# Expression of Estrogen Receptors in OSCC in Relation to Histopathological Grade

CHRISTIAN DOLL[1], RUZA ARSENIC[2], HERMANN LAGE[2], KORINNA JÖHRENS[2], STEFAN HARTWIG[1], KATJA NELSON[3] and JAN D. RAGUSE[1]

[1]*Department of Oral and Maxillofacial Surgery/Clinical Navigation, Campus Virchow-Clinic, Charité University Hospital, Berlin, Germany;*
[2]*Institute of Pathology, Campus Mitte, Charité University Hospital, Berlin, Germany;*
[3]*Department of Oral and Maxillofacial Surgery, University Hospital Freiburg, Freiburg, Germany*

**Abstract.** *Background/Aim: Estrogen receptor (ER)-mediated pathways are involved in the pathogenesis of several tumors. Preliminary studies have demonstrated a significant effect of ER agonists and antagonists on oral squamous cell carcinoma (OSCC) cell lines. Recent results suggest that ER subtype-specific expression patterns might depend on the grade of differentiation of OSCC. Therefore, the aim of the present study was to evaluate the expression of ERα and ERβ in OSCC and its correlation to histological tumor grade and gender. Materials and Methods: Tumor sections of 25 patients (13 males and 12 females) retrieved from OSCC databases with two different histological gradings (well-differentiated, poorly differentiated) were evaluated. The detection of ERα and ERβ expression in tumor cells and corresponding healthy mucosa adjacent to tumor was performed using immunohistochemistry. Results: Well-differentiated OSCC showed no significant difference between the expression of ERβ in tumor cells and corresponding mucosa. In poorly-differentiated OSCC the expression of ERβ was significantly higher in tumor cells than in corresponding mucosa. In patients without regular alcohol and/or nicotine abuse, there was no significant difference of ERβ expression in OSCC compared to corresponding healthy mucosa in contrast to patients having these risk factors. Expression of ERα was found in one tumor. Conclusion: ERβ is the predominant ER sub-type expressed significantly higher in poorly-differentiated OSCC tumors compared to healthy mucosa adjacent to the tumor. Different expression patterns in relation to histological grade might suggest an influential role of ERβ in tumor (de-) differentiation of OSCC.*

*Correspondence to:* Dr. Christian Doll, Department of Oral and Maxillofacial Surgery/Clinical Navigation, Campus Virchow-Clinic, Charité University Hospital, Augustenburger Platz 1 13353 Berlin Germany. Tel: +49 30450655267 Fax: +49 30450555914 e-mail: Christian.Doll@charite.de

*Key Words:* Oral squamous cell carcinoma, head and neck cancer, estrogen receptor, histopathologic grade, immunohistochemistry.

Oral and pharyngeal cancer is the sixth most common cancer in the world with more than 400,000 new cases reported annually. Squamous cell carcinoma represents the vast majority of all malignant lesions located in the oral cavity (1, 2). Despite improvements in therapeutic and diagnostic techniques in recent years, oral squamous cell carcinoma (OSCC) remains a lethal disease with a five-year survival rate of approximately 50%, urging the need for novel treatment modalities (3).

Estrogens influence various physiological processes by regulating growth and differentiation of cells. The effects are mediated through two different estrogen receptors (ER): estrogen receptor-alpha (ERα) and -beta (ERβ). ER-mediated signals are involved in the development and progression of several hormone-related cancers. Particularly for breast cancer, this causal relation is well-characterized (4). Selective estrogen receptor modulators (SERMs) like tamoxifen (TAM) have been successfully used to target ERs to inhibit cancer growth (5) and have become the gold standard of anti-estrogen treatment in breast cancer (6). Also for malignancies, which are considered not primarily hormone-dependent like colon cancer, glioma or lung cancer, ER-mediated influences in the pathogenesis are described (7-9). Therefore possible anti-tumor effects of estrogen-related therapies are currently under investigation (5, 10, 11).

Few studies have demonstrated a significant effect of ER agonists and antagonists on OSCC (12-18). In cultured OSCC cell lines, TAM seems to be able to induce growth inhibition (13, 15, 16, 18). In combination with cisplatin, TAM leads to an additive apoptotic effect compared to single use of the chemotherapeutic agent (14, 17). These facts indicate that ER might become a potential therapeutic target in OSCC.

Table I. *Clinical and histopathological characteristics of patients with OSCC.*

| No | Gender | Age | Tumor grade | Tumor localization | Risk factor* |
|---|---|---|---|---|---|
| 1 | m | 69.2 | G1 | Tongue | + |
| 2 | m | 55.9 | G1 | Tongue | – |
| 3 | m | 63.4 | G1 | Buccal mucosa | – |
| 4 | m | 62.6 | G1 | Tongue/floor of mouth | – |
| 5 | m | 63.2 | G1 | Tongue/floor of mouth | + |
| 6 | m | 61.2 | G1 | Alveolar process/crest | + |
| 7 | m | 42.5 | G3 | Floor of mouth | + |
| 8 | m | 32.9 | G3 | Tongue | – |
| 9 | m | 61.7 | G3 | Alveolar process/crest | + |
| 10 | m | 54.9 | G3 | Floor of mouth | + |
| 11 | m | 59.7 | G3 | Floor of mouth | + |
| 12 | m | 51.1 | G3 | Floor of mouth/tongue | + |
| 13 | m | 49.5 | G3 | Floor of mouth/tongue | + |
| 14 | f | 86.1 | G1 | Alveolar process/crest, floor of mouth | – |
| 15 | f | 79.8 | G1 | Tongue | – |
| 16 | f | 69 | G1 | Tongue | + |
| 17 | f | 63.3 | G1 | Tongue | – |
| 18 | f | 73 | G1 | Palate | – |
| 19 | f | 36 | G3 | Tongue | – |
| 20 | f | 91.7 | G3 | Alveolar process/crest, vestibule | – |
| 21 | f | 82.6 | G3 | Buccal mucosa | ** |
| 22 | f | 65.3 | G3 | Floor of mouth | + |
| 23 | f | 66 | G3 | Tongue | + |
| 24 | f | 63.8 | G3 | Floor of mouth | + |
| 25 | f | 65.8 | G3 | Floor of mouth/tongue | + |

m, Male; f, female; *anamnestic risk factors (tobacco and/or alcohol abuse). **Risk factors could not be assessed precisely.

There currently exists limited information on the expression pattern of ERα and ERβ in OSCC and still remains controversial. Immunohistochemical analysis of ERs in primary tissue deriving from patients with tongue cancer revealed that ERβ is the predominant sub-type expressed in the majority of all evaluated samples. Only few cases were positive for ERα (13, 19). In contrast, one study including patients with OSCC shows the predominant expression of ERα (12). Immunohistochemical detection of ER in OSCC cell lines revealed only ERβ expression, but not ERα (20). Then again studies using immunoblotting/PCR show both the expression of ERα and ERβ in many of the evaluated OSCC cell lines (12-15, 19, 20).

As described in typical hormone-dependent tumors recent results suggest ER subtype-specific expression patterns might depend on grade of differentiation of OSCC (16). To elucidate this assumption, we evaluated the expression of ERα and ERβ in relation to the different histological grade and gender of the patients.

## Materials and Methods

*Ethics statement*. This study was approved by the Ethics Committee of the Faculty of Medicine Charité, Berlin.

*Patients*. Tumor sections of 25 patients (13 males and 12 females, mean age 62.8 years, range=32.9-91.7) retrieved from OSCCs with two different histologic gradings (G1, well-differentiated: n=11; G3, poorly differentiated: n=14) were included in the study and evaluated for the expression of ERα and ERβ (Table I). Moderate differentiated tumors (G2) were excluded to have clearly distinguishable populations of well- and poorly-differentiated tumors. The histological grade of all tumor samples was extracted from pathological reports and confirmed by experienced pathologists. Patients were classified according to anamnestic risk factors - positive status was defined as regular tobacco and/or alcohol abuse.

*Immunohistochemistry*. Detection of ERα and ERβ expression in tumor cells and corresponding healthy mucosa adjacent to tumor was performed using immunohistochemistry (IHC). Sections of paraffin-embedded tissues were mounted on glass slides and heated at 60˚C. De-paraffinization and rehydration was performed in xylene and a series of ethanol concentrations (ranging from 100%-70%). For antigen retrieval, sections were heated in citrate buffer (pH 6.0) in a pressure cooker followed by cooling at room temperature. Endogenous peroxidase was inhibited with peroxidase blocking solution (Dako, Glostrup, Denmark). To minimize non-specific protein binding sections were incubated with normal goat serum (Invitrogen, Carlsbad, CA, USA). Primary antibodies against either human ERα (Clone SP1; Neomarkers, Fremont, CA, USA) or human ERβ (E3558-40; United States Biological, Swampscott, MA,

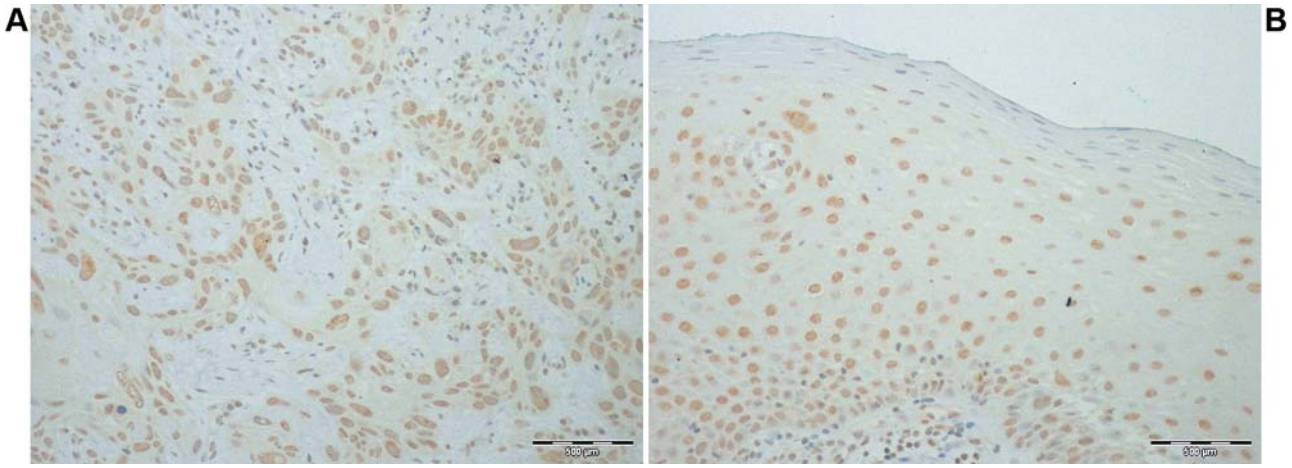


Figure 1. *Immunohistochemical staining of ERβ in OSCC. Brown color represents positive expression of ERβ. Moderate expression of ERβ (IRS 6) in poorly differentiated OSCC (Figure 1A) and weak expression (IRS 3) in corresponding healthy mucosa (Figure 1B) of patient number 23 (Table I).*

USA) were diluted (ERα 1:50, ERβ 1:600) in antibody diluent solution (Invitrogen) and applied for 1 hour on the target tissue. Incubation of diluted secondary antibody (P0448; Dako) was performed for 30 min. The signal was developed with a substrate-chromogen system (K3468; Dako). Sections were counterstained with Mayer's hematoxylin, dehydrated and finally covered with glass. For ERα, sections of breast carcinoma were used for positive and negative control. For ERβ, MCF7 was used for positive control. Isotype control (Invitrogen) was used to differentiate between nonspecific and specific antibody signal. The expression level was quantified by light microscopy (Leica Microsystems, Wetzlar, Germany) using the immunoreactive score (IRS) for estrogen receptor detection (21). Both the confirmation of the tumor grading and the evaluation of the expression levels were performed by experienced pathologists.

*Statistical analysis*. The collected data were analyzed by using SPSS Statistics (IBM Corporation, Armonk, NY, USA). To compare the results of ER expression between tumor cells and healthy mucosa the Wilcoxon signed-rank Test for paired samples was applied. The Mann-Whitney *U*-Test for un-paired samples was used to compare the data between the patients. The results were considered statistically significant at $p<0.05$.

## Results

In order to evaluate the expression of ERα and ERβ in OSCC, specific antibodies were used in tissue sections of OSCC. The expression level of ER for each tumor and corresponding healthy mucosa was quantified using the immunoreactive score (IRS). Expression of ERα was found only in one poorly differentiated tumor, however not in the corresponding healthy mucosa adjacent to the tumor. In contrast, expression of ERβ was observed in all evaluated samples (Figure 1).

The mean overall immunoreactive score in OSCC was 3.64±1.680 (standard deviation) and 2.52±1.503 in corresponding healthy mucosa adjacent to tumor. The expression of ERβ was significantly higher in tumor tissue compared to mucosa ($p<0.001$).

In well-differentiated OSCC, the mean IRS in tumor cells was 3.36±1.690 and 2.36±1.362 in mucosa. There was no significant difference between the expression of ERβ in tumor cells and corresponding mucosa, although a tendency of higher expression was seen ($p=0.059$). The mean IRS in poorly differentiated OSCC was 3.86±1.703 and 2.64±1.646 in mucosa. In contrast to well-differentiated OSCC, the expression of ERβ in poorly differentiated tumor cells was significantly higher than in corresponding mucosa ($p=0.002$). There is neither a significant difference between the expression of ERβ in well- and poorly-differentiated tumors ($p=0.557$), nor between its corresponding healthy mucosa ($p=0.529$).

In males, the mean IRS in tumor cells was 3.62±1.325 and 2.15±0.555 in mucosa and the expression of ERβ was significantly increased in tumor ($p=0.004$) compared to healthy mucosa. In females, the mean IRS in tumor cells was 3.67±2.060 and 2.92±2.065 in mucosa and again the expression of ERβ was significantly higher in tumor cells compared to mucosa ($p=0.028$). There was no different expression of ERβ in tumors ($p=0.743$) between the male and female population and no significant difference comparing the corresponding healthy mucosa between males and females ($p=0.528$).

Sub-group analysis in relation to gender and differentiation grade of the tumor (Table II) revealed that ERβ was not expressed significantly higher ($p=0.125$) in well-differentiated OSCC (3.67±1.506) compared to mucosa

Table II. *Clinical and histopathological characteristics of patient groups.*

| Group | N | Gender | Grade | Mean age | Range of age | Risk factors* |
|---|---|---|---|---|---|---|
| M/G1 | 6 | m | G1 | 62.6 | 55.9-69.2 | 3/6 (50%) |
| M/G3 | 7 | m | G3 | 50.3 | 32.9-61.7 | 6/7 (86%) |
| F/G1 | 5 | f | G1 | 74.2 | 63.3-86.1 | 1/5 (20%) |
| F/G3 | 7 | f | G3 | 67.3 | 36.0-91.7 | 4/6 (67%)** |

m, Male; f, female; *anamnestic risk factors (tobacco and/or alcohol abuse). **Risk factors of one patient could not be assessed precisely.

(2.00±0.632) in the male population. In contrast, a tendency of higher expression was seen in poorly differentiated male OSCCs ($p$=0.064). The mean IRS in tumor cells was 3.57±1.272 and 2.29±0.488 in mucosa. Comparing the well- and poorly differentiated tumors in males, there was no significant difference between the expression of ERβ ($p$=0.834). Also the corresponding healthy mucosa showed no significant difference ($p$=0.641) between these two populations.

In females, well-differentiated OSCC (3.00±2.000) did not show any significant difference ($p$=1.000) in comparison to healthy mucosa (2.80±1.924). However, poorly differentiated female OSCCs (4.14±2.116) revealed a tendency towards significance of higher expression of ERβ in tumor cells ($p$=0.061) compared to mucosa (3.00±2.309). There was no significant difference between the expression of ERβ from tumors of different histologic grade ($p$=0.309), nor from its corresponding healthy mucosa adjacent to the tumors ($p$=0.869).

In patients with positive anamnestic risk factors like alcohol and smoking, the mean IRS in tumor cells was 3.93±1.859 and 2.64±1.646 in corresponding healthy mucosa. The expression of ERβ was significantly higher in tumor cells compared to mucosa ($p$=0.004). In contrast, there was no significant difference ($p$=0.065) found between the expression of ERβ in OSCC (3.30±1.494) and corresponding mucosa (2.40±1.430) in patients without anamnestic risk factors, although a tendency for difference was seen.

## Discussion

Several studies have demonstrated a significant effect of ER agonists and antagonists on OSCC cell lines (12-18). In contrast to typical hormone-related cancers like breast carcinoma, biology and mechanisms of the ER and its ligands in OSCC cells are poorly understood. Recent results suggest ER subtype-specific expression patterns might depend on the grade of differentiation of OSCC (16), as seen in other (typical) hormone-related tumors (4). To elucidate this assumption, the expression of ERα and ERβ in relation to histopathological grade, typical risk factors as well as the gender of patients in OSCC and the corresponding healthy mucosa, was evaluated.

The present study showed that ERβ is the predominant subtype in all OSCC primary tissues of different anatomical sites as well as in healthy mucosa adjacent to tumors, consistent with results from previous studies using immunohistochemistry in SCC of the tongue and healthy oral mucosa (13, 19, 22). However the results obtained in the study are in conflict to the observations made by Egloff *et al.* in 2009 (12): immunohistochemical analysis of tissue microarrays from patients with head and neck squamous cell carcinoma (including 23 squamous cell carcinomas of the oral cavity of a total of 56 tumors) revealed that 95% of the evaluated tumors were positive for (nuclear) ERα and 44% for (nuclear) ERβ. Detailed information about these OSCCs including the grading and the exact locations of the tumors within the oral cavity are not mentioned in this study. Therefore, the comparability to the present study appears difficult.

As shown by Nelson *et al.* in an *in vitro* study (16), different progressive stages of SCC of the floor of mouth revealed different ER subtype-specific expression patterns. Apart from the fact that these cell lines derived from a patient with recurrent OSCC, additional factors like undergone radiation and/or chemotherapy treatment might lead to different ER expression.

To our knowledge, no difference in expression pattern in OSCC compared to healthy mucosa in relation of histopathologic grade has been described to date. As shown in this study, well-differentiated OSCC revealed no significant difference between the expression of ERβ in tumor cells and corresponding mucosa. However, in poorly differentiated OSCC the expression of ERβ in tumor cells was significantly higher than in corresponding mucosa. This demonstrates an association between ERß expression level and the grade of differentiation in OSCC. Thus, ERβ might have an influential role in tumor dedifferentiation.

Similar results for ER expression were observed in one study evaluating the ER expression in eosophageal carcinoma - ERβ is over-expressed only in poorly-differentiated squamous cell carcinoma compared to normal esophageal mucosa. ERα expression was not found in

eosophageal tumor cells (23), suggesting that estrogen might stimulate the growth of esophageal carcinoma through ERβ. Other studies show both the (immunohistochemical) expression of ERα and ERβ in esophageal squamous cell carcinoma cells, however ERβ was found to be associated with unfavorable prognosis (24, 25).

Also in lung cancer, ERβ represents the predominant ER sub-type. Several studies show contradictory results concerning the prognostic value of ERβ expression which might be (among other factors) due to different isoforms of the receptor (26). Siegfried *et al*. (26) postulate that local production of estrogens by macrophages/inflammatory cells through chronic infection in response to carcinogens, might be an important source of the hormone, independent of reproductive tissues.

In contrast to these maligancies, ERβ expression appears to be lost during the carcinogenic process in colon cancer. An ER-dependent prevention for certain patient populations might be possible at an early stage (26, 27).

As mentioned, ERα and ERβ are widely distributed in different tissues and the expression can be at similar levels or the ratio of ERα/ERβ can be shifted towards one sub-type. There are several isoforms for each ER (28). The change of ER expression pattern plays an important role in the development and progression of cancer (4). In breast cancer for example, ERα has been implicated in cancer progression whereas the expression of ERβ is lost during progression of the tumor, apparently by promoter methylation (5, 29).

The exact role of ERβ in cancer is only poorly understood. Different isoforms of the receptor seem to have different biological roles (4). For instance, down-regulation/loss of wildtype ERβ (ERβ1), mainly localized in the nucleus, is associated with poor differentiation of several tumors. However expression of ERβ2 and ERβ5, splice variants of ERβ1 and localized in the cytoplasm and the nucleus of cells, are associated with poor outcome in certain tumors (4). With regard to the OSCC it might be possible, that certain isoforms of ERβ be up-regulated during the process of de-differentiation. This might lead to the conclusion that (especially) patients with poorly differentiated tumors might profit from an anti-estrogen therapeutic approach. The important role of ERβ as a therapeutic target is highlighted by the observation made by Ishida *et al*. in 2007 (13): the inhibitory effect on the proliferation of SCC cell lines following knockdown of ERβ by small-interfering RNA seems to be more effective than the knockdown of ERα.

Typical risk factors of OSCC are tobacco and alcohol abuse (1, 2). Interestingly, the results of the present study show no significant difference of ERβ expression in OSCC compared to corresponding healthy mucosa in patients without these typical risk factors in contrast to patients with alcohol and/or tobacco abuse. It remains to be elucidated if this observation is a causal connection.

The results of the present study clearly show that there is a difference in the expression pattern of ERβ with regard to histopathological grade. Further studies are necessary to evaluate the role of ERα expression as this could not be elucidated within this study due to the limited number of patients. The gender and differentiation grade related sub-group analysis revealed no significant difference for the female and male population, although tendencies towards significance were seen. Therefore, evaluation of more sections of OSCC for ERβ might lead to further information.

In summary, the present study revealed that ERβ is the predominant ER-subtype expressed significantly higher in OSCC of different anatomical sites compared to healthy mucosa adjacent to the tumor. This investigation also showed different expression patterns for ERβ in OSCC in relation to histopathological grade suggesting an influential role in tumor de-differentiation. To receive a better understanding over the biology of OSCC further studies should focus on identifying ERβ isoforms in primary tumors.

## Conflicts of Interest

The Authors declare there exist no conflicts of interest.

## Acknowledgements

The Authors thank Gerda Siebert for her outstanding statistical assistance.

## References

1 Tsantoulis PK, Kastrinakis NG, Tourvas AD, Laskaris G and Gorgoulis VG: Advances in the biology of oral cancer. Oral Oncol *43*: 523-534, 2007.
2 Warnakulasuriya S: Global epidemiology of oral and oropharyngeal cancer. Oral Oncol *45*: 309-316, 2009.
3 Scully C and Bagan J: Oral squamous cell carcinoma overview. Oral Oncol *45*: 301-308, 2009.
4 Thomas C and Gustafsson JA: The different roles of ER subtypes in cancer biology and therapy. Nat Rev Cancer *11*: 597-608, 2011.
5 Shanle EK and Xu W: Selectively targeting estrogen receptors for cancer treatment. Adv Drug Deliv Rev *62*: 1265-1276, 2010.
6 Huang B, Warner M and Gustafsson JA: Estrogen receptors in breast carcinogenesis and endocrine therapy. Mol Cell Endocrinol, 2014.
7 Li W, Winters A, Poteet E, Ryou MG, Lin S, Hao S, Wu Z, Yuan F, Hatanpaa KJ, Simpkins JW and Yang SH: Involvement of estrogen receptor beta5 in the progression of glioma. Brain Res *1503*: 97-107, 2013.
8 Sato R, Suzuki T, Katayose Y, Miura K, Shiiba K, Tateno H, Miki Y, Akahira J, Kamogawa Y, Nagasaki S, Yamamoto K, Ii T, Egawa S, Evans DB, Unno M and Sasano H: Steroid sulfatase and estrogen sulfotransferase in colon carcinoma: regulators of intratumoral estrogen concentrations and potent prognostic factors. Cancer Res *69*: 914-922, 2009.
9 Nemenoff RA and Winn RA: Role of nuclear receptors in lung tumourigenesis. Eur J Cancer *41*: 2561-2568, 2005.

10 Miki Y, Abe K, Suzuki S, Suzuki T and Sasano H: Suppression of estrogen actions in human lung cancer. Mol Cell Endocrinol *340*: 168-174, 2011.
11 Sareddy GR, Nair BC, Gonugunta VK, Zhang QG, Brenner A, Brann DW, Tekmal RR and Vadlamudi RK: Therapeutic significance of estrogen receptor beta agonists in gliomas. Mol Cancer Ther *11*: 1174-1182, 2012.
12 Egloff AM, Rothstein ME, Seethala R, Siegfried JM, Grandis JR and Stabile LP: Cross-talk between estrogen receptor and epidermal growth factor receptor in head and neck squamous cell carcinoma. Clin Cancer Res *15*: 6529-6540, 2009.
13 Ishida H, Wada K, Masuda T, Okura M, Kohama K, Sano Y, Nakajima A, Kogo M and Kamisaki Y: Critical role of estrogen receptor on anoikis and invasion of squamous cell carcinoma. Cancer Sci *98*: 636-643, 2007.
14 Kim MJ, Lee JH, Kim YK, Myoung H and Yun PY: The role of tamoxifen in combination with cisplatin on oral squamous cell carcinoma cell lines. Cancer Lett *245*: 284-292, 2007.
15 Nelson K, Helmstaedter V and Lage H: The influence of tamoxifen on growth behavior and cell-cell adhesion in OSCC *in vitro*. Oral Oncol *43*: 720-727, 2007.
16 Nelson K, Helmstaedter V, Moreau C and Lage H: Estradiol, tamoxifen and ICI 182,780 alter alpha3 and beta1 integrin expression and laminin-1 adhesion in oral squamous cell carcinoma cell cultures. Oral Oncol *44*: 94-99, 2008.
17 Tavassoli M, Soltaninia J, Rudnicka J, Mashanyare D, Johnson N and Gaken J: Tamoxifen inhibits the growth of head and neck cancer cells and sensitizes these cells to cisplatin induced-apoptosis: role of TGF-beta1. Carcinogenesis *23*: 1569-1575, 2002.
18 Ku TK and Crowe DL: Coactivator-mediated estrogen response in human squamous cell carcinoma lines. J Endocrinol *193*: 147-155, 2007.
19 Marocchio LS, Giudice F, Correa L, Pinto Junior Ddos S and de Sousa SO: Oestrogens and androgen receptors in oral squamous cell carcinoma. Acta Odontol Scand *71*: 1513-1519, 2013.
20 Shatalova EG, Klein-Szanto AJ, Devarajan K, Cukierman E and Clapper ML: Estrogen and cytochrome P450 1B1 contribute to both early- and late-stage head and neck carcinogenesis. Cancer Prev Res (Phila) *4*: 107-115, 2011.
21 Remmele W and Stegner HE: Recommendation for uniform definition of an immunoreactive score (IRS) for immuno-histochemical estrogen receptor detection (ER-ICA) in breast cancer tissue. Pathologe *8*: 138-140, 1987.
22 Valimaa H, Savolainen S, Soukka T, Silvoniemi P, Makela S, Kujari H, Gustafsson JA and Laine M: Estrogen receptor-beta is the predominant estrogen receptor subtype in human oral epithelium and salivary glands. J Endocrinol *180*: 55-62, 2004.
23 Kalayarasan R, Ananthakrishnan N, Kate V and Basu D: Estrogen and progesterone receptors in esophageal carcinoma. Dis Esophagus *21*: 298-303, 2008.
24 Dong J, Jiang SW, Niu Y, Chen L, Liu S, Ma T, Chen X, Xu L, Su Z and Chen H: Expression of estrogen receptor alpha and beta in esophageal squamous cell carcinoma. Oncol Rep *30*: 2771-2776, 2013.
25 Zuguchi M, Miki Y, Onodera Y, Fujishima F, Takeyama D, Okamoto H, Miyata G, Sato A, Satomi S and Sasano H: Estrogen receptor alpha and beta in esophageal squamous cell carcinoma. Cancer Sci *103*: 1348-1355, 2012.
26 Siegfried JM and Stabile LP: Estrongenic steroid hormones in lung cancer. Semin Oncol *41*: 5-16, 2014.
27 Barzi A, Lenz AM, Labonte MJ and Lenz HJ: Molecular pathways: Estrogen pathway in colorectal cancer. Clin Cancer Res *19*: 5842-5848, 2013.
28 Dahlman-Wright K, Cavailles V, Fuqua SA, Jordan VC, Katzenellenbogen JA, Korach KS, Maggi A, Muramatsu M, Parker MG and Gustafsson JA: International Union of Pharmacology. LXIV. Estrogen receptors. Pharmacol Rev *58*: 773-781, 2006.
29 Zhao C, Dahlman-Wright K and Gustafsson JA: Estrogen receptor beta: an overview and update. Nucl Recept Signal *6*: e003, 2008.

*Received August 13, 2015*
*Revised September 19, 2015*
*Accepted September 23, 2015*