ANTICANCER RESEARCH *34*: 1547-1552 (2014)

Review

Effective Targeting of the Epidermal Growth Factor Receptor (EGFR) for Treating Oral Cancer: A Promising Approach

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Abstract. *Oral cancer is a serious problem growing in incidence in many parts of the world; it is considered the sixth most common cancer and despite sophisticated surgical and radiotherapeutic modalities, oral squamous cell carcinoma, which represents 90% of oral cancers, is characterized by poor prognosis and a low survival rate. The Epidermal growth factor receptor family of receptor tyrosine kinases (RTK) comprises of four distinct receptors: the EGFR (also known as ErbB-1/HER1), ErbB-2 (neu, HER2), ErbB-3 (HER3) and ErbB-4 (HER4). Several studies have been published on the role of EGFR in the pathogenesis of oral carcinoma. The aim of the present review is to describe the role of EGFR pathway in oral cancer with special focus on its role during the carcinogenesis process as a result of therapeutic approaches of EGFR in oral cancer. The EGFR is a 170-kDa cell-surface protein involved in many biological processes, such as proliferation, migration, DNA synthesis and adhesion. Overexpression of EGFR results in a poor prognosis in oral cancer and its activation is associated with the malignant phenotype, inhibition of apoptosis and increased metastatic potential. EGFR variations and mutations have been correlated with tumor formation, and possibly alter the therapeutic efficacy of EGFR inhibitors.*

Modern oncology focuses on signal transduction pathways to derive more knowledge on cancer development. One of the various molecules studied for this purpose is the Epidermal Growth Factor Receptor (EGFR), a tyrosine kinase receptor located at the cell membrane (1). This cell membrane tyrosine kinase is involved in a variety of cellular activities including proliferation, differentiation, survival and death, activating multiple downstream cell signaling pathways including the RAS/RAF/MEK/ERK1/2 pathway. Over-activation of this pathway is considered an etiological factor in human cancer, which contributes to cancer development, metastasis and resistance to chemotherapy (2-3).

It is well-known that cancer has been considered a major public health issue in the USA and other countries worldwide. Statistics show that one in four deaths for Americans can be attributed to cancer. It is estimated that approximately 569,490 americans died from cancer in 2010, with an average of 1,500 deaths per day (4). Oral cancer is a serious and rapidly-growing problem in many parts of the globe; it is considered the sixth most common cancer (5) and despite sophisticated surgical and radiotherapeutic modalities (6), oral squamous cell carcinoma, which represents 90% of oral cancers, is characterized by poor prognosis and a low survival rate (7). The leading cause of death for this type of cancer is metastasis, which occurs primarily by the lymphatic route and whose incidence is significantly correlated with clinical stage and localization of primary tumors (8).

Several studies have been published on the role of EGFR in the pathogenesis of oral carcinoma. The aim of the present review is to describe the role of EGFR pathway specifically in oral cancer with special focus on its role during the carcinogenesis process, thereby raising our understanding as an effective targeting for treating oral cancer.

Understanding the EGFR Pathway

The ErbB family of receptor tyrosine kinases (RTK) comprises of four distinct receptors: the EGFR (also known as ErbB-1/HER1), ErbB-2 (neu, HER2), ErbB-3 (HER3) and ErbB-4 (HER4) (9-10). In the present review, we focus largely on EGFR (Erb1/HER1) and its implications for oral cancer.

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Key Words: Epidermal growth factor receptor, oral cancer, review.

In the 1980s, the EGFR was cloned, sequenced and subsequently recognized as a receptor tyrosine kinase (RTK) (11). EGFR is best known for its classical function as a tyrosine kinase receptor localized on the plasma membrane and activated upon ligand binding. Activated EGFR recruits a number of downstream signaling molecules, leading to the activation of several major pathways crucial for tumor growth, progression and survival (12, 13). However, over the last decades efforts have been undertaken to better-understand the role of the EGFR in the nucleus (14), which was shown to regulate the biology of normal and malignantly-transformed cells (15). High levels of EGFR were also found in the nuclei of the many tumors, including those of skin breast (16), adrenocortical carcinoma (17), thyroid (18) and oral cavity (19).

Structurally, the ErbB family members consist of an extracellular ligand-binding domain, a single hydrophobic transmembrane domain and a cytoplasmatic tyrosine kinase-containing domain with several phosphorylation sites (20, 21). Studies on the crystal structures of EGFR, ErbB-2 and ErbB-3's extracellular domains have led to new insights in the process of ligand-induced receptor dimerization (22-24). Eleven ligands are known to bind the ERbB family of receptors. These can be classified into three groups of ligands that i: specifically bind to EGFR (including EGF, transforming growth factor-a, amphi-regulin and epigen, ii: those that bind to EGFR and ErbB4, including beta cellulin (BTC), heparin-biding EGF (HB-EGF) and epiregulin (EPR) which show dual specifity by binding both EGFR and erbB4, and iii: neuregulin (NRG) (also known as hereregulin) and can be divided in two sub-groups based upon their capacity to bind ErbB3 and ErbB4 (NRG-1 and NRG-2) or only ErbB4 (NRG-3 and NRG-4) (25-28). Furthermore, the extracellular region of EGFR, HER3 and HER 4 is sub-divided into four domains (I, II, III and IV) (24), these domains reveal two distinct conformations: a closed, inactive conformation, domains II and IV interact with each other at the molecular level, thus preventing domains I and III from interacting with their cognate ligand (22, 23, 29). Both the open and closed conformations remain in equilibrium with each other (30, 31). The structure of ErbB2 has a conformation that resembles the ligand-activated state with a protruding dimerization loop. In this conformation domains, L1 and L2 are very close and this interaction makes ligand binding impossible, explaining why ErbB2 has no ligand (32, 33).

Although these ligands show redundancy, heparin-binding–EGF is the only ligand whose absence in knock-out mice results in post-natal lethality as a result of heart and lung diseases. Null mutations for EGFR cause development defects in the epithelial structure of the skin, pancreas, gastrointestinal tract and central nervous system (25, 34).

Activation of EGFR signaling triggered by ligand-induced receptor dimerization following which the tyrosine residues present in the intrinsic kinase domain of one receptor cross phosphorylates specific residues in the C-terminal tail of the partnering receptor, thus providing a scaffold for the recruitment of effector proteins (35, 36). These phosphorylated tyrosines serve as specific binding sites for several adaptor proteins such as phospholipase Cg, CBL, GRB2, SHC and p85. Several signaling transducers bind to these adaptors to initiate multiple signal pathways, including mitogen-activated protein-kinase, phosphatidylinositol 3-kinase/AKT and the signal transducer and activator of transcription STAT3 and STAT5 pathways (37).

These cellular processes are often de-regulated in malignant cells and for tumor maintenance due to the several mutations present in various genes involved in these pathways (3).

EGFR Signaling and Oral Cancer

Recent advances in our understanding over the molecular progression of oral squamous cell carcinoma have revealed underlying genetic, epigenetic and metabolic alterations which disrupt cellular protein expression and function resulting in the appearance of abnormal histological phenotypes (38). EGFR biomarker detection in oral squamous cell carcinoma may fulfill multiple roles in cancer diagnostics, not only for early detection but at-diagnosis for prognostic evaluation and treatment selection (39). The relationship between EGFR and cancer prognosis has been studied in multiple solid tumor types. In particular, researchers have reported that overexpression of EGFR and other growth factors with similar structural and functional capacities are associated with several malignancies. EGFR has been correlated with poor prognosis in some human cancers and is apparently predictive of disease-free survival independent of cervical lymph node status (40).

Researchers also found an overexpression of EFGR in the plasma membrane correlated with poor prognosis in tongue cancer (41, 42). Grandis *et al*. showed that EGFR-overexpression provided independent prognostic value for both local control and survival (43). More than 80% of invasive squamous cell carcinoma cases of head and neck overexpressed EGFR, thus excess of EGFR is often linked to unfavorable clinical outcome, high recurrence and low survival rates (44, 45). Activation of EGFR signaling is associated with the malignant phenotype, manifested by angiogenesis, inhibition of apoptosis as well as increased metastatic potential (46). Chang *et al*. in 2013 demonstrated the role of pAKT and EGFRvIII in oral carcinomas, acting as a determinant factors for patient survival; these data suggested that both molecules could be used as prognostic biomarkers (47). However, Ulanovski *et al*. showed that overexpression of plasma membrane EGFR cannot serve as a prognostic factor nor as a predictor of survival and treatment success in squamous cell carcinoma of the tongue (41). Laimer *et al*. found a prognostic value of cytoplasmatic EGFR expression on a tissue microarray-based immunohistochemical analysis (48). Lo *et*

al. analyzed 37 cases of oral squamous cell carcinoma for nuclear EGFR using standard IHC methods. They found that 24% of cases analyzed had nuclear EGFR in more that 5% of tumor cells. Patients with high nuclear EGFR expression in this cohort demonstrated poor overall survival compared to patients with no/low levels of nuclear EGFR (16).

Although targeting membrane-bound EGFR has shown benefit as a new and emerging approach in tumor cells, an interesting case is being built regarding nuclear EGFR signaling networks, through which it participates in cancer progression, survival and response to chemotherapeutics (12). Furthermore, very little is known regarding the physiological function and cancer relevance of the nuclear EGFR pathway until recent years. EGFR has been consistently detected in the nuclei of cancer cells and primary tumor specimens of various origins as wells as in those of other highly proliferative tissues (49-52).

Certainly, the localization of the EGFR provides a better understanding over its role in cancer cells as well as prognosis and treatment.

EGFR Mutations and Polymorphims in Oral Cancer

Genetic variations in EGFR are a pivotal event that may alter protein function, and contribute to tumor formation, and possibly alter the therapeutic efficacy of EGFR inhibitors (53). Recent studies indicate that the incidence of EGFR mutations in oral carcinoma differs between ethnic groups, ranging from 0-4% in whites to 7% in Asians (54).

The first intron of the *EGFR* gene contains a highly polymorphic microsatellite sequence: 9-23 CA simple sequence repeat (SSR) close to a downstream enhancer sequence (55). It is located more than 1000 bp downstream of the promoter, helical conformation analyses have suggested a possible regulatory role of this polymorphic region on transcription. Indeed, it has been proposed that the number of CA repeats may be able to modify DNA conformation after binding of transcription factors (56). Into an inverse relation, experimental data showed when the transcription activity declines the number of repeats increases (57).

According to Perea *et al*., preliminary *in vitro* and clinical data indicated the influence of the CA repeat polymorphism on the efficacy of EGFR tyrosine kinase inhibitors (58). Studies with 13 cell lines demonstrated that the cell lines with short CA repeats exhibited higher EGFR mRNA and protein expression when compared to remaining cell lines with long CA repeats, being more susceptible to Erlotinib (58), that has been shown to induce apoptosis (59) and metabolic oxidative stress (60).

Several structural variants in EGFR were observed in human malignancies and according to Wikstrand *et al*. the most frequently detected genomic variant, termed EGFRvIII, is expressed in 42% of oral tumors. The EGFRvIII is a 145-kDa protein resulting from the deletion of amino acids 6-273 of the wild-type EGFR extracellular domain (61). The transmembrane domain of EGFRvIII is thought to be identical to that of the wild-type protein, a hydrophilic sequence of 23 amino acids with a yet-unknown role in receptor function (62). Following activation by EGFRvIII, the PI3K pathway initiates survival and anti-apoptotic signals that are not subject to the regulatory mechanisms that govern Ras-Raf-MEK-Erk/MAPK signaling (63).

Effective Targeting of EGFR for Oral Cancer Treatment

Treatment of tumor cells *in vitro* with an anti-EGFR antibody induces arrest of cells in G_1 phase with an increase in the cyclin-dependent kinase inhibitor p27kip1 and a decrease in retinoblastoma protein (Rb) phosphorylation (64). Moreover, synergy exists between EGFR inhibition, radiation and chemotherapy (65).

When oral squamous carcinoma cells were pre-treated with EGF, their sensitivity to radiation was enhanced in relation to the number of EGFRs on their surfaces (66). These findings indicate that EGFR expression and its signal transduction pathways may play an important role in determining the sensitivity to chemotherapeutic agents or irradiation, and alterations in receptor expression or function may influence response to these therapies (67).

According to Huang and O'Sullivan, treatment of oral cancer includes single-modality surgery, radiotherapy (external-beam radiotherapy (EBRT) and/or brachytherapy), or various combinations of these modalities with or without systemic therapy (chemotherapy and/or target agents) (68).

Because of the relationship between overexpression of EGFR and aggressive behavior of tumor cells, monoclonal antibodies directed against this receptor might prove to be effective therapeutic agents (67). The anti-EGFR monoclonal antibody named 225 was generated and has been shown to have an antitumor activity *in vitro* and in xenograft models (69-71). This antibody has been chimerized with human IgG1 (C225) (60) and was able to inhibit the growth of cultured EGFR-expressing tumor cell lines and to express the *in vivo* growth of these tumors when grown as xenografts in nude mice (72-74).

Another anti-EGFR antibody named Cetuximab was shown to induce autophagy in several cancer cell lines, including oral cancer *via* inhibition of EGFR signaling and subsequent down-regulation of the mammalian target of rapamycin (mTOR) and hypoxia inducible factor 1-α signaling pathways (75). The induction of autophagy has been shown to be cytoprotective in a variety of important processes related to cancer therapy including resistance to chemotherapeutics (76, 77), ionizing radiation (78), basement membrane detachment, growth factor deprivation and hypoxia (79-81).

Inhibition of EGFR plays an important role in tumor progression. There are two different ways of EGFR molecular-targeted drug interaction offering a more effective inhibition. The first one involves the connection of the drug to the extracellular domain of the receptor that inhibits the connection of the ligand. The second targets the intracellular portion that has tyrosine kinase activity and exerts its action by restricting ATP binding or binding to the active site of the enzyme (82, 83).

Erlotinib is an orally-active potent, selective inhibitor of the EGFR tyrosine kinase (84). In a phase II trial, single-agent Erlotinib demonstrated a low response rate in ~4% in patients with recurrent or metastatic oral squamous cell carcinoma. When Erlotinib was combined with cisplatin, a response rate of 21% was achieved in a phase I/II trial in a similar patient population, and rates of grade 3 and 4 toxicity were minimal (85).

Concluding Remarks

This review aimed to present the therapeutic approaches of EGFR in oral cancer. EGFR is a 170-kDa cell-surface protein involved in many biological processes, such as proliferation, migration, DNA synthesis and adhesion. Overexpression of EGFR provides a poor prognosis in oral cancer and its activation is associated with the malignant phenotype, inhibition of apoptosis and increased metastatic potential. EGFR variations and mutations have been correlated with tumor formation, and possibly alter the therapeutic efficacy of EGFR inhibitors.

Conflicts of Interest

None declared.

Acknowledgments

This work was supported by CAPES (Coordination for the Improvement of Higher Level -or Education- Personnel). DAR is a recipient of a CNPq (National Counsel of Technological and Scientific Development) fellowship.

References

1 Grandis J and Tweardy D: Elevated levels of transforming growth factor alpha and epidermal growth factor recetor messenger RNA are early markers of carcinogenesis in head and neck cancer. Cancer Res *53*: 3579-3584, 1993.

2 Okamoto I: Epidermal growth factor receptor in relation to tumor development: EGFR-targeted anticancer therapy. FEBS J *277*: 309-315, 2010.

3 Mitsudomi T and Yatabe Y: Epidermal growth factor receptor in relation to tumor development: EGFR gene and cancer. FEBS J *277*: 301-308, 2010.

4 Jemal D, Siegel R, Xu J and Ward E: Cancer statistics. CA Cancer J Clin *60*: 277-300, 2010.

5 Parkin D, Bray F, Ferlay J and Pisani P: Global cancer statistics. Cancer J Clin *55*: 74-108, 2005.

6 Xi S and Grandis J: Gene therapy for the treatment of oral squamous cell carcinoma. J Dent Res *82*: 11-16, 2003.

7 Magrath I and Litvak J: Cancer in developing countries: opportunity and challenge. J Nat Cancer Inst *85*: 862-874, 1993.

8 Berenson J, Yang I and Mickel R: Frequent amplification of the BCL-1 locus in head and neck squanous cell carcinomas. Oncogene *4*: 1111-1116, 1989.

9 Fergunson K, Berger M, Mendrola J, Cho H, Leahy D and Lemmon M: EGF activates its receptor by removing interactions that autoinhibit cetodomain dimerization. Mol Cell *11*: 507-517, 2003.

10 Yarden Y: The EGFR family and its ligands in human cancer. Signaling mechanisms and therapeutic opportunities. Eur J Cancer *4*: S3-S8, 2001.

11 Ullrich A, Coussens L, Hayflick J, Dull T, Gray A, Tam A, Lee J, Yarden Y, Libermann T and Schlessinge J: Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. Nature *309(5967)*: 418-425, 1984.

12 Lo H and Hung M: Nuclear EGFR signalling network in cancers: linking EGFR pathway to cell cycle progression, nitric oxide pathway and patient survival. Br J Cancer *94*: 184-188, 2006.

13 Yarden Y, Shilo B: SnapShot: EGFR signaling pathway. Cell *131*: 1018, 2007.

14 Campos ADA, Rodrigues M, Andrade CD, Goes AD, Nathanson M and Gomes D: Epidermal growth factor receptors destined for the nucleus are internalized *via* a clathrin-dependent pathway. Biochem Biophys Res Commun *412(2)*: 341-346, 2011.

15 Lo H: Nuclear Mode of the EGFR Signaling Network: Biology, Prognostic Value, and Therapeutic Implications. Discov Med July *10(50)*: 44-51, 2010.

16 Lo H, Xia W, Wei Y, Ali-Seyed M, Huang S and Hung M: Novel prognostic value of nuclear epidermal growth factor receptor in breast cancer. Cancer Res *65(1)*: 338-348, 2005.

17 Kamio T, Shigematsu K, Sou H, Kawai K and Tsuchiyama H: Immunohistochemical expression of epidermal growth factor receptors in human adrenocortical carcinoma. Hum Pathol *21(3)*: 277-282, 1990.

18 Marti U, Ruchti C, Kampf J, Thomas G, Williams E, Peter H, Gerber H and Burgi U: Nuclear localization of epidermal growth factor and epidermal growth factor receptors in human thyroid tissues. Thyroid *11(2)*: 137-145, 2001.

19 Psyrri A, Yu Z, Weinberger P, Sasaki C, Haffty B, Camp R, Rimm D and Burtness B: Quantitative determination of nuclear and cytoplasmic epidermal growth factor receptor expression in oropharyngeal squamous cell cancer by using automated quantitative analysis. Clin Cancer Res *11(16)*: 5856-5862, 2005.

20 Ferguson K, Berger M and Mendrola J: EGF activates its receptor by removing interactions that autoinhibit ectodomain dimerization. Mol Cell *11*: 507-517, 2003.

21 Olayioye M, Neve R, Lane H and Hynes N: The ErbB siganling network: receptor heterodimerization in development and cancer. EMBO J *19*: 3159-3167, 2000.

22 Cho H and Leahy D: Structure of the extracellular region of HER3 reveals an interdomain tether. Science *297*: 1330-1333, 2002.

23 Garrett TP, McKern NM, Lou M, Elleman TC, Adams TE, Lovrecz GO, Kofler M, Jorissen RN, Nice EC, Burgess AW and Ward CW: The crystal structure of a truncated ErbB2 ectodomain reveals an active conformation poised to interact with other ErbB receptors. Mol Cell *11*: 495-505, 2003.

24 Ogiso H, Ishitani R, Nureki O, Fukai S, Yamanaka M, Kim JH, Saito K, Sakamoto A, Inoue M, Shirouzu M and Yokoyama S: Crystal structure of the complex of human epidermal growth factor and receptor extracellular domains. Cell *110*: 775-787, 2002.

25 Schneider M and Wolf E: The epidermal growth factor receptor ligands at a glance. J Cell Physiol *218*: 460-466, 2009.

26 III KC, Weber J, Unger M, Ledesma J, Yu N, Gassmann M and Lai C: Neuregulin-2, a new ligand of ErbB3/ErbB4-receptor tyrosine kinases. Nature *387(6632)*: 512-516, 1997.

27 Chang H, 2nd DRD, Gilbert W, Stern D and McMahan U: Ligands for ErbB-family receptors encoded by a neuregulin-like gene. Nature *387(6632)*: 509-512, 1997.

28 Harari D, Tzahar E, Romano J, Shelly M, Pierce J, Andrews G and Yarden Y: Neuregulin-4: a novel growth factor that acts through the ErbB-4 receptor tyrosine kinase. Oncogene *18(17)*: 2681-2689, 1999.

29 Garrett TP, McKern NM, Lou M, Elleman TC, Adams TE, Lovrecz GO, Zhu HJ, Walker F, Frenkel MJ, Hoyne PA, Jorissen RN, Nice EC, Burgess AW and Ward CW: Crystal structure of a truncated epidermal growth factor receptor extracellular domain bound to transforming growth factor alpha. Cell *110*: 763-773, 2002.

30 Bouyain S, Longo P, Li S, Ferguson K and Leahy D: The extracellular region of ErbB4 adopts a tethered conformation in the absence of ligand. Proc Natl Acad Sci USA *102*: 15024-15029, 2005.

31 Dawson J, Berger M, Lin C, Schlessinger J, Lemmon M and Ferguson K: Epidermal growth factor receptor dimerization and activation require ligand-induced conformational changes in the dimer interface. Mol Cell Biol *25*: 7734-7742, 2005.

32 Garrett T, McKern N, Lou M, Elleman T, Adams T, Lovrecz G, Kofler M, Jorissen R, Nice E, Burgess A and Ward C: The crystal structure of a truncated ErbB2 ectodomain reveals an active conformation, poised to interact with other ErbB receptors. Mol Cell *11(2)*: 495-505, 2003.

33 Cho HS, Mason KX, Ramyar K, Stanley AM, Gabelli SB, Denney DW Jr. and Leahy DJ: Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. Nature *421(6924)*: 756-760, 2003.

34 Miettinen P, Berger J, Meneses J, Phung Y, Pedersen R, Werb Z and Derynck R: Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. Nature *376(6538)*: 337-341, 1995.

35 Siwak D, Carey M, Hennessy B, Nguyen C, Murray MM, Nolden L and Mills G: Targeting the epidermal growth factor receptor in epithelial ovarian cancer: current knowledge and future challenges. J Oncol 568938, 2010.

36 Tzahar E, Waterman H, Chen X, Levkowitz G, Karunagaran D, Lavi S, Ratzkin B and Yarden Y: A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor. Mol Cell Biol *16*: 5276-5287, 1996.

37 Hynes N and Lane H: ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev *5*: 341-354, 2005.

38. Brinkman B and Wong D: Disease mechanism and biomarkers of oral squamous cell carcinoma. Curr Opin Oncol *18(3)*: 228-233, 2006.

39 Weigum S, Floriano P, Christodoulides N and McDevitt J: Cell-based sensor for analysis of EGFR biomarker expression in oral cancer. Lab Chip *7*: 995-1003, 2007.

40 Tandon AK, Clark GM, Chamness GC, Ullrich A and McGuire WL: HER-2/neu oncogene protein and prognosis in breast cancer. J Clin Oncol *7*: 1120-1128, 1989.

41 Ulanovski D, Stern Y, Roizman P, Shpitzer T, Popovtzer A and Feinmesser R: Expression of EGFR and Cerb-B2 as prognostic factors in cancer of the tongue. Oral Oncology *40*: 532-537, 2004.

42 Yamada T, Takagi M, Shioda S: Evaluation of epidermal growth factor receptor in squamous cell carcinoma of the oral cavity. Oral Surg Oral Med Oral Pathol *73*: 67-70, 1992.

43 Rubin Grandis J1, Melhem MF, Barnes EL and Tweardy DJ: Quantitive immunohistochemical analysis of transformation growth factor-alpha and epidermal growth factor receptor in patients with squamous cell carcinoma in head and neck. Cancer J Clin *78(6)*: 1284-1292, 1996.

44 Chung C, Ely K, McGavran L, Varella-Garcia M, Parker J, Parker N, Jarrett C, Carter J, Murphy B, Netterville J, Burkey B, Sinard R, Cmelak A, Levy S, Yarbrough W, Slebos R and Hirsch F: Increased epidermal growth factor receptor gene copy number is associated with poor prognosis in head and neck squamous cell carcinomas. J Clin Oncol *24*: 4170-4176, 2006.

45 Temam S, Kawaguchi H, El-Naggar A, Jelinek J, Tang H, Liu D, Lang W, Issa J, Lee J and Mao L: Epidermal growth factor receptor copy number alterations correlate with poor clinical outcome in patients with head and neck squamous cancer. J Clin Oncol *25*: 2164-2170, 2007.

46 Modjtahedi H and Essapen S: Epidermal growth factor receptor inhibitors in cancer treatment: advances, challenges and opportunities. Anticancer Drugs *20*: 851-855, 2009.

47 Chang K, Tsai S, Chen S, Tsou H, Yen C, Liu K, Fang H, Wu H, Chou S, Tang C, Liu S, Lu P, Yen C and Chang J: Dissecting the EGFR-PI3K-AKT pathway in oral cancer highlights the role of the EGFR variant III and its clinical relevance. J Bio Med Sci *20*: 43, 2013.

48 Laimer K, Spizzo G, Gastl G, Obrist P, Brunhuber T, Fong D, Barbieri V, Jank S, Doppler W, Rasse M and Norer B: High EGFR expression predicts poor prognosis in patients with squamous cell carcinoma of the oral cavity and oropharynx: A TMA-based immunohistochemical analysis. Oral Oncology *43*: 193-198, 2007.

49 Lo H, Hsu S, Ali-Seyed M, Gunduz M, Xia W, Wei Y, Bartholomeusz G, Shih J and Hung M: Nuclear interaction of EGFR and STAT3 in the activation of iNOS/NO pathway. Cancer Cell *7*: 575-589, 2005.

50 Lo H, Hsu S and Hung M: EGFR signaling pathway in breast cancers: from traditional signal transduction to direct nuclear trans-localization. Breast Cancer Res Treat 1-8, 2005.

51 Lin S, Makino K, Xia W, Matin A, Wen Y, Kwong K, Bourguignon L and Hung M: Nuclear localization of EGF receptor and its potential new role as a transcription factor. Nat Cell Biol *3*: 802-880, 2001.

52 Cao H, Lei Z, Bian L and Rao C: Functional nuclear epidermal growth factor receptors in human choriocarcinoma JEG-3 cells and normal human placenta. Endocrinology *136*: 3163-3317, 1995.

53 Gebhardt F, Burger H and Brandt B: Modulation of EGFR gene transcription by a polymorphic repetitive sequence-a link between genetics and epigenetics. Int J Biol Markers *15(1)*: 105-110, 2000.

54 Schwentner I, Witsch-Baumgartner M, Sprinzl GM, Krugmann J, Tzankov A, Jank S, Zwierzina H and Loeffler-Ragg J: Identification of the rare EGFR mutation p.G796S as somatic and germline mutation in white patients with squamous cell carcinoma of the head and neck. Head Neck *30(8)*: 1040-1044, 2008.

55 Bornstein P, McKay J, Liska DJ, Apone S and Devarayalu S: Interactions between the pro-moter and first intron are involved in transcriptional control of alpha 1 (I) collagen gene expression. Mol Cell Biol *8*: 4851-4857, 1988.

56 Gebhardt F, Burger H and Brandt B: Modulation of EGFR gene transcription by secondary structures, a polymorphic repetitive sequence and mutations – a link between genetics and epigenetics. Histol Histopathol *15*: 929-936, 2000.
57 Gebhardt F, Zanker KS and Brandt B: Modulation of epidermal growth factor receptor gene transcription by a polymorphic dinucleotide repeat in intron 1. J Biol Chem *274*: 13176-13180, 1999.
58 Perea S, Oppenheimer D and Amador M: Genotypic bases of EGFR inhibitors pharmacological actions. Proc Am Soc Clin Oncol *23*: 196 (Abstr 3005), 2004.
59 Tang PA, Tsao M-S and Moore MJ: A review of erlo-tinib and its clinical use. Expert Opin Pharmacother *7*: 177-193, 2006.
60 Orcutt KP, Parsons AD, Sibenaller ZA, Scarbrough PM, Zhu Y, Sobhakumari A, Wilke WW, Kalen AL, Goswami P, Miller FJ Jr., Spitz DR and Simons AL: Erlotinib-mediated inhi-bition of EGFR signaling induces metabolic oxidative stress through NOX4. Cancer Res *71*: 3932-3940, 2011.
61 Wikstrand CJ, Reist CJ, Archer GE, Zalutsky MR and Bigner DD: The class III variant of the epidermal growth factor receptor (EGFRvIII): characterization and utilization as an immunotherapeutic target. J Neurovirol *4(2)*: 148-58, 1998.
62 Pedersen MW, Meltorn M, Damstrup L and Poulsen HS: The type III epidermal growth factor receptor mutation. Biological significance and potential target for anti-cancer therapy. Ann Oncol *12(6)*: 745-760, 2001.
63 Moscatello DK, Holgado-Madruga M, Emlet DR, Montgomery RB and Wong AJ: Constitutive activation of phosphatidylinositol 3-kinase by a naturally occurring mutant epidermal growth factor receptor. J Biol Chem *273(1)*: 200-206, 1998.
64 Huang S, Bock J and Harari P: Epidermal growth factor receptor blockade with C225 modulatesproliferation, apoptosis, and radiosensitivity in squamous cell carcinomas of the head and neck. Cancer Res *59*: 1935-1940, 1999.
65 Gilbert J, Rudek MA, Higgins MJ, Zhao M, Bienvenu S, Tsottles N, Chaudhry MA, Wahl R, Forastiere A and Gillison M: A phase I trial of erlotinib and concurrent chemoradiotherapy for stage III and IV (M0) squamous cell carcinoma of the head and neck. Clin Cancer Res *18(6)*: 1735-1742, 2012.
66 Kwok TT and Sutherland RM: Differences in, EGF-related radiosensitisation of human squamous carcinoma cells with high and low numbers of EGF receptors. Br J Cancer *64*: 251-254, 1991.
67 Shin DM, Donato NJ, Perez-Soler R, Shin HJ, Wu JY, Zhang P, Lawhorn K, Khuri FR, Glisson BS, Myers J, Clayman G, Pfister D, Falcey J, Waksal H Mendelsohn J and Hong WK: Epidermal growth factor receptor-targeted therapy with C225 and cisplatin in patients with head and neck cancer. Clin Cancer Res *7(5)*: 1204-1213, 2001.
68 Huang SH and O'Sullivan B: Oral cancer: Current role of radiotherapy and chemotherapy. Med Oral Patol Oral Cir Bucal 18(2):e233-240, 2013.
69 Sato JD, Kawamoto T, Le AD, Mendelsohn J, Polikoff J and Sato GH: Biological effects *in vitro* of monoclonal antibodies to human epidermal growth factor receptors. Mol Biol Med *1*: 511-529, 1983.
70 Kawamoto T, Sato JD, Le A, Polikoff J, Sato GH and Mendelsohn J: Growth stimulation of A431 cells by epidermal growth factor: identification of high-affinity receptors for epidermal growth factor by an antireceptor monoclonal antibody. Proc Natl Acad Sci USA *80*: 1337-1341, 1983.
71 Masui H, Kawamoto T, Sato JD, Wolf B, Sato G and Mendelsohn J: Growth inhibition of human tumor cells in athymic mice by anti-epidermal growth factor receptor monoclonal antibodies. Cancer Res *44*: 1002-1007, 1984.
72 Goldstein NI, Prewett M, Zuklys K, Rockwell P and Mendelsohn J: Biological efficacy of a chimeric antibody to the epidermal growth factor receptor in a human tumor xenograft model. Clin Cancer Res *1*: 1311-1318, 1995.
73 Masui H, Moroyama T and Mendelsohn J: Mechanism of anti-tumor activity in mice for anti-epidermal growth factor receptor mono-clonal antibodies with different isotypes. Cancer Res *46*: 5592-5598, 1986.
74 Fan Z, Masui H, Altas I and Mendelsohn J: Blockade of epidermal growth factor receptor function by bivalent and monovalent fragments of 225 anti-epidermal growth factor receptor monoclonal antibodies. Cancer Res *53*: 4322-4328, 1993.
75 Li X and Fan Z: The epidermal growth factor recep-tor antibody cetuximab induces autophagy in cancer cells by downregulating HIF-1alpha and Bcl-2 and activating the beclin 1/hVps34 complex. Cancer Res *70*: 5942-5952, 2010.
76. Ren JH, He WS, Nong L, Zhu QY, Hu K, Zhang RG, Huang LL, Zhu F and Wu G: Acquired cisplatin resistance in human lung adenocarcinoma cells is associated with enhanced autophagy. Cancer Biother Radiopharm *25*: 75-80, 2010.
77 Chen S, Rehman SK, Zhang W, Wen A, Yao L and Zhang J: Autophagy is a therapeutic target in anticancer drug resistance. Biochim Biophys Acta *1806*: 220-229, 2010.
78 Lomonaco SL, Finniss S, Xiang C, Decarvalho A, Umansky F, Kalkanis SN, Mikkelsen T and Brodie C: The induction of autophagy by gamma-radiation contributes to the radioresistance of glioma stem cells. Int J Cancer *125*: 717-722, 2009.
79 Fung C, Lock R, Gao S, Salas E and Debnath J: Induction of autophagy during extracellular matrix detachment promotes cell survival. Mol Biol Cell *19*: 797-806, 2008.
80 Lum JJ, Bauer DE, Kong M, Harris MH, Li C, Lindsten T and Thompson CB: Growth factor regulation of autoph-agy and cell survival in the absence of apoptosis. Cell *120*: 237-248, 2005.
81 Mazure NM, Pouysségur J: Hypoxia-induced autoph-agy: cell death or cell survival? Curr Opin Cell Biol *22*: 177-180, 2010.
82 Ciardiello F and Tortora G: A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. Clin Cancer Res *7*: 2958-2970, 2001.
83 Harari PM, Huang SM, Herbst RS and Quon H: Molecular Targeting of the Epidermal Growth Factor Receptor. *In*: Harrison LB, Session RB, Hong WK, editors. Head and Neck Cancer: A Multidisciplinary Approach. 2nded. Philadelphia: Lippincott, Williams and Wilkings, 2003.
84 Thomas F, Rochaix P, Benlyazid A, Sarini J, Rives M, Lefebvre JL, Allal BC, Courbon F, Chatelut E and Delord JP: Pilot study of neoadjuvant treatment with erlotinib in nonmetastatic head and neck squamous cell carcinoma. Clin Cancer Res *13*: 7086-7092, 2007.
85 Soulieres D, Senzer NN, Vokes EE, Hidalg M, Agarwala SS and Siu LL: Multicenter phase II study of erlotinib and oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with recurrent or metastatic squamous cell cancer of the head and neck. J Clin Oncol *22*: 77-85, 24.

Received February 2, 2014
Revised March 5, 2014
Accepted March 6, 2014