CLINICAL REVIEW

David W. Eisele, MD, *Section Editor*

BASIC EVIDENCE OF MOLECULAR TARGETED THERAPY FOR ORAL CANCER AND SALIVARY GLAND CANCER

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Abstract: *Background.* Recently, attention has been focused on molecular targeted cancer therapy in various tumors. Although there is no single consistent molecular target specific for oral squamous cell carcinoma (OSCC) and salivary gland cancer (SGC), there are a number of promising candidate proteins. The aim of this review is to introduce the basic evidences to support the molecular targeting for OSCC and SGC.

Methods. We focused on the 4 molecules, epidermal growth factor receptor (EGFR), cyclooxygenase-2 (COX-2), peroxisome proliferator-activated receptor γ (PPARγ), and progesterone receptor, that are, respectively, associated with the proliferation and the differentiation of OSCC and SGC.

Results. Gefitinib ("Iressa," ZD1839), a small molecule EGFR tyrosine kinase inhibitor, can inhibit the proliferation of OSCC cell lines in a dose- and time-dependent manner and lead to cell cycle arrest with accumulation of cells in the G1 phase, and a decrease of cells in S phase. The agent suppressed tumor metastasis in the animal model. Furthermore, a cooperative antiproliferative effect was obtained when cancer cells were treated with radiation followed by gefitinib. While radiation alone did not significantly affect p38 mitogen-activated protein kinase and MAP kinase kinase (MEK)1/2 autophosphorylation, the combination of gefitinib and radiation completely inhibited the downstream signaling of EGFR. Gefitinib enhanced tumor radioresponsiveness by multiple mechanisms, including the growth inhibition and effects on DNA repair after exposure to radiation. Next, the level of COX-2 expression correlated inversely with increased tumor radiation sensitivity. Treatment with celecoxib, a COX-2 selective inhibitor, enhanced the radioresponsiveness of HSC-2 cells, which constitutively expressed COX-2. Another promising molecular target is the PPARγ, which is a member of the nuclear receptor superfamily of ligand-activated transcription factors. Recent studies have demonstrated that PPARγ ligands induce cellular differentiation and inhibit cell growth in carcinomas of various types. These data suggest that synthetic PPARγ ligands may be useful for molecular targeting of oral cancer. Finally, the possibility of using molecular targeted therapy directed at hormone receptors in the treatment of advanced SGCs was described.

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Conclusion. The basic data strongly suggested the possibility of tumor suppression by targeting these molecules. Studies of different targeted agents alone or with more conventional treatment modalities are needed to fully determine what role the targeted therapy will play in the management of patients with OSCC and SGC. ©2008 Wiley Periodicals, Inc. *Head Neck* **30:** 800–809, 2008

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Despite multidisciplinary treatment with surgery, chemotherapy, and radiation, the overall survival rate has not improved significantly in patients with oral cancer. Novel therapeutic alternatives to standard therapy need to be established to improve the prognosis for patients with advanced oral cancer. Molecular targeted therapy is a treatment modality that targets molecules and proteins that are selectively expressed by cancer cells. These include growth factors and their receptors, signal transduction molecules, oncogenes, hormones, apoptosis-related molecules, angiogenesis-related factors, as well as inhibitors of cell motility, invasion, and proteolysis. Molecular targeted therapy has several potential advantages compared with conventional anticancer agents as summarized in Table 1. Some of the molecular targeted agents that are currently available are listed in Table 2. Here we review molecular targeted therapy and offer several examples of promising molecular targets in oral squamous cell carcinoma (OSCC) and salivary gland cancer (SGC), including the epidermal growth factor receptor (EGFR), cyclooxygenase-2 (COX-2), peroxisome proliferator-activated receptor γ (PPARγ), and the progesterone receptor (PR).

EPIDERMAL GROWTH FACTOR RECEPTOR

Targeting the EGFR Inhibits OSCC Proliferation. The EGFR and the cell cycle have been independently evaluated as targets for therapy, and there is evidence supporting a role for the inhibition of cell cycling through blockade of EGFR-mediated signals via small-molecule tyrosine kinase inhibitors (TKIs) of the cytosolic kinase domain or antibody targeting of the extracellular portions of the EGFR.[1–4] Gefitinib ("Iressa," ZD1839), a small-molecule EGFR TKI, can inhibit the proliferation of OSCC cell lines in a dose- and time-dependent manner and lead to cell cycle arrest with accumulation of cells in the G1 phase, and a decrease of cells in S phase as determined by flow cytometric analysis.[5]

C225, an anti-EGFR antibody, induces G1 arrest in human OSCC cell lines, via an upregulation of $p27^{KIP1}$ cyclin-dependent kinase inhibitor.[6] Gefitinib has also been shown to induce G1 arrest via levels of $p27^{KIP1}$ through modulation of ubiquitin-dependent protein degradation.[7,8] Cell growth is inhibited by an increase of the cell cycle inhibitor $p27^{KIP1}$ and a decrease of its ubiquitin ligase subunit.[5]

Blocking the EGFR can lead to inhibition of regional lymph node metastasis in OSCC, and the effect of gefitinib treatment on OSCC cells has also been examined in an orthotopic nude mouse model. Using an OSCC cell line with a high level of green fluorescent protein, (GFP)-SAS-L1, lymph node metastasis could be readily detected visually after orthotopic injection in the tongues of nude mice.[9] Using this model, treatment with gefitinib reduced the identification from all of 12 mice with metastases in the control group to 6 of 13 of gefitinib-treated animals with metastases (46.2%).[10]

Cell adhesion to the extracellular matrix (ECM) is a step involved in invasion and metastasis. The ability of stable transfectants to adhere to the ECM proteins has been investigated. Cells treated with gefitinib reduced attachment to fibronectin but not laminin, and it was also suggested

Table 1. Comparison of conventional agent and molecular targeting therapy.

	Conventional agent	Molecular targeting therapy
Target	DNA, protein	Specific molecule of cancer cell
Acting mechanism	Cytotoxic	Each/both of cytotoxic and cytostatistic
Optimal dose	Close to MTD	Not necessarily compatible to MTD
Endpoint of therapy	CR or PR of the tumor	CR or PR of the tumor, improvement of QOL
Accumulation	High	Little
Profile of toxicity	Characteristic to the structure of the agent	Characteristic to the target molecule
Bone marrow suppression	Frequent	Rare (depends on the target molecule)
Nausea, vomiting	Frequent	Rare (depends on the target molecule)

Abbreviations: MTD, maximum-tolerated dose; CR, complete remission; PR, partial remission; QOL, quality of life.

Table 2. Agents for molecular targeting therapy.

Generic name	Product name (manufacturer)	Molecular target	Indication
Imatinib	Glivec (Novartis Pharmaceuticals)	Bcr-Abl/TK	CML
Gefitinib	Iressa (AstraZeneca)	EGFR/TK	NSCLC
Erlotinib	Tarceva (Genentech, OSI Pharmaceuticals)	EGFR/TK	NSCLC, pancreatic cancer
Cetuximab	Erbitux (Bristol-Myers Squibb, ImClone Systems)	EGFR	Colon cancer
Trastuzumab	Herceptin (Genentech)	HER2	Breast cancer
Bevacizumab	Avastin (Genentech)	VEGF	Colon cancer
Rituximab	Rituxan (Genentech, Biogen Idec)	CD20	NHL
Gemtuzumab	Mylotarg (Wyeth Ayerst)	CD33	AML

Abbreviations: TK, tyrosine kinase; CML, chronic myelogenous leukemia; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; VEGF, vascular endothelial growth factor; CD, cluster of differentiation; NHL, non-Hodgkin's lymphoma; AML, acute myelogenous leukemia.

that the reduction of cell adhesion in OSCC is secondary to downregulation of integrin α3, αv, β1, β4, β5, β6 and focal adhesion kinase (FAK) phosphorylation by EGFR blockade therapy with gefitinib.[10] Previous reports described that some integrins, such as αvβ6, α5β1, αvβ1, contribute to SCC migration.[11,12] FAK is associated with integrins within focal adhesions, and integrin activation by ECM ligands is associated with increased tyrosine phosphorylation and kinase activity of FAK.[13,14]

In summary, several studies confirm that systemic administration of EGFR targeting inhibits metastasis of human OSCC implanted in the tongues of athymic nude mice. There are data to support that the selective downregulation of integrin expression and FAK phosphorylation by the tumor cells after gefitinib therapy leads to the reduction of cell adhesion to the ECM, thus contributing to the reduction in spontaneous metastasis from these highly metastatic tumors.[10]

Enhancement of Tumor Radiosensitivity by Combined Treatment with EGFR Targeted Agents. Recent studies have shown that molecular blockade of EGFR with either an EGFR monoclonal antibody or an EGFR TKI enhances the radiosensitivity of human squamous cell carcinomas.[15–17] There are extensive data showing that cetuximab (Erbitx, mC225) can sensitize OSCC to external beam radiation and this can lead to decreased clonogenic survival of tumor cells in vitro assays, and decreased tumor growth in vivo models. These observations have led to a series of clinical investigations that culminated in a phase III clinical trial for patients with locoregionally advanced OSCC, who were randomized to treatment with radiotherapy alone versus radiotherapy plus cetuximab. This landmark clinical trial showed statistically significant benefits in locoregional control and survival for patients who received the investigational agent, and subsequently led to the Food and Drug Administration approval of cetuximab for the treatment of locoregionally advanced OSCC.

The combination of radiotherapy given along with small-molecule TKIs has also been investigated. It was found that when the EGFR-TKI, gefitinib ("Iressa," ZD1839), was given in combination with radiation in vitro, a cooperative antiproliferative effect was obtained when cancer cells were treated with radiation followed by gefitinib. Cells treated with a combination of radiation and gefitinib were arrested in G1 and G2-M phases with a decrease in the S phase population.[18] While radiation alone did not significantly affect p38 mitogen-activated protein kinase and MAP kinase kinase (MEK)1/2 autophosphorylation, the combination of gefitinib and radiation completely inhibited the downstream signaling of EGFR. Results from DNA damage repair analysis in cultured OSCC cells demonstrated that gefitinib had a strong inhibitory effect on the DNA-dependent protein kinase complex pathways after radiation. Tumor xenograft studies demonstrated that the combination of gefitinib and radiation caused growth inhibition and tumor regression of well-established OSCC tumors in athymic mice (Figure 1). Immunohistochemical analysis of OSCC xenografts revealed that gefitinib caused a striking decrease in tumor cell proliferation when combined with radiotherapy. Overall, the investigators concluded that gefitinib enhances tumor radioresponsiveness by multiple mechanisms that involve antiproliferative growth inhibition and effects on DNA repair after exposure to radiation (Figure 2).[18]

CYCLOOXYGENASE-2

COX is a key enzyme in the conversion of arachidonic acid to prostaglandins (PGs). COX-1 is con-

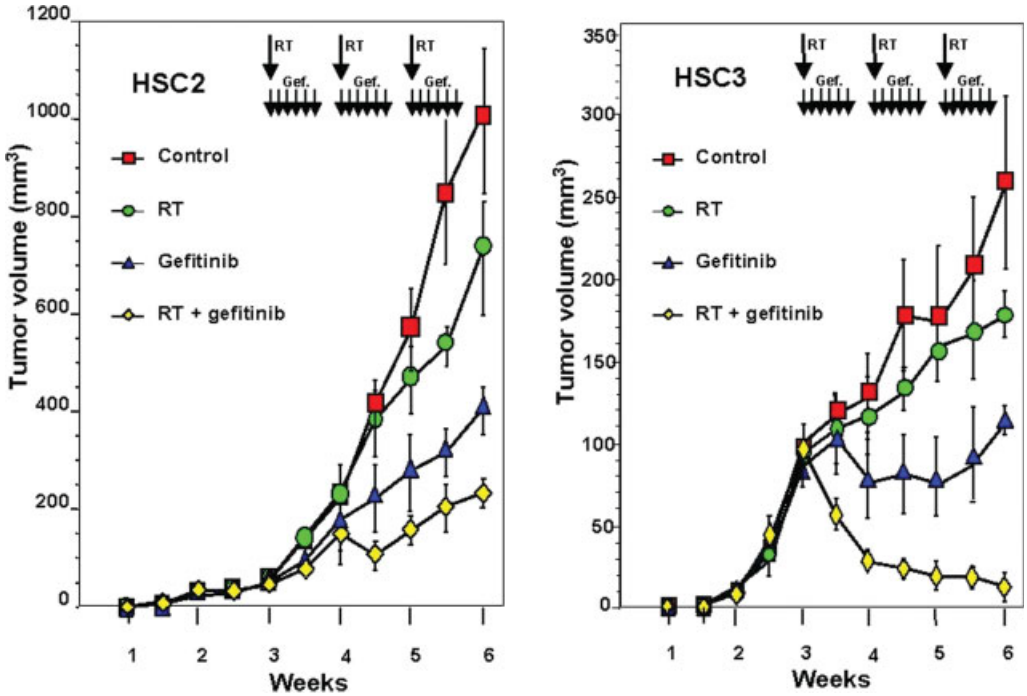

FIGURE 1. Tumor suppression by radiotherapy (RT) and gefitinib. Oral squamous cell carcinoma (OSCC) cells (HSC2 and HSC3) were treated with radiation (4 Gy), gefitinib (Gef; 1.0 μM), or a combination of the two treatments. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

stitutively expressed for the maintenance of homeostatic function in most of the cells, whereas COX-2 is induced during pathologic conditions such as inflammation and cancers. COX-2 levels have been found to be elevated in head and neck, esophageal, gastric, pancreatic, hepatocellular, colorectal, breast, and lung cancers, relative to the normal epithelia from which these tumors develop. COX-2 activation has been found to be an early event during carcinogenesis, and its

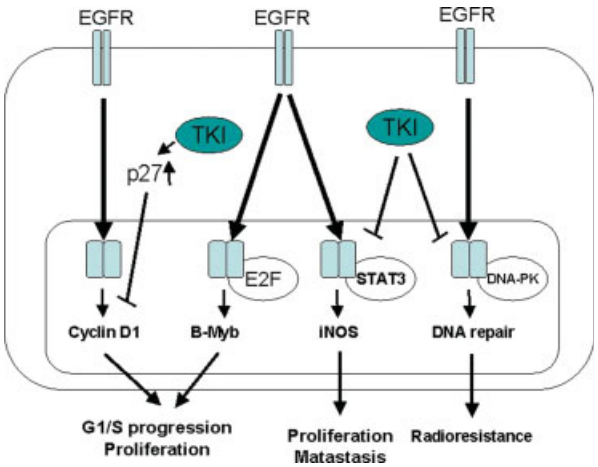

FIGURE 2. Epidermal growth factor receptor (EGFR) pathway. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

increased expression has been associated with the development of genomic instability. COX-2 plays an important role in tumor growth and spread of tumors by affecting mitogenesis, cellular adhesion, immune surveillance, apoptosis, and angiogenesis. In addition, inhibition of COX-2 increases radiation sensitivity without influencing normal tissue response to radiation. Terakado et al[19] have shown that the level of the COX-2 expression correlated inversely with increased tumor radiation sensitivity. Furthermore, treatment with celecoxib, a COX-2 selective inhibitor, enhanced the radioresponsiveness of HSC-2 cells, which constitutively expressed COX-2. The authors concluded that COX-2 expression levels correlate with radiation tolerance and COX-2 selective inhibition may be a potent enhancer of radiation therapy in OSCC.

There is an increasing amount of evidence revealing that a combined administration of nonselective COX1/COX-2 inhibitor and EGFR inhibitor prevents tumor progression in preclinical models. The molecular pathway of signal crosstalk between EGFR and COX-2 is becoming clearer. PGE2 transactivates and phosphorylates EGFR and triggers the extracellular signal-regulated kinase (ERK) 2-mitogenic signaling pathway.[20] PG E2 also activates the phosphatidyl inositol 3-kinase/Akt pathway and causes migra-

tion, invasion, and proliferation of cancer cells.[21] Tortora et al[22] reported that a combination of the COX-2 inhibitor, SC-236, ZD1839, and DNA/RNA-mixed backbone antisense oligonucleotide targeted against the RIα regulatory subunit of protein kinase A, showed prolonged tumor suppression of transplanted human colon cancer in nude mice. This method seems to be a promising treatment modality in the future, but the efficacy of combined molecular targeting therapy should be confirmed in the clinical setting.

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ

PPARγ is a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors, functioning as a regulator of lipid metabolism and adipocyte differentiation[23,24] and exists in 2 isoforms produced by the alternative splicing at the 5′ end of the gene. Compared with PPARγ1, PPARγ2 contains an N-terminal extension of 28 amino acids. Many tissues express PPARγ1 at a low level, but in adipose tissue PPARγ2 is expressed at unusually high levels.[25] PPARγ forms heterodimers with the retinoid X receptor[26] and can be activated by ligands. Synthetic PPARγ ligands are used clinically as orally active antidiabetic agents, for example, thiazolidinediones (TZDs) such as troglitazone (TRO), pioglitazone (PIO), and ciglitazone[27] or nonsteroidal anti-inflammatory drugs such as indomethacin and ibuprofen.[28] On the other hand, natural ligands are 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2),[29,30] polyunsaturated fatty acids and fish oil components, docosahexaenoic acid, and eicosapentaenoic acid.[31]

Numerous studies indicate that PPARγ ligands can induce the differentiation of human liposarcoma[32] and breast cancer cells,[33] and they can inhibit the cell growth of various carcinomas arising from the breast,[34] prostate,[35] lung,[36] colon,[37] stomach,[38] bladder,[39] and pancreas.[40] In a human colon cancer, mutations found in the PPARγ gene were associated with its loss of function.[41] PPARγ ligands can also significantly suppress the growth of human bladder carcinoma cells, and the loss of PPARγ expression is associated with the progression of this cancer.[42] These observations suggest that PPARγ may function as a tumor suppressor gene, and it is therefore a potential molecular target for cancer treatment.

In salivary gland tumors, the expression of PPARγ was demonstrated using reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemistry. PPARγ protein was detected in 3 of 5 pleomorphic adenomas, all of 7 adenoid cystic carcinomas, and in the 1 carcinoma in pleomorphic adenoma but not in 5 normal salivary gland tissues. Furthermore, the function of PPARγ in human SGC cells was investigated using 2 classes of ligands for this protein: (1) the naturally occurring ligand 15d-PGJ_2, and (2) the synthetic TZD derivatives, TRO and PIO. Both the synthetic ligands induced the transcriptional activity of intrinsic PPARγ, but the natural ligand, 15d-PGJ_2, could not activate PPARγ in human SGC cells.[43] Mutations within the ligand-binding domain of PPARγ can affect ligand-dependent transcriptional activity.[41,44] In a human colon cancer, 2 missense mutations have been detected in the ligand-binding domain of PPARγ, which impaired the function of the protein.[41] One of these mutations maintained the normal response to synthetic ligands, but transcription decreased on exposure to natural ligands. However, no mutations were detected in the total coding region of the PPARγ1 gene in human SGC cells.[43] The lack of response to 15d-PGJ_2 by human SGC cells may be associated with their expression profile of coactivators for PPARγ. Synthetic ligands can activate PPARγ regardless of coactivators, whereas natural ligands require some coactivators to achieve PPARγ activation.[45]

The synthetic ligands, TRO and PIO, both inhibit the growth of human SGC cells. Furthermore, overexpression of PPARγ1 or PPARγ2 suppressed significantly the growth of cancer cells regardless of the presence of synthetic ligands. Treatment of PPARγ1 or PPARγ2 transfectants with synthetic ligands had an additive inhibitory effect on growth.[43] Therefore, the antiproliferative effects of synthetic PPARγ ligands in human SGC cells were mediated at least in part by PPARγ.

The molecular mechanisms underlying the inhibitory effect on growth of PPARγ and its synthetic ligands are largely unknown. Several reports have indicated that PPARγ and its ligands can induce the expression of p21, p16, or p27 cyclin-dependent kinase inhibitor and thus inhibit cell growth.[39,40] In human SGC cells, synthetic PPARγ ligands arrested the cell cycle at G1 phase and induced the downregulation of S-phase kinase-associated protein (Skp) 2 protein and accumulation of $p27^{kip1}$ protein (Figure 3). Because SGC is generally resistant to chemotherapy and radiotherapy, the synthetic PPARγ ligands may be a useful molecular targeting drug for treatment of this cancer.

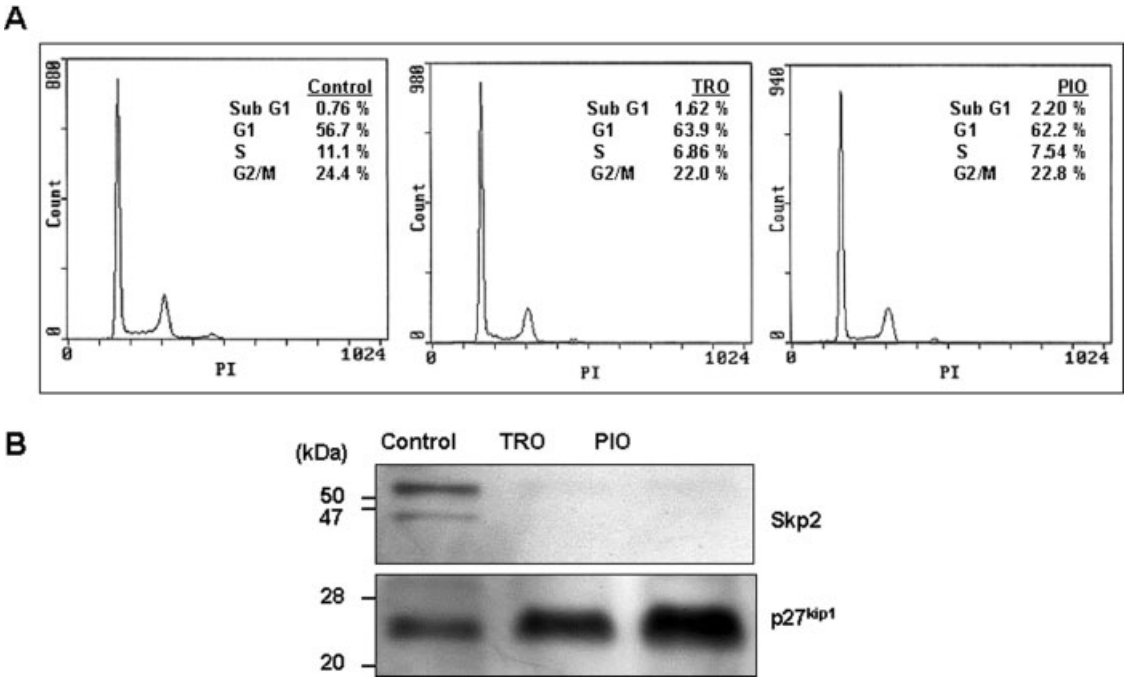

FIGURE 3. Cell cycle analysis for human salivary gland cancer (SGC) cells treated with thiazolidinediones (TZDs). (**A**) Cells were treated with troglitazone (TRO) or pioglitazone (PIO) at the concentration of 20 m*M* for 48 hours, and then cell cycle was analyzed using flow cytometry. Both induced G1 arrest. Expression of S-phase kinase-associated protein (Skp) 2 and p27kip1 proteins in human SGC cells treated with TZDs. (**B**) After treatment of TRO or PIO (20 m*M*) for 48 hours, the expression of Skp2 and p27kip1 proteins was examined using western blotting. Both reduced the expression of Skp2 protein and induced the accumulation of p27kip1.

In OSCC, PPARγ mRNA was detected in 17 of 28 cases using RT-PCR.[46] The function of PPARγ in human OSCC cells was also investigated using the synthetic TZD derivatives, TRO and PIO. Although the synthetic ligands, particularly TRO, significantly suppressed the growth of OSCC cells, they did not induce transcriptional activity of PPARγ even in human OSCC cells expressing PPARγ mRNA.[46] Loss of PPARγ expression and function may be associated with OSCC progression. It is possible that mutations in the PPARγ gene may affect ligand-dependent transcriptional activity.[41,44] However, no mutations were detected in the total coding region of the PPARγ1 gene in human OSCC cells.[46] According to a recent study, the antiproliferative effect of the TZDs is independent of PPARγ and mediated instead by the inhibition of translation initiation.[47] Furthermore, Nikitakis et al[48] have reported that neither rosiglitazone nor ciglitazone inhibits cell growth in other human OSCC cells. These results suggest that the growth-inhibiting action of synthetic ligands may depend on some other mechanism without affecting PPARγ activation. Xin et al[49] reported that vascular endothelial cells express PPARγ and its ligands are potent inhibitors of angiogenesis both in vitro and in vivo.[49] Based on these observations, we suggest that the synthetic PPARγ ligands, especially TRO, may be useful agents for the treatment of OSCC regardless of PPARγ expression (Figure 4).

HORMONE THERAPY

In the reproductive organs, hormonal stimulation is critically involved in carcinogenesis. For

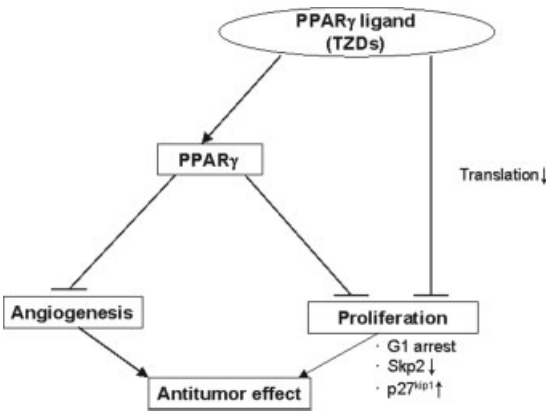

FIGURE 4. Antitumor effect of synthetic peroxisome proliferator-activated receptor (PPAR) γ ligands (thiazolidinediones, TZDs). PPARγ is expressed in many cancers including salivary gland and oral epithelium. Synthetic PPARγ ligands such as TZDs are generally antiproliferative in these cancers. TZDs can suppress tumor growth via PPARγ-dependent or -independent pathways.

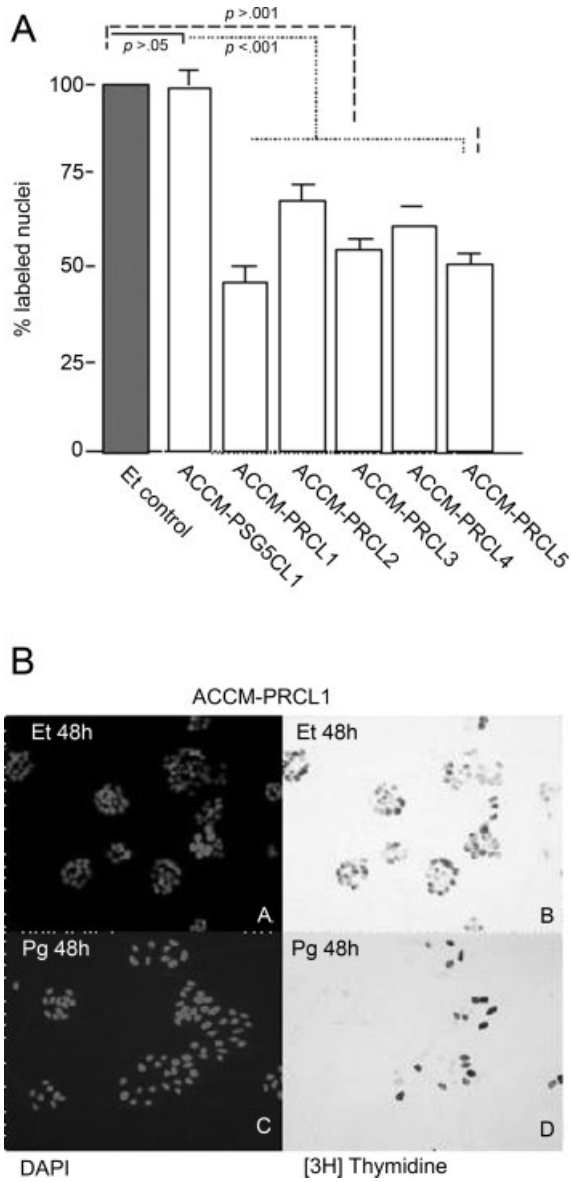

FIGURE 5. The effect of progesterone (Pg) on cell proliferation in progesterone receptor (PR) transfectants. (**A**) The cells were cultured in 5% serum with either control solvent (Et) or Pg for 48 hours. [3H]Thymidine was added during the last 16 hours. The percent of labeled nuclei of all the cells treated with the control solvent was normalized as 100%. ACCM-pSG5CL1 cells are ACCM cells, which are originated from human adenoid cystic carcinoma, transfected with pSG5 empty vector. ACCM-PRCL1, 2, 3, 4, and 5 cells are cell populations which express PR. (**B**) The number of labeled nuclei is significantly decreased in the Pg-treated PR-transfected clones, as seen in panel D.

instance, the sex steroid hormones, estrogen and progesterone, play an important role in normal mammary gland development, and it is believed that breast cancer progression is influenced by these hormones and their receptors.[50–53] Human SGC has been reported to have some similarity with mammary gland tumor with regard to its histology and steroid hormone receptor status.[54] Moreover, some studies have shown the possibility of the involvement of steroid hormone receptor in SGC progression.[55–58] On the other hand, it is considered that these hormones basically do not have a role in OSCC, although some studies suggest the possibility of a response to steroid hormones.[59] Therefore, in this section, we focus on the possibility of a hormonal therapy for SGC.

First, the progesterone–progesterone receptor (Pg-PR) system plays an important role in various gynecologic malignant tumors.[60–62] In patients with breast cancer, the level of these steroid hormone receptors is a strong prognostic factor and has been used in clinical management as an indicator of endocrine responsiveness.[52,53] However, depending on the tissue type, progesterone is classified as a hormone involved in proliferation or differentiation.[63,64] It was already reported that in human aggressive breast cancer cells without PR, reintroduction of PR after progesterone treatment is sufficient to reduce the malignant phenotypes.[65] Hence, it is hypothesized that PR also plays an important role in SGC. This is because some investigators have reported that SGC often expressed PR.[54,56–58] Following progesterone treatment, the PR transfected SGC cells showed drastic morphological change; the transfectants appeared more flattened and spread out when compared with the control cells.[66] Furthermore, a significant reduction in the proliferative activity of the transfectants was also observed after Pg treatment (Figure 5A). The percentage of labeled nuclei reduced significantly in the PR transfectants (Figure 5B). The growth-inhibitory effect of progesterone in the PR-transfected SGC cells was associated with dose-dependent reductions in the percentage of the S-phase cells along with an increase in the G0-G1 phase cells,[66] the downregulation of Id-1 and c-myc proteins, and the upregulation of p21 as shown in Figure 6.

Estrogen receptor (ER) also has a role in SGC cells. Ohshiro et al[67] reported that estrogen induced cell migration of ER-positive SGC cells, and this effect was blocked by the pure antiestrogen and MAP/ERK kinase inhibitor. Basically, the Et-ER system is expected to possess the opposite effects to Pg-PR system. This kind of phenomenon is often observed in the cells derived from malignant tumors of the reproductive organs in females.

Moreover, the androgen-androgen receptor (AR) system was also reported to offer a possibility

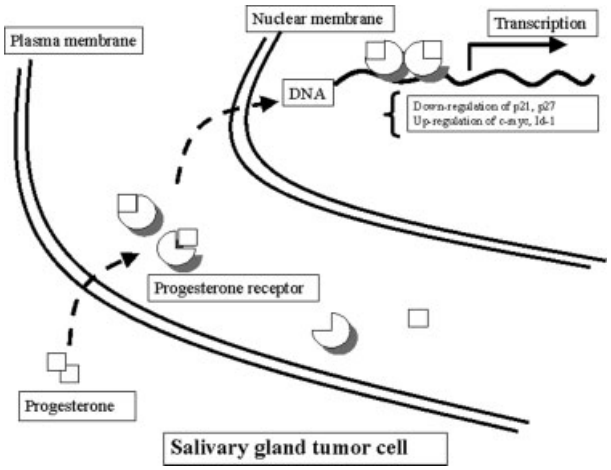

FIGURE 6. Progesterone-Progesterone receptor (Pg-PR) system in human salivary gland cancer (SGC) cells. SGC cells which express the PR show the upregulation of p21, p27, and the downregulation of Id-1 and c-myc proteins.

for hormonal therapy for SGC. It is reported that some kinds of SGCs, such as carcinoma and pleomorphic adenomas, salivary duct carcinomas, and basal cell adenocarcinomas, express AR. Locati et al[68] reported the complete remission with androgen-deprivation therapy in a recurrent AR-expressing adenocarcinoma of the parotid gland. This report suggests that a similar mechanism to prostate tumors may be implicated in AR-positive SGC.

However, the expression of sex steroid hormone receptor in clinical samples of SGC is still controversial. The expression pattern of the receptor is totally different between several reports.[69–72] These discrepancies have to be overcome, and it is necessary to confirm the effect of these hormones via its receptor by in vivo introduction of the receptor using cultured SGT cells.

Some new strategies for the treatment of SGC have been proposed. For example, it was reported that differentiation therapy,[73] adoptive immunotherapy,[74] and gene therapy[75] might be new aspects in the treatment of SGC. There is a place for new treatment modalities in patients with SGC. Hormonal therapy based on sex steroid hormones may be a completely new therapeutic option for SGC.

CONCLUSION

EGFR inhibitors (gefitinib, erlotinib, and cetuximab), COX-2 inhibitors (celecoxib), synthetic PPARγ ligands, and hormonal therapy have been demonstrated to be promising molecular targeting agents against oral malignant neoplasms.

Combined therapies using these molecules may improve the outcome of these patients. However, more translational research, and subsequently, randomized clinical trials are needed before these therapies can indeed be introduced in the clinical practice.

REFERENCES

1. Sato JD, Kawamoto T, Le AD, Mendelsohn J, Polikoff J, Sato GH. Biological effects in vitro of monoclonal antibodies to human epidermal growth factor receptors. Mol Biol Med 1983;1:511–529.
2. Chou JL, Fan Z, DeBlasio T, Koff A, Rosen N, Mendelsohn J. Constitutive overexpression of cyclin D1 in human breast epithelial cells does not prevent G1 arrest induced by deprivation of epidermal growth factor. Breast Cancer Res Treat 1999;55:267–283.
3. Wu X, Fan Z, Masui H, Rosen N, Mendelsohn J. Apoptosis induced by an anti-epidermal growth factor receptor monoclonal antibody in a human colorectal carcinoma cell line and its delay by insulin. J Clin Invest 1995;95: 1897–1905.
4. Peng D, Fan Z, Lu Y, DeBlasio T, Scher H, Mendelsohn J. Anti-epidermal growth factor receptor monoclonal antibody 225 up-regulates p27KIP1 and induces G1 arrest in prostatic cancer cell line DU145. Cancer Res 1996;56:3666–3669.
5. Shintani S, Li C, Mihara M, et al. Gefitinib ('Iressa', ZD1839), an epidermal growth factor receptor tyrosine kinase inhibitor, up-regulates $p27^{KIP1}$ and induces G1 arrest in oral squamous cell carcinoma cell lines. Oral Oncol 2004;40:43–51.
6. Di Gennaro E, Barbarino M, Bruzzese F, et al. Critical role of both p27KIP1 and p21CIP1/WAF1 in the antiproliferative effect of ZD1839 ('Iressa'), an epidermal growth factor receptor tyrosine kinase inhibitor, in head and neck squamous carcinoma cells. J Cell Physiol 2003; 195:139–150.
7. Malek NP, Sundberg H, McGrew S, Nakayama K, Kyriakides TR, Roberts JM. A mouse knock-in model exposes sequential proteolytic pathways that regulate p27Kip1 in G1 and S phase. Nature 2001;413:323–327.
8. Montagnoli A, Fiore F, Eytan E, et al. Ubiquitination of p27 is regulated by Cdk-dependent phosphorylation and trimeric complex formation. Genes Dev 1999;13:1181–1189.
9. Shintani S, Mihara M, Nakahara Y, Aida T, Tachikawa T, Hamakawa H. Lymph node metastasis of oral cancer visualized in live tissue by green fluorescent protein expression. Oral Oncol 2002;38:664–669.
10. Shintani S, Li C, Mihara M, Nakashiro K, Hamakawa H. Gefitinib ('Iressa'), an epidermal growth factor receptor tyrosine kinase inhibitor, mediates the inhibition of lymph node metastasis in oral cancer cells. Cancer Lett 2003;201:149–155.
11. Koivisto L, Grenman R, Heino J, Larjava H. Integrins α5β1, αvβ1, and αvβ6 collaborate in squamous carcinoma cell spreading and migration on fibronectin. Exp Cell Res 2000;255:10–17.
12. Thomas GJ, Lewis MP, Whawell SA, et al. Expression of the αvβ6 integrin promotes migration and invasion in squamous carcinoma cells. J Invest Dermatol 2001;117: 67–73.
13. Burridge K, Turner CE, Romer LH. Tyrosine phosphorylation of paxillin and pp125FAK accompanies cell adhe-

sion to extracellular matrix: a role in cytoskeletal assembly. J Cell Biol 1992;119:893–903.
14. Guan JL, Shalloway D. Regulation of focal adhesion-associated protein tyrosine kinase by both cellular adhesion and oncogenic transformation. Nature 1992;358: 690–692.
15. Raben D, Helfrich BA, Chan D, Johnson G, Bunn PA Jr. ZD1839, a selective epidermal growth factor receptor tyrosine kinase inhibitor, alone and in combination with radiation and chemotherapy as a new therapeutic strategy in non-small cell lung cancer. Semin Oncol 2002;29 (Suppl 4):37–46.
16. Huang S, Li J, Harari PM. Modulation of radiation response and tumor-induced angiogenesis following EGFR blockade by ZD1839 (Iressa) in human squamous cell carcinomas. In: Proceedings of the 12th NCI-EORTC-AACR Symposium on Molecular Targets and Cancer Therapeutics, Miami Beach, Florida, Oct. 29 – Nov. 2, 2001, Abstract 259.
17. Huang SM, Li J, Armstrong EA, Harari PM. Modulation of radiation response and tumor-induced angiogenesis after epidermal growth factor receptor inhibition by ZD1839 (Iressa). Cancer Res 2002;62:4300–4306.
18. Shintani S, Li C, Mihara M, et al. Enhancement of tumor radioresponse by combined treatment with gefitinib ('IRESSA', ZD1839), an epidermal growth factor receptor tyrosine kinase inhibitor, is accompanied by inhibition of DNA damage repair and cell growth in oral cancer. Int J Cancer 2003;107:1030–1037.
19. Terakado N, Shintani S, Yano J, et al. Overexpression of cyclooxygenase-2 is associated with radioresistance in oral squamous cell carcinoma. Oral Oncol 2004;40:383–389.
20. Pai R, Soreghan B, Szabo IL, Pavelka M, Baatar D, Tarnawski AS. Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. Nat Med 2002;8:289–293.
21. Buchanan FG, Wang D, Bargiacchi F, DuBois RN. Prostaglandin E2 regulates cell migration via the intracellular activation of the epidermal growth factor receptor. J Biol Chem 2003;278:35451–35457.
22. Tortora G, Caputo R, Damiano V, et al. Combination of a selective cyclooxygenase-2 inhibitor with epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 and protein kinase A antisense causes cooperative antitumor and antiangiogenic effect. Clin Cancer Res 2003;9: 1566–1572.
23. Zhu Y, Alvares K, Huang Q, Rao MS, Reddy JK. Cloning of a new member of the peroxisome proliferator-activated receptor gene family from mouse liver. J Biol Chem 1993;268:26817–26820.
24. Tontonoz P, Hu E, Spiegelman BM. Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor. Cell 1994;79:1147–1156.
25. Fajas L, Auboeuf D, Raspe E, et al. The organization, promoter analysis, and expression of the human PPARγ gene. J Biol Chem 1997;272:18779–18789.
26. Kliewer SA, Umesono K, Noonan DJ, Heyman RA, Evans RM. Convergence of 9-cis retinoic acid and peroxisome proliferator signaling pathways through heterodimer formation of their receptors. Nature 1992;358: 771–774.
27. Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ). J Biol Chem 1995;270: 12953–12956.
28. Lehmann JM, Lenhard JM, Oliver BB, Ringold GM, Kliewer SA. Peroxisome proliferator-activated receptor α and γ are activated by indomethacin and other non-steroidal anti-inflammatory drugs. J Biol Chem 1997;272: 3406–3410.
29. Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM. 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 is a ligand for the adipocyte determination factor PPARγ. Cell 1995;83:803–812.
30. Kliewer SA, Lenhard JM, Willson TM, Patel I, Morris DC, Lehmann JM. A prostaglandin J_2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation. Cell 1995;83:813–819.
31. Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev 1999;20:649–688.
32. Tontonoz P, Singer S, Forman BM, et al. Terminal differentiation of human liposarcoma cells induced by ligands for peroxisome proliferator-activated receptor γ and the retinoid X receptor. Proc Natl Acad Sci U S A 1997;94: 237–241.
33. Mueller E, Sarraf P, Tontonoz P, et al. Terminal differentiation of human breast cancer through PPARγ. Mol Cell 1998;1:465–470.
34. Elstner E, Müller C, Koshizuka K, et al. Ligands for peroxisome proliferator-activated receptor γ and retinoic acid receptor inhibit growth and induce apoptosis of human breast cancer cells in vitro and in BNX mice. Proc Natl Acad Sci U S A 1998;95:8806–8811.
35. Kubota T, Koshizuka K, Williamson EA, et al. Ligand for peroxisome proliferator-activated receptor γ (Troglitazone) has potent antitumor effect against human prostate cancer both in vitro and in vivo. Cancer Res 1998;58:3344–3352.
36. Tsubouchi Y, Sano H, Kawahito Y, et al. Inhibition of human lung cancer cell growth by the peroxisome proliferator-activated receptor-γ agonists through induction of apoptosis. Biochem Biophys Res Commun 2000;270:400–405.
37. Sarraf P, Mueller E, Jones D, et al. Differentiation and reversal of malignant changes in colon cancer through PPARγ. Nat Med 1998;4:1046–1052.
38. Sato H, Ishihara S, Kawashima K, et al. Expression of peroxisome proliferator-activated receptor (PPAR) γ in gastric cancer and inhibitory effects of PPARγ agonists. Br J Cancer 2000;83:1394–1400.
39. Guan YF, Zhang YH, Breyer RM, Davis L, Breyer MD. Expression of peroxisome proliferator-activated receptor γ (PPARγ) in human transitional bladder cancer and its role in inducing cell death. Neoplasia 1999;1:330–339.
40. Motomura W, Okumura T, Takahashi N, Obara T, Kohgo Y. Activation of peroxisome proliferator-activated receptor γ by troglitazone inhibits cell growth through the increase of $p27^{Kip1}$ in human pancreatic carcinoma cells. Cancer Res 2000;60:5558–5564.
41. Sarraf P, Mueller E, Smith WM, et al. Loss-of-function mutations in PPARγ associated with human colon cancer. Mol Cell 1999;3:799–804.
42. Nakashiro K, Hayashi Y, Kita A, et al. Role of peroxisome proliferator-activated receptor γ and its ligands in non-neoplastic and neoplastic human urothelial cells. Am J Pathol 2001;159:591–597.
43. Begum NM, Nakashiro K, Kawamata H, et al. Expression of peroxisome proliferator-activated receptor γ and the growth inhibitory effect of its synthetic ligands in human salivary gland cancer cells. Int J Oncol 2002;20: 599–605.
44. Barroso I, Gurnell M, Crowley VE, et al. Dominant negative mutations in human PPARγ associated with severe insulin resistance, diabetes mellitus and hypertension. Nature 1999;402:880–883.
45. Kodera Y, Takeyama K, Murayama A, Suzawa M, Masuhiro Y, Kato S. Ligand type-specific interactions of peroxisome proliferator-activated receptor γ with transcriptional coactivators. J Biol Chem 2000;275:33201–33204.

46. Nakashiro K, Begum NM, Uchida D, et al. Thiazolidinediones inhibit cell growth of human oral squamous cell carcinoma in vitro independent of peroxisome proliferator-activated receptor γ. Oral Oncol 2003;39:855–861.
47. Palakurthi SS, Aktas H, Grubissich LM, Mortensen RM, Halperin JA. Anticancer effects of thiazolidinediones are independent of peroxisome proliferator-activated receptor γ and mediated by inhibition of translation initiation. Cancer Res 2001;61:6213–6218.
48. Nikitakis NG, Siavash H, Hebert C, Reynolds MA, Hamburger AW, Sauk JJ. 15-PGJ_2, but not thiazolidinediones, inhibits cell growth, induces apoptosis, and causes downregulation of Stat3 in human oral SCCa cells. Br J Cancer 2002;87:1396–1403.
49. Xin X, Yang S, Kowalski J, Gerritsen ME. Peroxisome proliferator-activated receptor γ ligands are potent inhibitors of angiogenesis in vitro and in vivo. J Biol Chem 1999;274:9116–9121.
50. Clarke CL, Sutherland R. Progestin regulation of cellular proliferation. Endocr Rev 1990;11:266–301.
51. Kester HA, van der Leede BM, van der Saag PT, van der Burg B. Novel progesterone target genes identified by an improved differential display technique suggest that progestin-induced growth inhibition of breast cancer cells coincides with enhancement of differentiation. J Biol Chem 1997;272:16637–16643.
52. Horwitz KB. Mechanisms of hormone resistance in breast cancer. Breast Cancer Res Treat 1993;26:119–130.
53. Knight WA III, Osborne CK, Yochmowitz MG, McGuire WL. Steroid hormone receptors in the management of human breast cancer. Ann Clin Res 1980;12:202–207.
54. Wick MR, Ockner DM, Mills SE, Ritter JH, Swanson PE. Homologous carcinomas of the breasts, skin, and salivary glands. A histologic and immunohistochemical comparison of ductal mammary carcinoma, ductal sweat gland carcinoma, and salivary duct carcinoma. Am J Clin Pathol 1998;109:75–84.
55. Toida M, Shimokawa K, Makita H, et al. Intraoral minor salivary gland tumors: a clinicopathological study of 82 cases. Int J Oral Maxillofacial Surg 2005;34:528–532.
56. Shick PC, Riordan GP, Foss RD. Estrogen and progesterone receptors in salivary gland adenoid cystic carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995;80:440–444.
57. Teymoortash A, Lippert BM, Werner JA. Steroid hormone receptors in parotid gland cystadenolymphoma (Warthin's tumour). Clin Otolaryngol 2001;26:411–416.
58. Ozono S, Onozuka M, Sato K, Ito Y. Immunohistochemical localization of estradiol, progesterone, and progesterone receptor in human salivary glands and salivary adenoid cystic carcinomas. Cell Struct Funct 1992;17:169–175.
59. Ku TK, Crowe DL. Coactivator-mediated estrogen response in human squamous cell carcinoma lines. J Endocrinol 2007;193:147–155.
60. McGuire WL, Clark GM. Role of progesterone receptors in breast cancer. Semin Oncol 1985;12(Suppl 1):12–16.
61. Munstedt K, Steen J, Knauf AG, Buch T, von Georgi R, Franke FE. Steroid hormone receptors and long-term survival in invasive ovarian cancer. Cancer 2000;89: 1783–1791.
62. Kauppila A, Kujansuu E, Vihko R. Cytosol estrogen and progestin receptors in endometrial carcinoma of patients treated with surgery, radiotherapy, and progestin. Clinical correlates. Cancer 1982;50:2157–2162.
63. Groshong SD, Owen GI, Grimison B, et al. Biphasic regulation of breast cancer cell growth by progesterone: role of the cyclin-dependent kinase inhibitors, p21 and p27(Kip1). Mol Endocrinol 1997;11:1593–1607.
64. Graham JD, Clarke CL. Physiological action of progesterone in target tissues. Endocr Rev 1997;18:502–519.
65. Sumida T, Itahana Y, Hamakawa H, Desprez PY. Reduction of human metastatic breast cancer cell aggressiveness on introduction of either form A or B of the progesterone receptor and then treatment with progestins. Cancer Res 2004;64:7886–7892.
66. Yoshimura T, Sumida T, Liu S, et al. Growth inhibition of human salivary gland tumor cells by introduction of progesterone (Pg) receptor and Pg treatment. Endocr Relat Cancer 2007;14:1107–1116.
67. Ohshiro K, Rayala SK, Williams MD, Kumar R, El-Naggar AK. Biological role of estrogen receptor β in salivary gland adenocarcinoma cells. Clin Cancer Res 2006;12:5994–5999.
68. Locati LD, Quattrone P, Bossi P, Marchiano AV, Cantu G, Licitra L. A complete remission with androgen-deprivation therapy in a recurrent androgen receptor-expressing adenocarcinoma of the parotid gland. Ann Oncol 2003;14:1327–1328.
69. Vadlamudi RK, Balasenthil S, Sahin AA, et al. Novel estrogen receptor coactivator PELP1/MNAR gene and ERβ expression in salivary duct adenocarcinoma: potential therapeutic targets. Hum Pathol 2005;36:670–675.
70. Dori S, Trougouboff P, David R, Buchner A. Immunohistochemical evaluation of estrogen and progesterone receptors in adenoid cystic carcinoma of salivary gland origin. Oral Oncol 2000;36:450–453.
71. Jeannon JP, Soames JV, Bell H, Wilson JA. Immunohistochemical detection of oestrogen and progesterone receptors in salivary tumours. Clin Otolaryngol 1999;24:52–54.
72. Lewis JE, McKinney BC, Weiland LH, Ferreiro JA, Olsen KD. Salivary duct carcinoma. Clinicopathologic and immunohistochemical review of 26 cases. Cancer 1996; 77:223–230.
73. Sato M. Differentiation therapy for salivary gland tumors. Gan To Kagaku Ryoho 1993;20:1028–1036 [in Japanese].
74. Ueta E, Osaki T, Yamamoto T, Yoneda K. Induction of differentiation in maxillary adenoid cystic carcinomas by adoptive immunotherapy in combination with chemoradiotherapy. Oral Oncol 1998;34:105–111.
75. Zhang Y, Wang H, Toratani S, et al. Growth inhibition by keratinocyte growth factor receptor of human salivary adenocarcinoma cells through induction of differentiation and apoptosis. Proc Natl Acad Sci U S A 2001;98:11336–11340.