Regulation of estrogen receptor α function in oral squamous cell carcinoma cells by FAK signaling

Yi-Lin Chang1,*, Yu-Kan Hsu3,*, Tsung-Fan Wu1, Chieh-Ming Huang1,2, Li-Yin Liou1, Ya-Wen Chiu1, Yu-Hsuan Hsiao1, Fuh-Jinn Luo2 and Ta-Chun Yuan1

1Department of Life Science, Institute of Biotechnology, National Dong Hwa University, Hualien 97401, Taiwan
2Department of Pathology, and 3Department of Dentistry, Mennonite Hospital, Hualien 970, Taiwan, Republic of China
*(Y-L Chang and Y-K Hsu contributed equally to this work)

Abstract

Estrogen receptor α (ERA) is a DNA-binding transcription factor that plays an important role in the regulation of cell growth. Previous studies indicated that the expression of ERα in cell lines and tumors derived from oral squamous cell carcinoma (OSCC). The aim of this study was to examine the activity and function of ERα in OSCC cells and the mechanism underlying ERα activation. Immunochemical analyses in benign (n = 11) and malignant (n = 21) lesions of the oral cavity showed that ERα immunoreactivity was observed in 43% (9/21) of malignant lesions, whereas none of benign lesions showed ERα immunoreactivity. The ERα expression was also found in three OSCC cell lines and its transcriptional activity was correlated with cell growth. Addition of estradiol stimulated cell growth, whereas treatment of tamoxifen or knockdown of ERα expression caused reduced cell growth. Interestingly, the expression and activity of focal adhesion kinase (FAK) were associated with the phosphorylation of ERα at serine 118 in OSCC cells. Elevated expression of FAK in the slow-growing SCC25 cells caused increases in ERα phosphorylation, transcriptional activity, and cell growth rate, whereas knockdown of FAK expression in the rapid-growing OECM-1 cells led to reduced ERα phosphorylation and activity and retarded cell growth. Inhibition of the activity of protein kinase B (AKT), but not ERK, abolished FAK-promoted ERα phosphorylation. These results suggest that OSCC cells expressed functional ERα, whose activity can be enhanced by FAK/AKT signaling, and this was critical for promoting cell growth. Thus, FAK and ERα can serve as the therapeutic targets for the treatment of OSCC.

Key Words

- oral squamous cell carcinoma
- estrogen receptor α
- focal adhesion kinase

Introduction

Estrogen receptors (ERs), including ERα and ERβ are nuclear receptors that function as transcription factors to active gene expression for regulating cell proliferation and survival. Upon ligand binding, ERs undergo phosphorylation and conformational changes that lead to the dissociation of heat shock proteins and subsequent receptor dimerization. The receptor dimers then undergo nuclear translocation and bind to estrogen-responsive elements (EREs) present in the promoter of estrogen-responsive genes. Once bound to EREs, ERs recruit coactivators or corepressors to form a complex that regulates the transcription of estrogen-responsive genes.

Correspondence should be addressed to T-C Yuan
Email yuan415@mail.ndhu.edu.tw
and thus modulates a variety of physiological functions. Instead of binding to DNA, ligand-activated ERs can function as coactivators to regulate gene transcription by interacting with certain transcription factors, such as activating protein 1 and specificity protein 1 (Osborne & Schiødt 2005, Thomas & Gustafsson 2011). Alternatively, ER functions can be regulated by intracellular signaling via phosphorylation in the absence of ligands. For example, phosphorylation of ERα plays critical roles in the regulation of ERα transcriptional activity, stability, and DNA binding (Weigel & Moore 2007, Murphy et al. 2011). Although ERα can be phosphorylated at multiple sites, serine 118 (Ser118) is one of the most reported phosphorylation sites with clinical significance (Murphy et al. 2009). Phosphorylation of ERα at Ser118 promotes the recruitment of coregulators to ERα-regulated genes and modulates estrogen-induced gene expression (Duplessis et al. 2011).

Accumulated evidence demonstrates the oncogenic roles of ERs in hormone-regulated cancers, such as breast, prostate, ovarian, or endometrial cancers; however, the role of ERs in oral squamous cell carcinoma (OSCC) is less understood. Previous studies have shown that the expression of ERα and ERβ in OSCC tumors and cell lines, and expression with tamoxifen significantly, inhibits cell proliferation and invasion (Ishida et al. 2007, Kim et al. 2007, Ku & Crowe 2007, Lukits et al. 2007). The expression of ERα and ERβ is also found in head and neck squamous cell carcinoma (HNSCC) tumors, but the nuclear levels of ERα, but not ERβ, in tumor lesions are significantly higher than that in paired adjacent mucosa. Importantly, patients with high nuclear ERα expression in tumors have a diminished survival rate compared with patients with low nuclear ERα (Egloff et al. 2009). These results suggest that HNSCC cells express ERα, the activities of which are associated with tumor development. However, the activation status of ERα and the regulatory mechanism of ERα activation in OSCC cells are mostly unknown.

Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase and is ubiquitously expressed in all tissues and cell types. It plays important roles in the mediation of adhesion-dependent and growth factor-dependent signalings through the activation of the MEK/ERK and the PI3K/AKT pathways, regulating a variety of cellular functions including proliferation, survival, and motility. In contrast, deregulated FAK signaling causes uncontrolled proliferation and enhanced migration, promoting the malignant transformation of tumor cells (Zhao & Guan 2009). A large number of studies have shown an increased expression of FAK (RB1CC1) mRNA and/or protein in different human cancers (Golubovskaya et al. 2009). In OSCC, the elevated expression and phosphorylation of FAK have been observed (Kornberg 1998, Schneider et al. 2002, Aronsohn et al. 2003). Over-expression of FAK in low-invading HNSCC cells leads to an elevated growth rate and an enhanced invasive capability (Schneider et al. 2002). Despite intensive studies on FAK functions in different cancers, the oncogenic role of FAK in OSCC cells is not fully understood.

The aim of this study was to analyze the expression of ERα in OSCC tumors and cell lines and to examine whether activated FAK signaling is the underlying mechanism responsible for ERα activation in OSCC cells, causing enhanced cell growth.

Materials and methods

Reagents and antibodies

Cell culture media and supplements were purchased from Invitrogen. The primary antibodies used for immunoblotting were epidermal growth factor receptor (EGFR), FAK, ERK, AKT, ERα, β-actin (Santa Cruz Biotechnology), p-Tyr1173 EGFR, ERα, ERβ, p53 (Epitomics, Burlingame, CA, USA), p-Ser118 ERα, p-Thr202/Tyr204 ERK, p-Ser473 AKT, p-Tyr397 FAK (Cell Signaling, Danvers, MA, USA), GAPDH, and lamin B1 (GeneTex, Irvine, CA, USA). Horseradish peroxidase (HRP)-conjugated secondary antibodies were obtained from Santa Cruz Biotechnology. MEK inhibitor U0126 and PI3K inhibitor LY294002 were obtained from Calbiochem (Darmstadt, Germany). All chemicals were purchased from Sigma.

Cell culture

Human tongue SCC cell lines (SCC4 and SCC25) and an ER-positive breast cancer cell line (MCF-7) were obtained from the Biosource Collection and Research Center (BCRC, Hsinchu, Taiwan) which routinely authenticates these lines by short tandem repeat (STR) analysis. A human gingival SCC cell line (OECD-1; Meng et al. 1998) was obtained from Dr K W Chang (National Yang-Ming University, Taipei, Taiwan). The STR profiling of OECD-1 cells was carried out at the BCRC. All experiments were carried out by each cell line at passages below 30. While the SCC4 and SCC25 cells were maintained in DMEM/F12 medium, the OECD-1 and MCF-7 cells were cultured in RPMI 1640 and DMEM medium respectively. Each medium was supplemented with 5% fetal bovine serum (FBS), 2 mM glutamine, and 0.05 mg/ml of gentamicin.
Luciferase reporter assay

An aliquot of $1 \times 10^4$ cells/well were plated into 24-well plates. After a 48 h incubation, the cells were cotransfected with 1 μg of pGL2-ERE-luc plasmids (Addgene, Cambridge, MA, USA) and 0.2 μg of Renilla luciferase expressing vector using a TransLT1-transfection reagent (Mirus, Madison, WI, USA). After an additional 48 h incubation, the cells were lysed, and the ratio of the two luciferase activities was analyzed using the Dual-luciferase Reporter Assay Kit (Promega) and a 2020n luminometer (Turner BioSystems, Sunnyvale, CA, USA).

Lentiviral infection

For stably expressing FAK proteins in SCC25 cells, 293FT cells (Invitrogen) were co-transfected with the pCDH-CMV-MCS-EF1-Puro (pCDH) vector (SBI, Mountain View, CA, USA) containing the WT FAK cDNA plus packaging plasmids, using TransLT1-transfection reagent (Mirus). The control cells were co-transfected with the pCDH vector alone plus packaging plasmids. For gene knockdown experiments, lentiviral vectors carrying shRNAs against the PTK2, ESR1, or ESR2 genes were obtained from the RNAi Core at Academic Sicina (Taipei, Taiwan). For the preparation of the shRNA-containing lentiviruses, 293FT cells were co-transfected with the lentiviral expression vector, containing the gene-targeted shRNA and with packaging plasmids using the PolyJet Transfection Reagent (SignaGen Lab., Ijamsville, MD, USA). The cells expressing shRNA against the gene encoding firefly luciferases or β-galactosidases served as a control (shCt). After a 72 h incubation, the culture medium containing lentiviruses was harvested. For infection, cells grown in six-well plates were incubated with viruses in the presence of 8 μg/ml of polybrene and were centrifuged at 840 g for 1 h at 37 °C. The infected cells were then gene knockdown experiments, lentiviral vectors carrying shRNAs against the PTK2, ESR1, or ESR2 genes were obtained from the RNAi Core at Academic Sicina (Taipei, Taiwan). For the preparation of the shRNA-containing lentiviruses, 293FT cells were co-transfected with the lentiviral expression vector, containing the gene-targeted shRNA and with packaging plasmids using the PolyJet Transfection Reagent (SignaGen Lab., Ijamsville, MD, USA). The cells expressing shRNA against the gene encoding firefly luciferases or β-galactosidases served as a control (shCt). After a 72 h incubation, the culture medium containing lentiviruses was harvested. 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NaF, followed by vigorously vortexing for 30–60 s. The supernatant containing nuclear proteins was collected by centrifuging at 16 000 g for 10 min at 4 °C.

**Tissue microarray and immunohistochemistry**

Tissue specimens including 11 benign and 21 malignant lesions of the oral cavity were collected from patients who underwent surgical resection at Mennonite Christian Hospital (Hualien, Taiwan). This procedure was approved by the Institutional Review Board of the Buddhist Tzu Chi General Hospital (#IRB101-115), and written informed consents were obtained from all participants. Tissue specimens were incorporated into a tissue microarray. The sections were deparaffinized in xylene, rehydrated with graded ethanol, and then boiled in 1 mM EDTA (pH 8.0) for 5 min in an autoclave. After three washes with 3% H2O2, the sections were blocked with a protein block serum (Dako, Glostrup, Denmark) for 5 min. Subsequently, the sections were incubated with an anti-ERα MAB (clone EP1; Dako) at 1:100 dilution for 30 min, and the signal was then amplified using an Envision kit (Dako) according to manufacturer’s protocol. All sections were counterstained with hematoxylin for 2.5 min. The expression of ERα was evaluated using a light microscope, and the scores were divided into three grades: negative (0), low (+1), and high (+2/+3). Tumor cores with >10% of cells staining +1 or greater were defined as positive.

**Statistical analysis**

Significance for group comparisons was assessed by Student’s two-tailed t-test or a one-way ANOVA followed by Tukey’s or Dunnett’s post hoc comparison. The χ² test was used for comparison between categorical variables. A P value <0.05 was considered significant. All statistical analyses were performed using Prism (GraphPad Software, La Jolla, CA, USA).

**Results**

**Expression of ERα in benign and malignant lesions of the oral cavity**

We initially examined the expression of ERα in benign and malignant lesions of the oral cavity by immunohistochemistry. Representative images of ERα immunostaining for each lesion are shown in Fig. 1. The immunoreactivity of ERα in the malignant tissue (Fig. 1B) was higher than that in the benign lesion (Fig. 1A). Unexpectedly, the OSCC tissue shows cytoplasmic ERα staining. Table 1 summarizes the intensity data of the ERα immunostaining in the series of 11 benign and 21 malignant lesions of the oral cavity. In benign lesions, we failed to detect any ERα staining. In contrast, ERα immunoreactivity was found in nine (9/21, 43%) of the malignant tissues. The results of a χ² test indicate the significant difference in the intensity of ERα staining between benign and malignant lesions of the oral cavity (P=0.013).

**OSCC cells express activated ERα correlated with their growth**

To investigate the oncogenic function of ERα in OSCC cells, we examined the expression of ERα in OSCC cell lines. As shown in Fig. 2A, three OSCC cell lines expressed differential levels of ERα proteins. OECM-1 cells expressed
a higher level of ERα compared with SCC4 and SCC25 cells; however, the expression level of ERα in OECM-1 cells was much lower than that in the ER-positive breast cancer MCF-7 cells. We further analyzed the transcriptional activity of ERα in OSCC cells by the luciferase reporter assay. We found that the ERα activity in OECM-1 cells was significantly higher than that in SCC4 or SCC25 cells ($P<0.001$; Fig. 2B). The results of subcellular fractionation analyses further revealed that OECM-1 cells contained a higher level of nuclear ERα compared with a low or undetectable level of nuclear ERα in SCC4 and SCC25 cells (Fig. 2C). Importantly, the expression and activity of ERα in OSCC cells were correlated with their growth rates. On day 4, the ratio of growth in OECM-1 cells was approximately four times higher than that of SCC4 or SCC25 cells ($P<0.001$). These data suggest that OSCC cells expressed ERα proteins and the differential expression and activity of ERα were correlated with their growth.

### Modulation of ERα activity or expression regulates the growth of OSCC cells

To examine whether ERα is functionally involved in the regulation of the growth of OSCC cells, we initially analyzed the growth-stimulating effects of E2 on the slow-growing SCC4 and SCC25 cells, because both cells expressed inactive ERα with low transcriptional activities. Under a hormone-deprived condition, both cells were cultured in the presence or absence of 10 nM E2 and the growth rate was then analyzed. Compared with the vehicle-treated cells, treatment with E2 led to increased cell growth of ~1.7 times (Fig. 3A). In contrast, tamoxifen, an ER antagonist, significantly inhibited the growth of OECM-1 cells ($P<0.001$). As shown in Fig. 3B, treating OECM-1 cells with 1 or 2 μM tamoxifen resulted in ~40 or 80% suppression of cell growth respectively. Because E2 and tamoxifen can modulate both ERα and ERβ activities, we further clarified ERα function in promoting cell growth by performing shRNA-mediated gene knockdown. To show the impacts of ERα knockdown on the cell growth, the fast-growing OECM-1 cells which exhibited a high level of ERα were used for the experiments. As shown in

### Table 1  ERα expression in benign and malignant lesions of the oral cavity

<table>
<thead>
<tr>
<th>Tissue types</th>
<th>n</th>
<th>ERα-negative (%)</th>
<th>ERα-positive (%)</th>
<th>P value</th>
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<tbody>
<tr>
<td>Benign</td>
<td>11</td>
<td>11 (100%)</td>
<td>0 (0%)</td>
<td>0.013</td>
</tr>
<tr>
<td>Malignant</td>
<td>21</td>
<td>12 (57%)</td>
<td>9 (43%)</td>
<td></td>
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Fig. 3C (upper panel), ERβ-depleted OECM-1 cells expressed an undetected level of ERβ and a slightly decreased level of ERβ compared with that in the shCt control cells. After 3 days of culture, ERβ-knockdown OECM-1 cells showed the morphological feature of cell shrinkage (Fig. 3C, middle panel) and exhibited a slow growth rate, ~80% lower than that of shCt cells (Fig. 3C, lower panel). These results suggest that OSCC cells expressed functional ERβ, whose expression or activity was important for promoting their growth.

**FAK activity correlates with ERβ expression and Ser118 phosphorylation in OSCC cells**

To determine the molecular mechanism(s) of how ERβ was activated in OSCC cells, the expression or phosphorylation levels of EGFR and FAK, two important tyrosine kinases in the malignancy of HNSCC, were analyzed in three OSCC cell lines. As shown in Fig. 4A, OECM-1 cells had lower expression and phosphorylation levels of EGFR, but exhibited higher FAK expression and phosphorylation, compared with SCC4 and SCC25 cells. Importantly, FAK expression and phosphorylation did not correlate with the expression or activity of ERKs and p53 proteins, but were positively correlated with the activities of protein kinase B (PKB/AKT). Compared with the relatively low level of AKT phosphorylation in SCC4 or SCC25 cells, OECM-1 cells exhibited activated AKT, which was associated with high levels of ERβ expression and phosphorylation at Ser118 (Fig. 4A). We further examined the impact of inhibiting FAK activity on ERβ Ser118 phosphorylation by treating OECM-1 cells with PF-431496, a FAK inhibitor (Buckbinder et al. 2007). Compared with the vehicle control, treatment of 1 or 5 µM PF-431496 caused decreases in the phosphorylation of FAK, AKT, and ERβ in a dose-dependent manner. However, PF-431396 had no inhibitory effect on ERK phosphorylation (Fig. 4B).

**FAK regulates ERβ phosphorylation and activity and the growth of OSCC cells**

We next examined the impact of elevated FAK expression on ERβ phosphorylation, transcriptional activity, and cell growth. To reduce the hormonal effect on ERβ phosphorylation and activity, FAK-overexpressed SCC25 cells were cultured in a hormone-deprived condition. Compared with the vector control, FAK-overexpressed cells exhibited higher phosphorylation levels of FAK itself, ERK, AKT, and ERβ (Fig. 5A). Importantly, elevated FAK expression also led to increases in ERβ transcriptional...
activity and cell growth in a ligand-independent manner (Fig. 5B and C). In contrast, the knockdown of FAK expression in OECM-1 cells resulted in reduced phosphorylation of ERK, AKT, and ERα that was correlated with decreased ERα activity, ~50% lower than that in the shCt control cells (Fig. 5D and E). In parallel with decreased ERα phosphorylation and activity, FAK-depleted cells exhibited a slow growth rate, about 60% lower than that in the shCt control cells (Fig. 5F). To further examine the role of ERK or AKT in mediating FAK-promoted ERα Ser118 phosphorylation, FAK-overexpressed SCC25 cells were treated with U0126 or LY294002, a MEK or a PI3K inhibitor, respectively, under a SR condition. As shown in Fig. 5G, treatment with U0126 led to the inhibition of ERK phosphorylation, promoting ERα activation and cell growth.

**Discussion**

The oncogenic role of ERα is well established in hormone-related cancers. In OSCC, evidence from cell-based or clinical studies indicates the expression of ERα; however, the functional role of ERα in OSCC malignancy and the mechanism underlying ERα activation in OSCC cells are not fully elucidated. In this study, we confirmed the expression of ERα in OSCC tumors and cell lines as reported in previous studies. Importantly, our main finding was that OSCC cells expressed functional ERα whose Ser118 phosphorylation and transcriptional activity were enhanced by FAK/AKT signaling, leading to promoted cell growth. To our knowledge, this is the first report to demonstrate the molecular mechanism of ligand-independent ERα activation in OSCC cells.

The functional properties of ERα are associated with its expression and activity. In agreement with a previous report (Colella et al. 2011), our data show that OSCC tumors expressed a high level of ERα compared with the benign tissues of the oral cavity. Furthermore, three OSCC cell lines expressed functional ERα and its activity was correlated with the cell growth. This notion was supported by results from E2 or tamoxifen treatment. However, studies also suggest the functional involvement of ERβ in OSCC cell proliferation and invasion (Ishida et al. 2007, Kim et al. 2007, Nelson et al. 2007). Ishida et al. (2007) further shows that knockdown of ERβ expression in an OSCC cell line, SCCNK, leads to a more prominent effect on growth inhibition than that of knocking down ERα. Differing from the observation, our data show that knocking down ERα expression in OECM-1 cells resulted in retarded cell growth, and no growth inhibition was observed in ERβ-knockdown cells (Supplementary Fig. 1, see section on supplementary data given at the end of this article). Although the contradictory results might be due to cell-type specificity, our data are consistent with the observations in other cancer cells that ERα, but not ERβ, plays a predominant role in the promotion of cell growth and survival (Thomas & Gustafsson 2011). While a few reports propose the proliferative and anti-apoptotic roles of ERβ in cancer cells, the tumor suppressor properties of ERβ have been demonstrated by the fact that overexpression of ERβ results in anti-proliferative responses, whereas knockdown of ERβ promotes the proliferative properties in different cancer cell types (Treeck et al. 2007, ...
FAK regulates ERα phosphorylation and activity and the growth of OSCC cells. (A) SCC25 cells stably expressing the vector or the WT FAK were cultured in a steroid-deprived medium for 48 h, and the cell lysates were used for immunoblotting with antibodies against the expression or phosphorylation of FAK, ERK, AKT, and ERα proteins. The luciferase reporter assay (B) and cell growth assay (C) of the vector- or FAK-expressed SCC25 cells were carried out as described in ‘Materials and methods’ section. OE CM-1 cells were infected with viruses carrying control or PTX2-targeted shRNA, i.e., shCt, or shFAK respectively. After an additional 72-h incubation, cells were harvested for western blot analyses (D), the luciferase reporter assay (E), or the cell growth assay (F). All the data were expressed as the mean ± S.E.M. from three independent experiments. Each set of experiments was conducted in duplicate. *P < 0.05, **P < 0.01, and ***P < 0.001 vs the ratios of vector or shCt control cells. (G) FAK-overexpressed SCC25 cells (SCC25-FAK) were treated with 2.5 or 5 μM U0126 or LY294002 for 16 h under a steroid-deprived condition. The cells treated with an equal volume of solvent (DMSO) served as a control. Total lysates were used for western blot analyses. regarding the relationship between the phosphorylation of ERK, AKT, and ERα were carried out in breast tumors or cell lines, especially MCF-7 cells. In OSCC cells, our data clearly show that the expression and activity of FAK were associated with the phosphorylation levels of AKT and ERα at Ser118. Although a correlation between FAK and ERK was observed, inhibition of FAK activity by PF-431396 had no effect on ERK activity. Similarly, treatment with PF-431396 in B cells can abolish anti-immunoglobulin-induced FAK phosphorylation but has no inhibitory effect on ERK activation (Tse et al. 2009). Our data further show that LY294002 can abolish FAK-promoted Ser118 phosphorylation, whereas U0126 failed to show any inhibitory effect on the phosphorylation level of Ser118. A similar observation in HER2 (ERBB2)-overexpressed MCF-7 cells is that U0126 has no effect on HER2-activated Ser118 phosphorylation, although the cells contain highly activated ERK (Weitsman et al. 2006). Thus, our data support the notion that in OSCC cells, FAK signaling via AKT activation promoted ERα phosphorylation at Ser118 in a ligand-independent manner, leading to ERα activation and enhanced cell growth.

In addition to Ser118 phosphorylation, other phosphorylation sites of ERα may involve the FAK-regulated ERα activation. Studies in breast cancer cells show that activated ribosomal protein S6 kinase 1 (S6K1) or c-Src can phosphorylate ERα at Ser167 or Tyr537, respectively, which causes ERα activation (Arnold et al. 1995, Paruthiyil et al. 2011, Bossard et al. 2012). Moreover, a study of 24 OSCC specimens shows that the frequency of ERα expression (50%) is higher than that of ERβ expression (33%) (Lukits et al. 2007). In a separate study of HNSCC tumors, the nuclear level of ERα, but not ERβ, is significantly higher in the tumor lesions than in paired adjacent normal mucosa (Egloff et al. 2009). Thus, our data support the functional significance of ERα in OSCC cells.

FAK is a key regulator to mediate integrin signaling. A line of evidence indicates that integrin signaling can regulate ERα expression and activity (Novaro et al. 2003). Our data further suggest that FAK can modulate ERα phosphorylation at Ser118. Although FAK can interact with ERα (Planas-Silva et al. 2006), it cannot directly phosphorylate ERα. Therefore, FAK may regulate Ser118 phosphorylation through the downstream serine/threonine kinases. It is of interest to note that two key protein kinases, ERK and AKT, which function as the downstream molecules of FAK signaling, are greatly involved in ERα phosphorylation in a ligand-dependent or ligand-independent manner. Studies in clinical specimens suggest the positive correlation between the phosphorylation levels of Ser118 and Ser167 of ERα, ERK, and AKT (Jiang et al. 2007, Yamashita et al. 2008).

Evidence from in vitro studies revealed that activated ERK phosphorylates Ser118, whereas AKT is predominantly responsible for Ser167 phosphorylation (Kato et al. 1995, Campbell et al. 2001). However, most studies in vitro
As FAK can modulate the activities of S6K1 and c-Src (Schlaepfer & Hunter 1997, Crossland et al. 2013), whether both kinases are involved in FAK-regulated ERα activation in OSCC cells deserves further study. Our results also indicate the role of ERα in FAK-regulated cell growth. However, a line of evidence shows that FAK facilitates cell survival through enhanced degradation of WT p53 proteins (Lim et al. 2008). In our study, three OSCC cell lines expressed an undetectable level or mutated form of p53 proteins (Sakai & Tsuchida 1992, Lin et al. 2004), and knockdown of FAK expression in OECM-1 cells had no effect on the expression of p53 (Supplementary Fig. 2, see section on supplementary data given at the end of this article). Thus, our data suggest that the regulatory effect of FAK signaling on the growth of OSCC cells was not mediated by p53 degradation.

Our data have shown that E2 and tamoxifen can modulate the growth of OSCC cells; however, it is of interest to know whether E2 or tamoxifen can regulate FAK activation. Previous studies have shown the effects of E2 or tamoxifen on FAK activation in different cell types, but the results are inconsistent. In breast cancer cells, treatment of E2 leads to a decrease in FAK phosphorylation in MCF-7 cells but causes increased phosphorylation in T47D cells (Bartholomew et al. 1998, Sanchez et al. 2010). In ovarian cancer PA-1 and HeyA8 cells, treatment of E2 or propylpyrazole triol, an ERα agonist, enhances FAK phosphorylation (Hung et al. 2014). In endometrial cancer RL95-2 and Ishikawa cells, both E2 and tamoxifen treatment can promote FAK phosphorylation (Tsai et al. 2013). Importantly, FAK expression and phosphorylation in OSCC cells can be inhibited by treatment of tamoxifen but are not affected by E2 stimulation (Ishida et al. 2007). Although the impact of E2 or tamoxifen on FAK activation was not examined in our study, whether FAK is involved in E2- or tamoxifen-regulated growth in OSCC cells deserves further investigation.

The aberrant expression or activation of FAK and ERα proteins are risk factors for promoting the malignant progression of various cancers. In the head and neck region, the increased expression of FAK or ERα is found in malignant tissues, which is significantly correlated with reduced progression-free survival in HNSCC patients (Egloff et al. 2009, de Vicente et al. 2013). Thus, FAK and ERα can serve as potential targets for the treatment of HNSCC. Indeed, tamoxifen combined with chemotherapeutic agents has been applied to treat HNSCC patients in a clinical trial with phase II completed (NCT00002608; http://www.clinicaltrials.gov). In addition, PF-00562271, a selective FAK inhibitor, is undergoing a phase I trial for HNSCC treatment (NCT00666926). Although there is no report to show the correlation between ERα and FAK expression in OSCC tissues, and no evidence to prove the therapeutic efficacy of tamoxifen or FAK inhibitors in OSCC patients, Kim et al. (2007) first showed that tamoxifen has a cytotoxic effect on OSCC cell lines, and the effect can be enhanced by combination with cisplatin. Our data further show that tamoxifen or PF-431396 significantly inhibited the growth of OECM-1 or FAK-overexpressed SCC25 cells. The combination of both inhibitors had a synergistic effect on the growth inhibition compared with each treatment alone, despite the lack of statistical significance (Supplementary Fig. 3).

In summary, we have demonstrated that the expression of ERα in OSCC tumors and cell lines, and the Ser118 phosphorylation and activity of ERα in OSCC cells were enhanced by FAK/AKT signaling, leading to increased cell growth. Thus, our data strongly suggest that FAK and ERα can serve as therapeutic targets for the treatment of OSCC patients.

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**Supplementary data**

This is linked to the online version of the paper at http://dx.doi.org/10.1530/ERC-14-0102.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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