The EGFR Inhibitor Gefitinib Enhanced the Response of Human Oral Squamous Cell Carcinoma to Cisplatin In Vitro

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Abstract

Introduction The epidermal growth factor receptor (EGFR) is highly expressed in a variety of solid tumors including oral cavity squamous cell carcinoma (OSCC) and has been implicated in the resistance of these tumors to cisplatin. This study was performed to determine if the EGFR tyrosine kinase inhibitor gefitinib could enhance the cytotoxic effect of cisplatin on OSCC cells in vitro.

Methods The expression of EGFR and the phosphorylation of its downstream signaling to ERK, and AKT pathway were detected by Western blotting. Cell proliferation and survival were determined by AlamarBlue and colony formation assay respectively. Cells apoptosis were determined by Western blotting for cleaved PARP protein and by flowcytometry of cells stained with Annexin V and PI.

Results Cal27, OSC19, and SCC25 cells treated with gefitinib 1 μM demonstrated reduced phosphorylation of EGFR, AKT, and ERK proteins with very limited inhibition of proliferation. Cisplatin inhibited proliferation of the same cell lines in a dose-dependent manner. The concentration producing 50% inhibition (IC50) for cisplatin decreased in the presence of gefitinib 1 μM, and a combination of cisplatin 5 μM and gefitinib 1 μM caused synergistic growth inhibition and synergistic reduction in cell survival. The growth inhibitory effect of the combination was associated with reduced ERK and AKT activation, increased poly ADP ribose polymerase (PARP) cleavage, and increased apoptosis.

Conclusion Thus, in OSCC cells in vitro, inhibition of EGFR activity with gefitinib enhances the apoptotic effect of cisplatin. This has potential implications for enhancement of cisplatin effectiveness in tumors that over-express the EGFR.

Key Points

Oral cavity squamous cell carcinoma (OSCC) cell lines Cal27, OSC19, and SCC25 express epidermal growth factor receptor (EGFR) at high levels with low basal phosphorylated EGFR (pEGFR).

OSCC cell lines have functional EGFR-ERK and EGFR-AKT signaling pathways. At 1 μM, gefitinib reduces AKT and ERK activation in unstimulated and EGF-stimulated cells.

Cisplatin inhibits OSCC cell growth, proliferation, and survival in a dose-dependent manner.

Combination of cisplatin with gefitinib enhances the cytotoxicity of cisplatin. This is associated with increased poly ADP ribose polymerase (PARP) cleavage and increased apoptotic cell populations.
1 Introduction

The epidermal growth factor receptor (EGFR) has been implicated in the survival and proliferation of cancer cells. EGFR is highly expressed in human oral cavity squamous cell carcinomas (OSCCs). High EGFR expression has been associated with resistance to chemotherapeutic agents used in the treatment of OSCCs such as cisplatin, 5-fluorouracil (5FU), cyclophosphamide, and doxorubicin [1–3].

Via downstream signaling through extracellular signal-regulated kinase (ERK) and AKT, the EGFR is implicated in multiple aspects of cancer cell physiology, including survival, proliferation, invasion, metastasis, angiogenesis, and apoptosis [4–6]. EGFR has already been recognized as a therapeutic target in head and neck squamous carcinomas, and a variety of EGFR inhibitors are currently used in the treatment of several human cancers [7–11].

Gefitinib is a low molecular weight tyrosine kinase inhibitor [12] that competes for ATP binding to the catalytic kinase domain of EGFR, thus inhibiting phosphorylation of EGFR and its downstream signaling pathways. Preclinical in vitro studies showed that EGFR inhibition with gefitinib results in decreased cell proliferation, survival, and migration with sensitivity to the drug (concentration producing 50% inhibition [IC50] ranged from <1 to 13 μM) depending on the cancer cell type and the presence or absence of a sensitizing mutation in the EGFR protein [13]. Early clinical trials showed that gefitinib is generally well tolerated in patients with a wide range of solid tumor types including lung, head and neck, colon, breast, and prostate cancers [14–16]. Since the introduction of tyrosine kinase inhibitors (TKIs) in clinical use for solid tumors in 2003, several molecular biomarkers, including gene mutations, EGFR protein expression, and EGFR gene copy number, have been identified and suggested to have potential value in predicting responses to TKI treatment [17–21].

Cisplatin is a chemotherapeutic cytotoxic DNA-damaging alkylating drug used in the treatment of various solid tumors, often in combination with other chemotherapeutic agents. In addition to playing a key role in the therapy of many other cancers, cisplatin is a crucial component in the treatment of head and neck cancers, including OSCC [20, 22]. Intrinsic and acquired drug resistance is a major drawback of cisplatin in clinical use. The molecular mechanisms of cisplatin resistance remain indistinct, but increased expression and activation of EGFR signaling pathways is associated with decreased cellular sensitivity to cisplatin. It has been noted that EGFR inhibitors can overcome some cisplatin insensitivity in EGFR overexpressing cancers [23–25]. In animal models and in in vitro studies, the combination of an anti-EGFR monoclonal antibody with cisplatin has shown synergism in inhibiting cell proliferation and inducing apoptosis in some cisplatin-resistant OSCC cell lines [26].

In the present study, we evaluate the effect of combining the EGFR-TKI gefitinib with cisplatin on in vitro proliferation, survival, cellular signaling, and apoptosis of OSCC cell lines Cal27, OSC19, and SCC25. These cell lines are known to express wild-type EGFR and have intact downstream ERK and AKT signaling pathways, which make them suitable models to study the effects of EGF-TKIs in OSCC.

2 Materials and Methods

2.1 Tissue Culture and Reagents

Cal27 and SCC25 cells were obtained from American Type Culture Collection. OSC19 cells were kindly provided by Dr. Jeffrey Myers (The University of Texas MD Anderson Cancer Center, Houston, TX, USA). Cells were grown in Dulbecco’s Modified Eagle’s Medium/F12 supplemented with 5% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C and 5% CO2. As extended in vitro cell culture and clonal expansion can lead to the emergence of new genotypes and altered cellular phenotypes over time, all experiments were performed on early passage cells between passage number 3 and 10. Chemical reagents were obtained as follows: cisplatin (purity >99.9%), crystal violet, and sodium orthovanadate from Sigma-Aldrich (St. Louis, MO, USA); gefitinib (purity >99%) from AstraZeneca (Macclesfield, UK); recombinant human epidermal growth factor (rhEGF) from Cell Sciences (Canton, MA, USA); anti-EGFR, anti-phosphorylated EGFR (pEGFR), anti-ERK1/2, anti-pERK1/2, anti-poly ADP ribose polymerase (PARP), anti-β-actin, and anti-γH2AX from Cell Signaling (Beverly, MA, USA); annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) from Millipore (Bedford, MA, USA); alamarBlue and trypan blue from Invitrogen (Carlsbad, CA, USA); and dimethylsulfoxide (DMSO), dithiothreitol (DTT), FBS and methanol from Thermo Fisher Scientific (Waltham, MA, USA).

2.2 Immunoblot

Treated cells in 6-cm dishes were washed with ice-cold phosphate-buffered saline (PBS) containing sodium orthovanadate 2 mM (to preserve the protein tyrosyl phosphate state in cells and cells lysates) [25], collected, resuspended in lysis buffer, and processed as previously described [15, 16]. Briefly, lysates were vortexed, incubated at 4 °C for 30 min, and centrifuged at 13,000 rpm.
for 15 min at 4 °C. The supernatant was collected and sample buffer containing DTT 0.1 M was added. Proteins were resolved by SDS-PAGE on a 12% polyacrylamide gel and then electrophoretically transferred to a polyvinyliden fluoride (PVDF) membrane (Millipore) using a semidry transfer device (Trans-Blot, Bio-Rad, Hercules, CA, USA). Membranes were subsequently blocked with casein 1% in PBS buffer (Bio-Rad, Hercules, CA, USA) for 2 h at room temperature, incubated with the appropriate primary antibody at 1:1000 dilution overnight at 4 °C. Membranes were incubated with infrared-labeled secondary antibodies (anti-mouse 680 Alexa, Molecular Probes, Eugene, OR, USA) or anti-rabbit IRDYE 800 (Rockland Immunochemicals, Gilbertsville, PA, USA) at 1:15,000 dilutions for 2 h at room temperature. Specific protein bands were detected and quantified using Odyssey Infrared Imaging System and Software version 1.2 from Li-Cor Biosciences (Lincoln, NE, USA).

2.3 alamarBlue Cell Proliferation Assay

Cells were plated at 5000 cells per well in a 96-well plate, grown for 24 h, and treated with cisplatin and/or gefitinib dissolved in DMSO added directly into the media. Because previous studies had shown that metabolic hydrolysis of cisplatin could occur within 5 min, cisplatin was added after gefitinib [27]. Control untreated cells were treated with an equal concentration of DMSO that did not exceed 0.1%; DMSO alone at 0.1% had no effect. All conditions were assessed in triplicate. Plates were incubated for 72 h at 37 °C. alamarBlue was added to each well according to the manufacturer’s protocol. Cells were incubated for 3 h at 37 °C and the fluorescence at 540 nm was recorded. Data were expressed as means ± standard error of the mean (SEM).

2.4 Colony Formation Assay

Ca127 and OSC19 cells were seeded at 250 cells per well in 6-well plates and incubated overnight at 37 °C. Cells were treated in triplicate with gefitinib 1 μM and/or cisplatin 5 μM. Plates were incubated for 14 days; during this period the medium was changed twice weekly with the appropriate concentration of each drug. Plates were washed with ice-cold PBS. Colonies were then fixed with methanol for 15 min, stained with 2% crystal violet and counted. Colonies consisting of ≥50 cells were scored.

2.5 Flow Cytometry

Ca127, OSC19, and SCC25 cells were grown in 6-cm dishes to 70% confluence in 5% FBS. Cells were incubated with the desired concentration of cisplatin and/or gefitinib for 72 h. The conditioned medium from each well was collected; cell monolayers were trypsinized, resuspended in the corresponding conditioned medium, centrifuged at 3000 rpm for 3 min at 4 °C, washed once with cold PBS, and resuspended in annexin V-FITC and PI according to the manufacturer’s recommendations (Millipore). Flow cytometry was conducted in the UVA Flow Cytometry Core Facility with marker combinations as follows: cells that are viable are both annexin V and PI negative; cells in early apoptosis are annexin V positive and PI negative; cells in late apoptosis are annexin V and PI positive; necrotic/dead cells are annexin V negative and PI positive.

2.6 Statistics

All statistical analyses were performed using Prism 3 (GraphPad Software, San Diego, CA, USA). Comparisons of conditions were assessed using a two-tailed Student’s t test with results considered statistically significant when p < 0.05.
3 Results

3.1 EGFR and pEGFR Levels in OSCC Cell Lines

EGFR and pEGFR levels in Cal27, OSC19 and SCC25 cells were characterized by immunoblot analysis of cell lysates obtained from subconfluent dishes either unstimulated or after stimulation with rhEGF 10 nM. The three cell lines express high levels of EGFR with low basal pEGFR (Fig. 1a); EGFR phosphorylation increase by 20-fold after treatment with rhEGF (Fig. 1b).

3.2 Gefitinib Inhibits EGFR Activation and Signaling in OSCC Cells

In Cal27, OSC19, and SCC25 cells, stimulation of EGFR with rhEGF 10 nM resulted in increased EGFR, ERK, and AKT phosphorylation; ERK activation was more prolonged than AKT activation. One hour after treatment with gefitinib 0.25, 0.5, or 1 μM, there was dose-dependent inhibition of EGF-stimulated EGFR, ERK, and AKT phosphorylation (Fig. 2a). EGFR phosphorylation was completely abolished by gefitinib 0.5 μM, which also

Fig. 2 Effect of gefitinib on OSCC cells signaling and growth. a Cal27, OSC19, and SCC25 cells were treated with gefitinib 0.25, 0.5, or 1 μM for 1 h, then rhEGF 10 nM for 10 min. Total cellular protein was isolated and immunoblot performed using the antibodies shown. β-Actin was used to indicate equal protein loading. b Cal27, OSC19, and SCC25 were grown in 96-well plates and treated with gefitinib for 72 h with the concentrations shown. Proliferation was measured using the alamarBlue assay, with relative net fluorescence used as a surrogate for cell number. Relative net fluorescence is expressed as a percentage of untreated cells. Values represent means ± standard error of the mean (SEM) of three independent triplicate experiments. *p < 0.05 compared with control. EGFR epidermal growth factor receptor, Gef gefitinib, OSCC oral cavity squamous cell carcinoma, pEGFR phosphorylated EGFR, rhEGF recombinant human epidermal growth factor

△Adis
suppressed the downstream phosphorylation of AKT; this concentration, however, did not eliminate ERK phosphorylation. Gefitinib 1 μM was required to eliminate EGFR-stimulated ERK activation. At this concentration, basal pERK was also eliminated, suggesting that the activation of ERK in unstimulated cells is a result of basal EGFR activation.

3.3 Gefitinib Inhibits OSCC Cell Proliferation

Cal27, SCC25, and OSC19 cells were treated with increasing concentrations of gefitinib and proliferation was assessed using the alamarBlue assay (Fig. 2b). Gefitinib exhibited a dose-dependent inhibition of proliferation. Cal27 was more resistant to the growth inhibitory effect of gefitinib than OSC19 and SCC25. Gefitinib 1 μM resulted in only 10% growth inhibition of Cal27 cells but 25 and 30% growth inhibition of OSC19 and SCC25 cells, respectively. The IC$_{50}$ of gefitinib was 6 μM for Cal27 cells and 2.5 μM for OSC19 and SCC25 cells. Complete growth inhibition was noted at 10 μM in all cell lines.

3.4 Gefitinib Enhances Growth Inhibitory Effect of Cisplatin on OSCC Cells

Cal27 cells were treated with cisplatin 1.5, 3.5, 6.5, 12.5, and 25 μM in the absence and presence of gefitinib 1 μM (Fig. 3a). Cell proliferation was determined at 72 h using alamarBlue. Treatment with cisplatin alone caused a dose-dependent reduction in cell number with an IC$_{50}$ of 12.5 μM. Combination with gefitinib resulted in enhanced growth inhibition by cisplatin with an IC$_{50}$ of 5 μM. At each concentration, there was a significant difference between the growth inhibition caused by cisplatin/gefitinib combination treatment compared with cisplatin alone ($p < 0.05$).

SCC25 and OSC19 cells were treated with cisplatin 5 μM and/or gefitinib 1 μM (Fig. 3b). Cell proliferation was determined at 72 h using alamarBlue. Gefitinib alone reduced cell number by approximately 20%, which was not...
statistically significant, and cisplatin alone reduced cell number by approximately 30% \((p < 0.05)\). The combination of cisplatin and gefitinib resulted in a 70–75% reduction in cell number \((p < 0.05)\) indicating a synergistic growth inhibitory effect.

The EGFR expression level was very similar in the three cell lines studied (Fig. 1), thus no correlation could be identified between EGFR expression level and response to gefitinib.

### 3.5 Gefitinib Enhances Cisplatin Inhibition of Clonogenic Survival of OSCC Cells

The effect of chronic exposure (14 days) to gefitinib 1 \(\mu\)M and/or cisplatin 5 \(\mu\)M on clonogenic survival was evaluated using a colony formation assay (Fig. 4). Gefitinib 1 \(\mu\)M caused a small reduction in the number of colonies formed; this was not statistically significant. Cisplatin 5 \(\mu\)M resulted in a 20% reduction in colony formation compared with untreated cells \((p < 0.05)\). Treatment with combined cisplatin and gefitinib resulted in a 65% reduction in colony formation \((p < 0.05)\), again indicating a synergistic effect.

### 3.6 Signaling Effects of Combined Treatment with Cisplatin and Gefitinib in OSCC Cells

Cal27 cells either untreated or pretreated with gefitinib 1 \(\mu\)M for 1 h were incubated with 1.5, 3.5, 6.25, or 12.5 \(\mu\)M cisplatin for 24 h followed by immunoblot analysis of whole cell lysates (Fig. 5a, b). PARP cleavage was assessed as a marker of apoptosis while pERK and pAKT were evaluated as indicators of pro-survival/anti-apoptotic signaling. In Cal27 cells, treatment with cisplatin alone did not cause significant PARP cleavage. Treatment with gefitinib alone caused an increase in PARP cleavage, which

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**Fig. 5** Impact of cisplatin and gefitinib co-treatment on signaling in OSCC. Cal27 (a) and SCC25 (c) cells were pretreated with gefitinib for 1 h and then with cisplatin for 24 h as indicated. Both adherent and non-adherent cells were collected and subjected to immunoblot with the indicated antibodies. \(\beta\)-Actin was used to indicate equal protein loading. b, d Summary of densitometric analysis of cleaved PARP as a percentage of total PARP. OSCC oral cavity squamous cell carcinoma, PARP poly ADP ribose polymerase
was augmented in a dose-dependent fashion by cisplatin. Cisplatin treatment induced a dose-dependent increase in ERK phosphorylation; this effect was inhibited by co-treatment with gefitinib.

SCC25 cells were similarly incubated with cisplatin 5 μM and/or gefitinib 1 μM followed by immunoblot analysis of whole cell lysates (Fig. 5c, d). Gefitinib alone and cisplatin alone caused small increases in PARP cleavage; combined gefitinib and cisplatin resulted in substantially greater PARP cleavage than either drug alone.

3.7 Gefitinib Enhances the Apoptotic Effect of Cisplatin on OSCC Cell Lines

Induction of apoptosis in Cal27, OSC19, and SCC25 cells was examined by flow cytometry. Cells were incubated...
with cisplatin 5 µM either alone or in combination with gefitinib 1 µM for 72 h and subsequently processed for flow cytometry using annexin V-FITC and PI as described in the Methods section. Figure 6a demonstrates the cell populations in early (lower right quadrant) and late (upper right quadrant) apoptosis. The results are summarized in Table 1 and Fig. 6b. The apoptotic cell population averaged 7, 3, and 6% in untreated Cal27, OSC19, and SCC25 cells, respectively. This population increased to 14, 20, and 9% (p < 0.05), respectively, upon gefitinib treatment and 19, 28, and 17% (p < 0.05), respectively, upon cisplatin treatment. Combined treatment with both cisplatin and gefitinib resulted in apoptosis in 40, 43, and 32% (p < 0.05), respectively.

4 Discussion

Targeted therapies using small molecules that inhibit key signaling pathways involved in tumor growth and proliferation have shown promising results against various malignant tumors in preclinical and clinical studies [28, 29]. Combining these targeted therapies to increase efficacy and reduce toxicity is an emerging therapeutic strategy [30]. One such approach is the combination of cisplatin and an EGFR inhibitor [31]. In the present study, the ability of the EGFR-TKI gefitinib to enhance the apoptotic action of cisplatin was evaluated in vitro in the OSCC cell lines Cal27, OSC19, and SCC25. The results demonstrate that, at a concentration that inhibits basal EGFR activity but does not significantly impact cell growth, gefitinib treatment significantly enhances the pro-apoptotic and anti-proliferative effects of cisplatin.

Compared with cisplatin alone, the combination of gefitinib and cisplatin led to significantly reduced cell numbers and colony formation in all three OSCC cell lines. This coincided with decreased ERK and AKT signaling, increased PARP cleavage, and increased apoptosis. These effects appeared to be synergistic. Treatment with cisplatin alone resulted in ERK activation in Cal27 cells, which could contribute to resistance to its growth inhibitory effect. This cisplatin-induced ERK activation was abolished by the addition of gefitinib 1 µM, a concentration that did not, by itself, cause growth inhibition. Thus, the increase in ERK signaling in response to cisplatin treatment may be EGFR-dependent.

These results are in agreement with numerous studies demonstrating that gefitinib inhibits cancer cell growth by mechanisms involving the inhibition of EGFR and its downstream ERK and AKT pathways [32, 33]. Consistent with the present findings, chemo-sensitizing effects of gefitinib have been reported in drug-resistant breast cancer cell lines [19, 34–37].

In this study, the expression level of EGFR in Cal27, OSC19, and SCC25 cells was high. No correlation was noted between EGFR expression level and sensitivity to gefitinib or synergism between gefitinib and cisplatin. Prior efforts to establish correlation between EGFR expression levels and the response to cetuximab or EGFR-TKI in upper aero-digestive tract squamous cell carcinoma (SCC) have been inconsistent and thus inconclusive [38]. In vitro work in head and neck SCC (HNSCC) suggests that high EGFR expression may correlate with response to EGFR-TKI [39] and high EGFR copy number has also been reported to correlate with erlotinib response in HNSCC patients [24]. On the other hand, a clinical trial of erlotinib in HNSCC showed that EGFR protein levels are not indicative of erlotinib response [40]. Further, a clinical study of combining gefitinib with cisplatin and radiation therapy failed to uncover EGFR protein expression as a predictive biomarker for gefitinib responsiveness [31, 41]. Finally, in vitro studies with erlotinib or cetuximab (a monoclonal antibody against the EGFR) showed that the total levels of EGFR protein correlate with EGFR inhibitor sensitivity; in this study, EGFR was downregulated in cetuximab- and erlotinib-resistant cells [18, 42].

EGFR-activating mutations had been established as a predictive biomarker to EGFR-TKI response, particularly in non-small-cell lung cancer (NSCLC), where the in-frame deletion of exon 19 and the L858R substitution in exon 21 account for 90% of the drug-sensitive mutations. The mutations of the TK domain affect amino acids near

| Table 1  | Apoptotic and necrotic cell populations after gefitinib and cisplatin treatment of OSCC |
|----------|------------------------------------------------------------------------------------------|
|          | Cal27                                                                                     |
|          | OSC19                                                                                     |
|          | SCC25                                                                                     |
| Apoptosis| Early | Late | Early | Late | Early | Late |
|----------|------|------|------|------|------|------|
| Untreated| 2.2  | 0.3  | 4.6  | 0.5  | 1.2  | 0.2  |
| Gefitinib 1 µM| 5.8  | 0.7  | 8.2  | 0.7  | 9.7  | 0.7  |
| Cisplatin 5 µM| 7.1  | 0.5  | 11.5 | 1.7  | 11.7 | 0.9  |
| Gefitinib + cisplatin| 15.0 | 0.9  | 25.1 | 0.8  | 23.4 | 1.4  |

M mean, OSCC oral cavity squamous cell carcinoma, SEM standard error of the mean.
the ATP-binding pocket that interfere with binding of gefitinib, and thus can be used a predictor of TKI response [43]. However, the incidence of such EGFR mutations are noticeably less common in OSCC, ranging from 0–8% depending on the ethnicity and geographical distribution, and these mutations appear different from NSCLC as it involves exon 20 more frequently [44, 45]. Therefore, EGFR mutation in OSCC does not carry the significance that it does in NSCLC [46]. Moreover, EGFR-TKI responders have been noted in some HNSCCs in the absence of EGFR mutation [24]. A phase II study of gefitinib 500 mg daily in combination with cisplatin in Asian patients with locally advanced HNSCC failed to identify predictive biomarkers of favorable outcome with the addition of gefitinib [42, 43, 47, 48]. EGFR FISH, protein expression, and mutational status did not predict for response or survival outcome of patients [24, 49, 50]. It is clear that biomarkers predictive of response to gefitinib in HNSCC are not identical to mutations affecting the EGFR gene or its products as in NSCLC [51].

Due to the complexity of factors involved in the EGFR signaling and cross talk it should be emphasized that there are limitations involved in this type of in vitro work utilizing cancer cell lines as these cell lines may harbor genetic mutations or other genetic anomalies that could alter the experimental outcomes. For example, it was reported that the SCC25 cell line harbors a deletion mutation in cyclin dependent kinase, Cdk1, and Cal27 cells are known to harbor a nonsense mutation in SMAD4, which modulates the transforming growth factor (TGF) signaling pathway and cell growth [46, 52, 53]. Nevertheless, these cell lines are considered reliable investigative models in the search for novel and targeted therapies to treat head and neck cancer.

In clinical studies of HNSCC, hepatocellular carcinoma, and NSCLC, addition of EGFR-TKIs showed no survival benefit over placebo when combined with cisplatin [54, 55]. Moreover, gefitinib did not improve time to progression or objective tumor response over chemotherapy alone. In a recent study of advanced HNSCC requiring postoperative chemoradiation therapy, patients were randomized to receive gefitinib or placebo in addition to cytotoxic chemotherapy. In the overall cohort, addition of gefitinib was not beneficial, but when the population was categorized according to the expression of the insulin-like growth factor-1 receptor (IGF1R), addition of gefitinib was noted to produce a statistically significant improvement in survival in patients with tumors expressing high IGF1R levels. Given the present results, it is possible that high IGF1R expression identified tumors that would benefit from gefitinib enhancement of cisplatin cytotoxicity, while in tumors with low IGF1R expression this enhancement did not occur, resulting in a lack of perceived benefit in the overall study cohort [56].

Identifying tumors in which cisplatin treatment efficacy is improved with gefitinib co-treatment could be important not only for improving cancer survival, but also for toxicity reduction if the same tumor response can be achieved with lower doses of cisplatin. This could have substantial importance in patients with OSCC, who are often malnourished and unable to tolerate maximal doses of cytotoxic drugs.

5 Conclusion

The EGFR-TKI gefitinib sensitizes OSCC Cal27, OSC19, and SCC25 cells to the growth inhibitory and pro-apoptotic effects of cisplatin. These in vitro observations demonstrate the need for a more thorough understanding of the molecular crosstalk in HNSCC. If a similar effect is noted in vivo, it is possible that, in appropriate settings, co-treatment with gefitinib could enhance cisplatin effects or reduce required cisplatin doses and thus toxicity. It will therefore be crucial to identify human tumors in which this effect can be observed and to find a predictor of this effect.

Compliance with Ethical Standards

Conflict of interest Ashraf Khalil declares no potential conflicts of interest; Mark J. Jameson declares no potential conflicts of interest.

Grant support The study was supported by NIH Grant K08-DE019477 and American Head and Neck Society pilot Grant to M. J. Jameson.

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