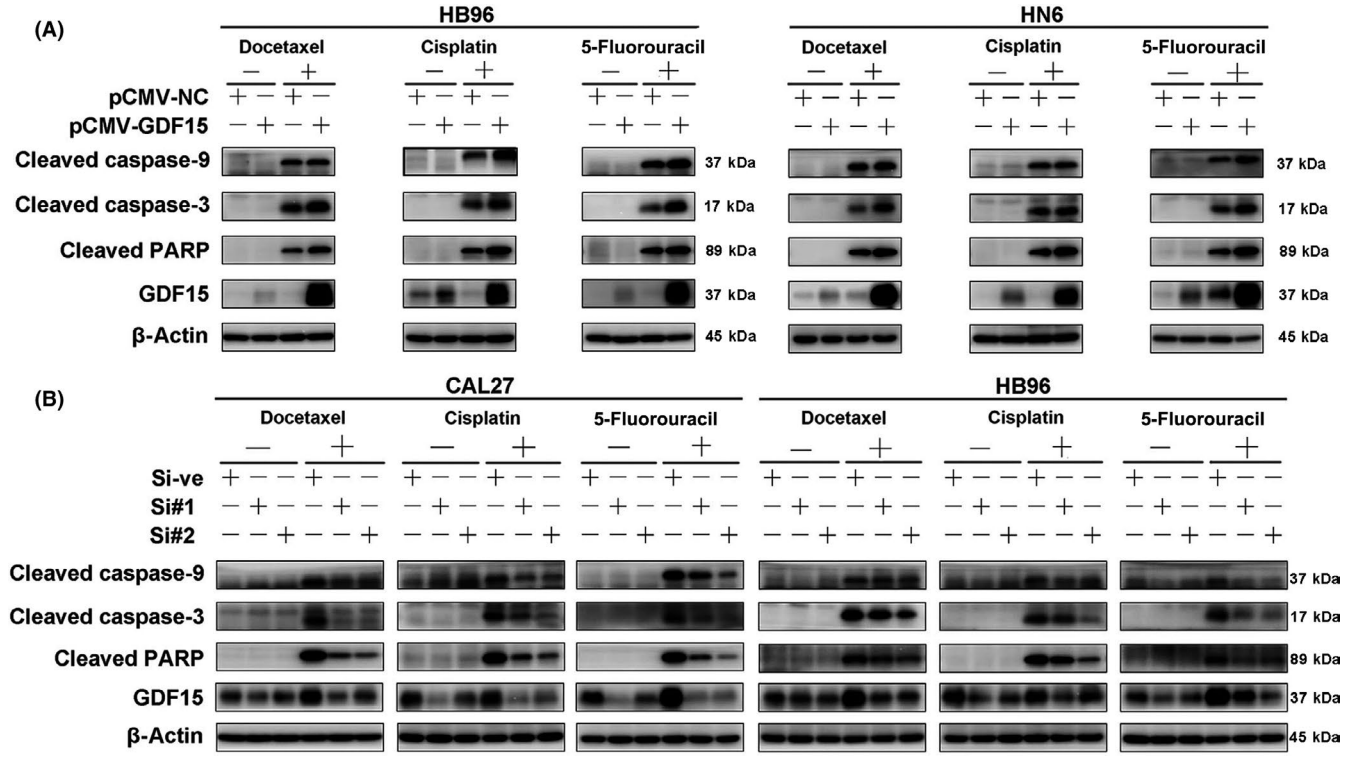


FIGURE 2 Growth differentiation factor 15 (GDF15) intervention on the cytotoxicity of chemoagents through caspase-9-dependent apoptosis in vitro. A, Increased expression of cleaved poly(ADP-ribose) polymerase (PARP), caspase-3, and caspase-9 in GDF15-overexpressing HB96 and HN6 cells when treated with 12.5 nmol/L docetaxel for 72 h, 10 μmol/L cisplatin for 72 h, or 50 μmol/L 5-fluorouracil for 48 h. B, Decreased expression of cleaved PARP, caspase-3, and caspase-9 in GDF15-silenced CAL27 and HB96 cells when treated with 12.5 nmol/L docetaxel for 72 h, 10 μmol/L cisplatin for 72 h, or 50 μmol/L 5-fluorouracil for 48 h

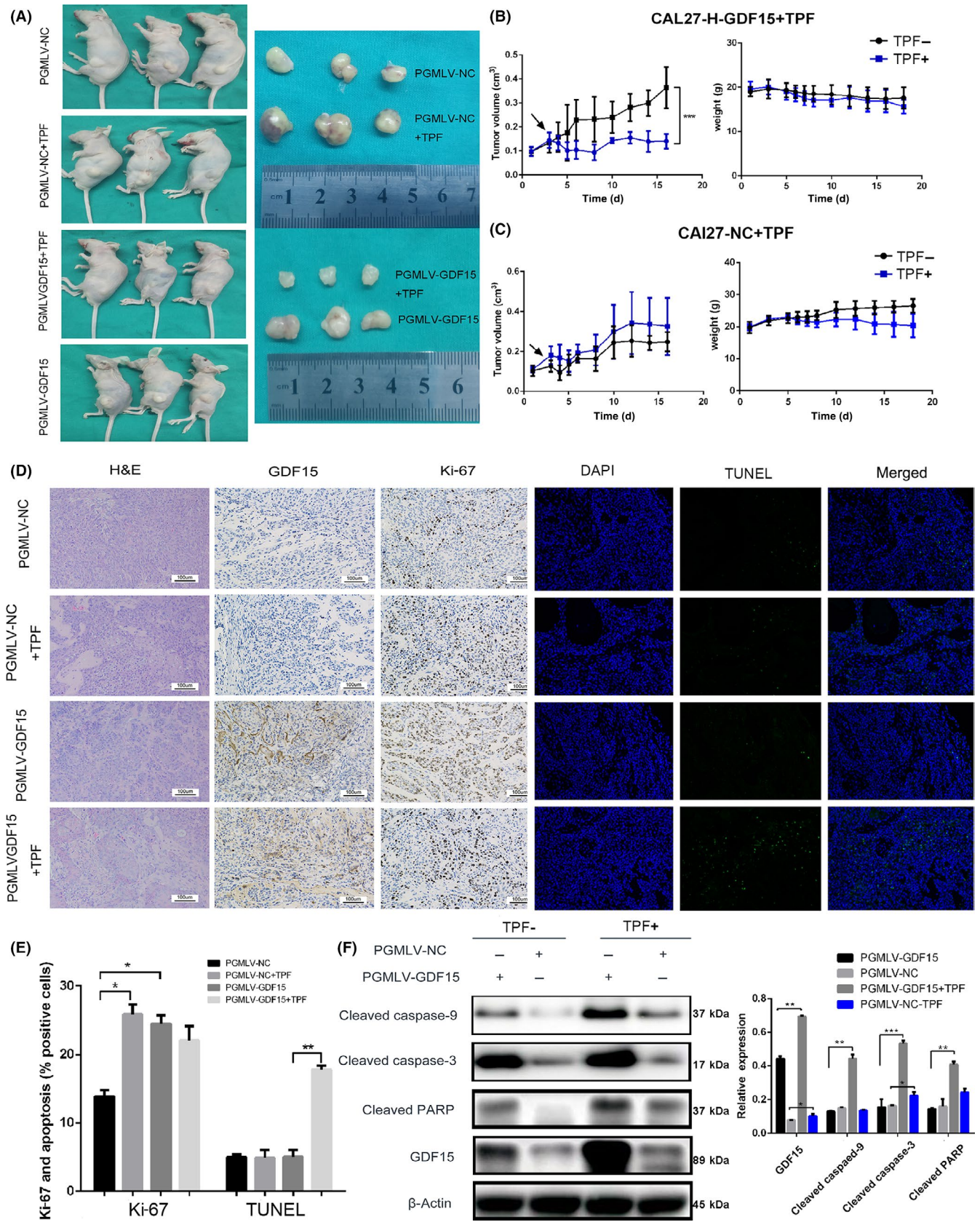
differential bands were then cut and digested for an LC-MS/MS analysis. A total of 669 potentially related proteins were identified, and 28 proteins larger than 100 kDa were screened by molecular weight for the Score Sequest HT sequence (Table S1, Figure S3). In the functional screen, only ErbB2 was identified to be a cell surface receptor protein in the LC-MS/MS data (Table S1). The binding relationship between erbB2 and GDF15 was further confirmed by two co-IP assays (Figure 4B).

As shown in the western blot analysis, after GDF15 was overexpressed or silenced, the expression of erbB2 did not change substantially, whereas the level of phosphorylated erbB2 was significantly altered. Phosphorylation of erbB2 was significantly upregulated in the GDF15-overexpressing HB96 and HN30 cells (Figure 4C) and downregulated in the GDF15-silenced CAL27 and HN6 cells (Figure 4D). Moreover, the level of p-AKT and p-Erk1/2 was much higher in the GDF15-overexpressing HB96 and HN30 cells compared to the control. The opposite results were observed in the GDF15-silenced CAL27 and HN6 cells (Figure 4C,D). To confirm the correlation between GDF15 overexpression and an increased level of phosphorylated erbB2, AKT, and Erk expression in vivo, xenografts in the CAL27-PGMLV-GDF15 and CAL27-PGMLV-NC groups were used for detection. Both immunohistochemical and western blot results show obvious increased phosphorylation of erbB2, AKT, and Erk in GDF15-overexpressing tumors compared with the control group (Figure 4E-G).

ErbB2 signaling pathways play a significant role in human cancer cell proliferation by regulating the PI3K/AKT and MAPK signaling pathways. Thus, we detected p-PDK1, p-AKT, and p-GSK3 of the PI3K signaling pathway and p-c-Raf, p-MEK1, p-Erk, p-P90RSK, and p-MSK1 of the MAPK signaling pathway in GDF15-overexpressing HB96 and HN30 cells. Both the PI3K/AKT and MAPK signaling pathways were activated (Figure S4).

3.4 | Treatment with CI-1033 blocks GDF15-overexpressing OSCC cell proliferation both in vivo and in vitro

We aimed to examine the in vitro inhibitory effect of the pan-erbB tyrosine kinase inhibitor CI-1033 on GDF15-overexpressing OSCC cells. A CCK-8 assay was used to evaluate the viability of non-GDF15-overexpressing and GDF15-overexpressing cells. The effect of CI-1033(1, 10, 100, 1000, 10 000, and 100 000 nmol/L) on proliferation was detected at 24, 48, and 72 hours. The IC_{50} value was calculated as 10.84 μmol/L in the GDF15-overexpressing HB96 cells and 19.37 μmol/L in the GDF15-overexpressing HN30 cells (Figure 5A). Compared with normal tumor cells, GDF15-overexpressing cells showed higher sensitivity to CI-1033. A concentration gradient colony formation experiment showed that


(A)
PGMLV-NC
PGMLV-NC+TPF
PGMLVGDF15+TPF
PGMLV-GDF15
PGMLV-NC
PGMLV-NC +TPF
PGMLV-GDF15 +TPF
PGMLV-GDF15
(B)
CAL27-H-GDF15+TPF
Tumor volume (cm3)
Time (d)
weight (g)
TPF–
TPF+
***
(C)
CAI27-NC+TPF
(D)
H&E
GDF15
Ki-67
DAPI
TUNEL
Merged
PGMLV-NC
PGMLV-NC +TPF
PGMLV-GDF15
PGMLVGDF15 +TPF
100um
(E)
Ki-67 and apoptosis (% positive cells)
PGMLV-NC
PGMLV-NC+TPF
PGMLV-GDF15
PGMLV-GDF15+TPF
Ki-67
TUNEL
(F)
TPF-
TPF+
PGMLV-NC
PGMLV-GDF15
Cleaved caspase-9
Cleaved caspase-3
Cleaved PARP
GDF15
β-Actin
37 kDa
17 kDa
37 kDa
89 kDa
45 kDa
PGMLV-GDF15
PGMLV-NC
PGMLV-GDF15+TPF
PGMLV-NC-TPF
Relative expression
GDF15
Cleaved caspaed-9
Cleaved caspase-3
Cleaved PARP

when the concentration of CI-1033 was 1 μmol/L or 10 μmol/L, the colony number was significantly reduced in the GDF15-overexpressing HB96 and HN30 cells compared with the control group (Figure 5B,C). These results also confirmed the CCK-8 results.

To verify the inhibitory effect of CI-1033 on erbB2-related pathways, HB96 and HN30 cells overexpressing GDF15 were treated with a CI-1033 concentration gradient for 1 hours. Phosphorylation of erbB2 was significantly inhibited by CI-1033 in a concentration-dependent manner (Figure 5D). Beginning with a concentration of CI-1033 greater than 10 μM, the level of erbB2 phosphorylation and downstream AKT and Erk activation was significantly inhibited (Figure S5). Furthermore, p-erbB2 and downstream AKT and Erk were inhibited by CI-1033 at different time points (0.5–12 hours) (Fig. S6).

The in vivo growth of xenografts was significantly inhibited after the use of CI-1033 in mice. Although the PGMLV-GDF15-CI-1033 group was associated with significantly smaller tumor volumes compared to the control group, the weights of the mice did not change (Figure 6A,B). An immunohistochemical analysis of the xenografts revealed a significant decrease in the level of p-erbB2 and Ki-67 index expression (Figure 6C,D). Western blot results indicated that the level of erbB2, AKT, and Erk phosphorylation was significantly inhibited by CI-1033 in the GDF15-overexpressing xenografts (Figure 6E,F).

Figure 6G shows a schematic diagram of the mechanism by which overexpression of GDF15 affects OSCC. The overexpression of GDF15 promotes OSCC proliferation through the phosphorylation of erbB2 and, subsequently, the PI3K/AKT and MAPK signaling pathways. CI-1033 was observed to block the growth of GDF15-overexpressing OSCC cells both in vitro and in vivo through inhibiting erbB2 phosphorylation, as well as downstream AKT and Erk activation.

3.5 | Treatment with TPF inhibited phosphorylation of the erbB2/AKT/Erk signaling pathways in GDF15-overexpressing OSCC

We evaluated the effect of TPF chemotherapy drugs on the erbB2 signaling pathway of GDF15-overexpressing OSCC. Compared to the control, when GDF15-overexpressing HB96 cells were treated with 12.5 nmol/L docetaxel for 72 hours, 10 μmol/L cisplatin for 72 hours, or 50 μmol/L 5-fluorouracil for 48 hours, the expression of erbB2, AKT, and Erk did not change substantially, whereas the level of phosphorylated erbB2, AKT, and Erk was significantly downregulated (Figure S7A). In addition, the level of erbB2, AKT, and Erk phosphorylation was significantly inhibited by TPF chemoagents in the GDF15-overexpressing xenografts (Figure S7B).

4 | DISCUSSION

In the present study, we confirmed that OSCC cells overexpressing GDF15 are sensitive to TPF chemoagents through a caspase-9 dependent pathway both in vivo and in vitro. The overexpression of GDF15 promotes OSCC proliferation through the phosphorylation of erbB2, and subsequently, the PI3K/AKT and MAPK signaling pathways. Both the in vitro and in vivo experimental results indicate that the erbB2 phosphorylation inhibitor CI-1033 can significantly inhibit cellular proliferation, colony formation, and xenograft formation of OSCC cells overexpressing GDF15. The results indicate that CI-1033 could be used as a potential targeted drug or alternative for OSCC patients with GDF15 overexpression who are resistant to TPF chemoagents.

The function of GDF15 in cancer development and progression is highly complex and remains poorly understood. The antitumor or protumor effects of GDF15 appear to be related to the tumor type and stage. In studies investigating *GDF15* as a tumor suppressor gene, many chemotherapy drugs have been found to induce the expression of GDF15 in some differentiated cells.[11] Moreover, studies on the associated mechanisms revealed that some known tumor suppressor genes (eg, *p53*, *GSK-3β*, and *C/EBPβ*) could regulate the expression of GDF15.[12,13] In studies regarding the mechanism of *GDF15* as an oncogene, GDF15 has been reported to stimulate cancer cell growth through AKT and Erk signaling in cervical and esophageal cancers.[14,15] Our previous study found the same result in oral cancer.[8] Furthermore, the activation of AKT and Erk by treatment with recombinant GDF15 has been found in gastric and breast cancer cells overexpressing erbB2.[16] Our present study provides direct evidence that GDF15 overexpression in OSCC cells activates erbB2 phosphorylation, further activating the AKT and Erk pathways. ErbB2 could represent one of the key binding proteins that regulates the growth and progression of OSCC with GDF15 overexpression. However, the identification of the detailed binding sites between GDF15 and erbB2 requires further study. Recent research on the GDF15 receptor has found that GDF15 mediates its function through the recently identified GFRAL, which signals through the

FIGURE 3 Growth differentiation factor 15 (GDF15) overexpression promotes sensitivity to docetaxel, cisplatin, and 5-fluorouracil (TPF) chemotherapy drugs in oral squamous cell carcinoma. A, Images of two groups of nude mice with tumors derived from CAL-27-PGMLV-GDF15 and their control cells (NC) treated with TPF chemotherapy drugs or not. B, Tumor growth curves and weight of the mice with CAL-27-PGMLV-GDF15 tumors treated with or without TPF drugs. Tumor volumes and weights were measured every 2 d after the onset of treatment. C, Tumor growth curves and weights of mice with CAL-27-PGMLV-NC tumors treated with or without TPF drugs. D, H&E, immunohistochemical staining against Ki-67, GDF15, and TUNEL assay in xenografts (original magnification, ×200). E, Comparison of the Ki-67 index and TUNEL-positive cells. F, Analysis of caspase activation-dependent proteins in the four xenograft groups, Quantification of the proteins relative to β-actin OD values is presented. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$. PARP, poly(ADP-ribose) polymerase

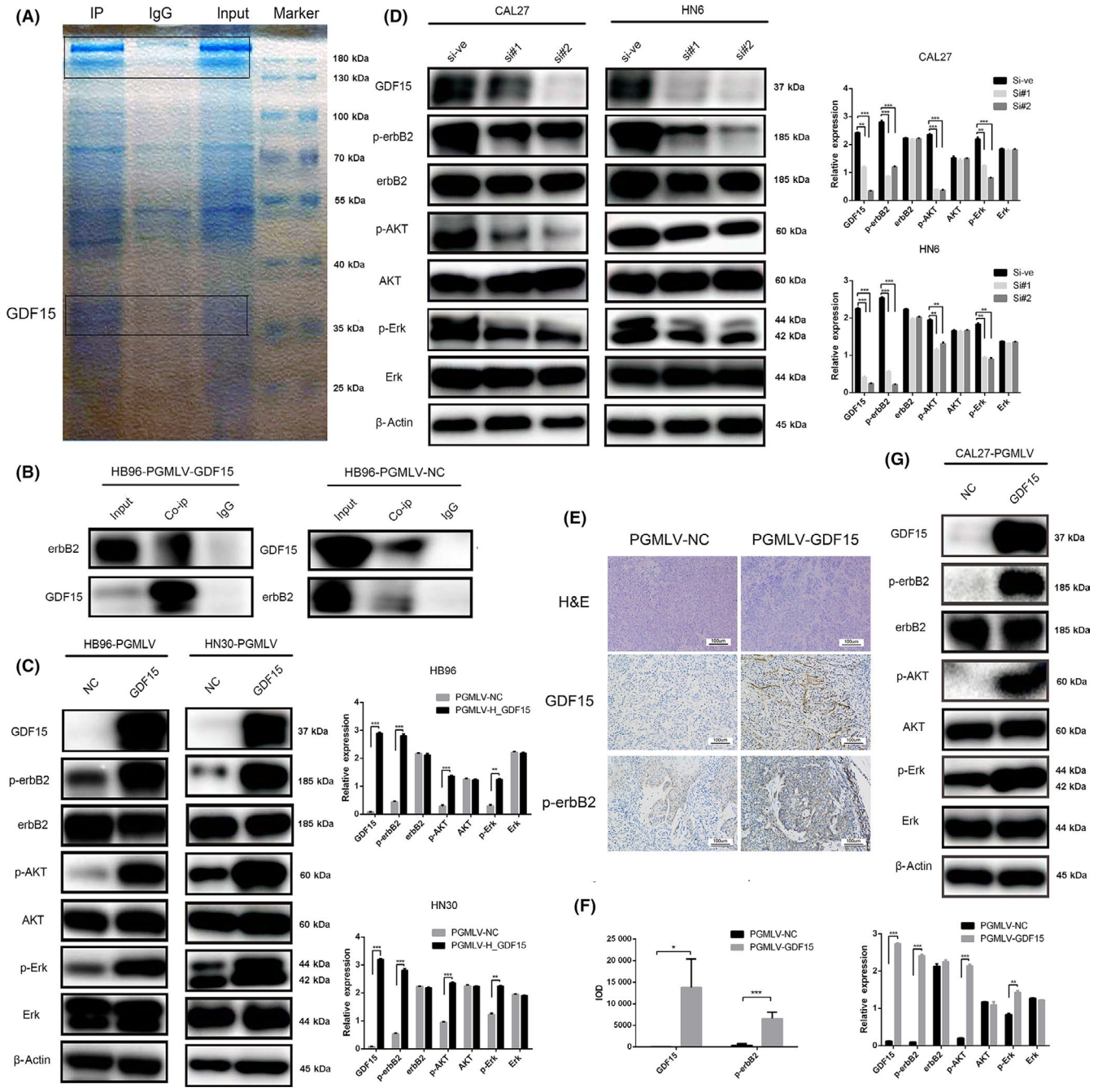


FIGURE 4 Growth differentiation factor 15 (GDF15) binding to erbB2 activates the AKT and Erk pathways. A, SDS-PAGE with 10% (w/v) polyacrylamide gels. Lanes: 1, immunoprecipitation (IP) protein; 2, IgG protein; and 3, input protein. Coomassie blue R-250 was used to stain the gel. B, GDF15 was immunoprecipitated from GDF15-overexpressing cell lysates (HB96-PGMLV-GDF15); erbB2 was immunoprecipitated from non-GDF15-overexpressing cell lysates (HB96-PGMLV-NC). Immunoblot of anti-ErbB2 Ab and anti-GDF15 Ab and the IP product. C, Western blot of the GDF15-overexpressing HB96 and HN30 cell lysates. D, Western blot analysis of CAL27 and HN6 cell lysates following treatment with siGDF15. E, H&E and immunohistochemical staining against GDF15 and p-erbB2 in xenografts (original magnification, ×200). F, Comparison of GDF15 and p-erbB2 integrated optical density (IOD) values. G, Harvested tumors were subsequently collected and a western blot detected the level of erbB2, AKT, and Erk expression and phosphorylation. Quantification of the proteins relative to β-actin optical density values is presented. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$

tyrosine kinase receptor, Ret, to regulate energy homeostasis.[17-19] Unfortunately, in this study, GFRAL/Ret did not appear in the IP combined mass spectrometry results. One possible reason for this discrepancy might be the specific location of GFRAL expression in the nervous system in both humans and mice.[20]

In patients with locally advanced OSCC, low levels of GDF15 expression in biopsy samples have been shown to be a prognostic biomarker that can predict better clinical outcomes; therefore, the standard treatment of radical surgical resection followed by postoperative radiation or chemoradiation for these patients could achieve

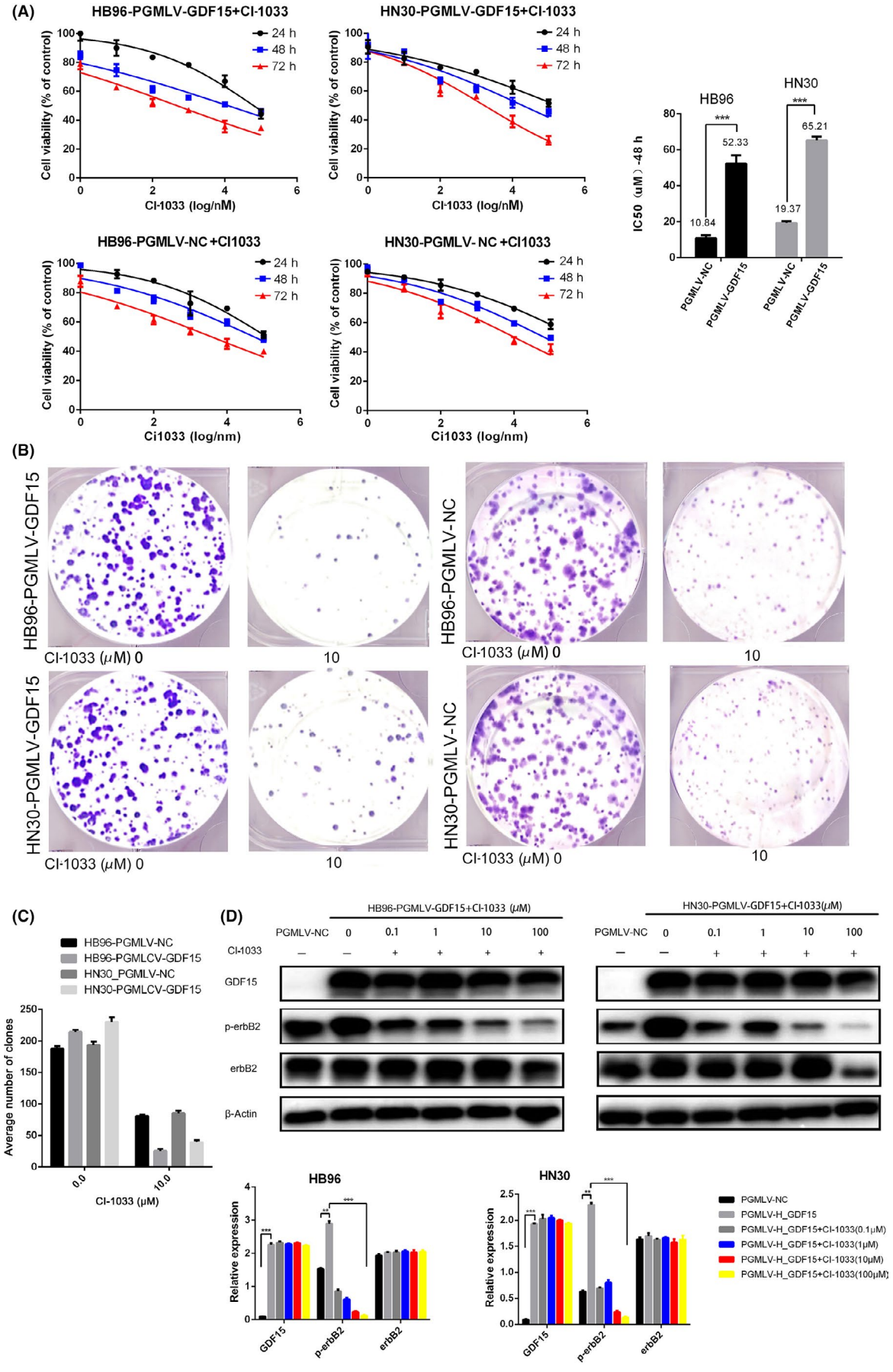


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FIGURE 5 Compared to normal tumor cells, growth differentiation factor 15 (GDF15)-overexpressing cells (HB96 and HN30) showed higher sensitivity to CI-1033. A, Oral squamous cell carcinoma cells were incubated with CI-1033 at various concentrations (1, 10, 100, 1000, 10 000, and 100 000 nmol/L) for 24, 48, and 72 h. A CCK-8 assay was used to detect cell viability. The IC_{50} values in HB96 and HN30 cells incubated with CI-1033 for 48 h were compared with vehicle-treated controls (PGMLV-NC). Calculations were carried out using Prism 7.0 (GraphPad). B, C, Colony formation experiment treated with CI-1033 (10 μmol/L) in non-GDF15-overexpressing cells and GDF15-overexpressing cells (HB96 and HN30). D, Expression of GDF15, erbB2, and p-erbB2 was detected. Quantification of the proteins relative to β-actin optical density values is presented. *$P < .05$, **$P < .01$, ***$P < .001$

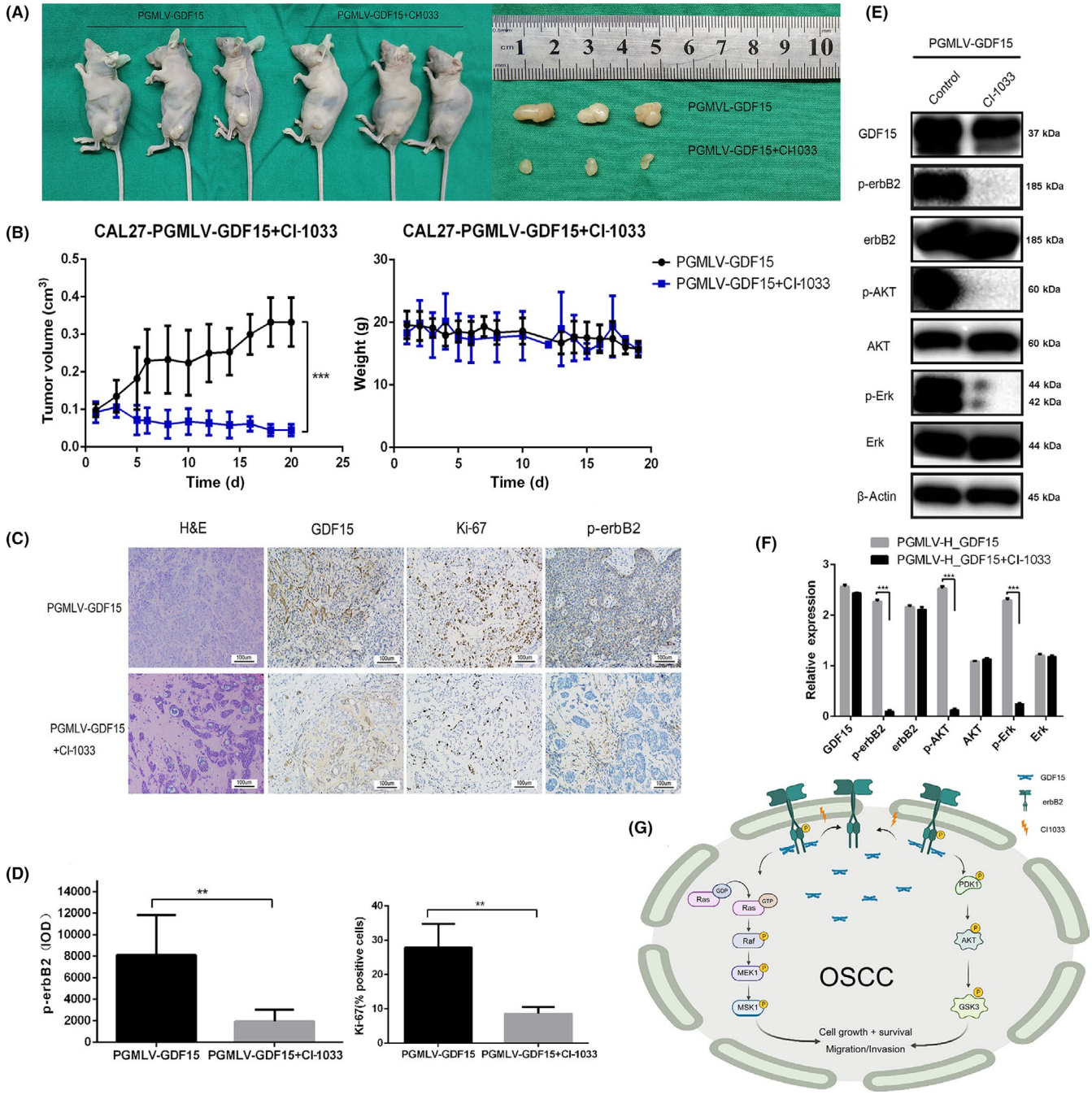

FIGURE 6 CI-1033 inhibited the growth of xenografts derived from growth differentiation factor 15 (GDF15)-overexpressing CAL27 cells. A, Comparison between nude mice with tumors derived from CAL-27-PGMLV-GDF15 cells treated with CI-1033 (5 mg/kg for 2 wk) or not. B, Comparison of tumor growth curves and mouse weights between the groups treated with or without CI-1033. Tumor volumes and weights were determined every 2 d after treatment initiation. C, H&E and immunohistochemical staining against GDF15, p-erbB2, and Ki-67 in xenografts (original magnification, ×200). D, Comparison of integrated optical density (IOD) values of p-erbB2 and the Ki-67 index. E, F, Harvested tumors were subsequently collected. Western blot analysis was used to detect the level of phosphorylated erbB2, AKT, and Erk expression. Quantification of the proteins relative to β-actin OD values is presented. *$P < .05$, **$P < .01$, ***$P < .001$. G, Schematic diagram of the mechanism by which GDF15 overexpression affects oral squamous cell carcinoma (OSCC)

good clinical outcomes. In contrast, as the clinical outcomes are relatively poor in patients with high GDF15 expression, an optimized treatment protocol should be considered. Our previous study reported that OSCC patients with cT3/4N0M0 and with high levels of GDF15 expression could benefit from an initial inductive TPF chemotherapy and subsequent radical surgery and radiotherapy, compared with those treated with the standard protocol. Moreover, a prospective phase II randomized clinical trial (NCT02285530) is currently ongoing to verify retrospective results. However, there continue to be requests to improve the treatment protocol to achieve better clinical outcomes in patients with high GDF15 expression, but not cT3/4N0M0. In addition, increased GDF15 expression has been reported to be induced by chemoagents and radiation, which resulted in chemoresistance and radiation resistance in prostate and oral cancers.[21,22] Increased GDF15 expression has also been reported to strengthen chemotherapeutic efficacy by inhibiting adipogenesis in acute myeloid leukemia.[23] In our previous studies and the present study,[9] both the in vitro and in vivo findings demonstrated that the overexpression or downregulation of GDF15 expression changed the chemotherapeutic sensitivity induced by TPF on apoptosis, with increasing or decreasing PARP, caspase-3, and caspase-9 activation. Growth differentiation factor 15-overexpressing cancer cells are sensitive to TPF chemoagents through a caspase-9 dependent pathway. Although GDF15 is entirely overexpressed in OSCC, patients or xenografts with relatively low GDF15 expression are not sensitive to TPF chemoagents; only cT3/4N0M0 patients and xenografts with GDF15 overexpression can benefit from treatment with TPF chemoagents. In this study, we also provide the feasibility of new treatment for patients with high GDF15 expression using erbB2 inhibitors.

ErbB2 is a 185-kDa receptor tyrosine kinase. The erbB signaling pathway regulatory network primarily includes the PI3K/AKT, RAS/RAF/MEK/Erk1/2, and phosphoinositol-specific phospholipase (PLC) gamma signaling pathways.[24] In this study, the upregulation or downregulation of GDF15 expression did not change the amount of erbB2 expression, but altered erbB2 phosphorylation and subsequently the phosphorylation of AKT and Erk signaling pathways. Therefore, we selected the pan-erbB receptor tyrosine-kinase inhibitor, CI-1033,[25] and found that it could effectively inhibit the AKT and Erk signaling pathways and inhibit the proliferation of OSCC cells with high GDF15 expression. As previously reported, CI-1033 could be clinically useful to inhibit advanced solid cancers (eg, renal cancer, breast cancer, and oropharyngeal cancer), especially when combined with docetaxel.[26-28] In the present study, CI-1033 was observed to block the growth of GDF15-overexpressing OSCC cells both in vitro and in vivo through inhibiting erbB2 phosphorylation, as well as downstream AKT and Erk activation. Furthermore, CI-1033 is not the only agent to inhibit the growth of OSCC with GDF15 overexpression through the erbB2 signaling pathway. Thus, any agent inhibiting erbB2 phosphorylation should be investigated. These agents alone or in combination with TPF chemoagents might benefit OSCC patients with high GDF15 expression who are not T3/4N0M0, which needs to be confirmed in further translational studies.

Based on our present and previous studies of GDF15 in OSCC, GDF15 could represent an effective biomarker for individualized therapy. For locally advanced OSCC patients with low levels of GDF15 expression, standard treatment (radical surgery and postoperative radiotherapy/chemoradiotherapy) could achieve good clinical outcomes. However, for cT3/4N0M0 OSCC patients with high levels of GDF15 expression, initial TPF induction chemotherapy followed by standard treatment could represent a more effective treatment strategy. Additionally, locally advanced OSCC patients (not cT3/4N0M0) with high GDF15 expression might benefit from treatment with an erbB2 inhibitor alone or in combination with TPF induction chemotherapy, by achieving better clinical outcomes. However, further clinical trials are recommended to verify this individualized therapy protocol in patients with locally advanced OSCC.

Our present results indicate that OSCC tumors overexpressing GDF15 are sensitive to TPF chemoagents through the caspase-9-dependent pathway both in vitro and in vivo. Overexpression of GDF15 promotes the cellular proliferation of OSCC through erbB2 phosphorylation. ErbB2 inhibitors might be used as potential targeted drugs or an alternative for OSCC patients with GDF15 overexpression. Our results suggest a theoretical individualized therapy protocol based on the level of GDF15 expression in biopsies obtained from patients with locally advanced OSCC.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

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SUPPORTING INFORMATION

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