Dysregulation and detection methods of EGFR in oral cancer. A narrative review.

Abstract: Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein, with an intracellular domain and tyrosine kinase function (TK) involved in cell proliferation. Dysfunctions in EGFR signaling pathways have been associated with oral malignant tumors such as oral squamous cell carcinoma (OSCC). Dysfunctions of EGFR may result from: increased EGF ligand; EGFR overexpression and copy number gain of the EGFR gene (EGFR CNG); EGFR mutations; failure in the downregulation of EGFR; and EGFR crosstalk. Of these alterations, overexpression of EGFR is by far the most studied dysfunction in OSCC. Clinicians should identify possible alterations of EGFR in the oral mucosa of patients, as EGFR can act as a biomarker for the diagnosis and prognosis of OSCC. Currently, there are several methods and techniques for detecting EGFR. Immunohistochemistry (IHC), fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR), are used to identify overexpression of EGFR, EGFR CNG and EGFR mutations, respectively. Detection of EGFR as a biomarker is key to identify any oral malignant transformation. Consequently, it becomes imperative to implement a non-invasive and inexpensive method of early diagnosis for OSCC in clinical practice.

Keywords: Epidermal Growth Factor Receptor, Mouth Neoplasm, Mutation.

INTRODUCTION.

In 1962, Dr. Cohen studied a protein extracted from the submandibular glands responsible for the early growth of the incisors and the eyelid in mice. In 1979, Cohen and Carpenter named this protein epidermal growth factor (EGF). EGFR binds to its EGFR receptor by means of a covalent bond type. EGFR is a protein encoded by a gene located on the short arm of chromosome 7 (182-184), region p14-p12. Its protein is a transmembrane glycoprotein receptor with 1186 amino acids and 170 kDa. Through the TK cytoplasmic domain, they transduce signals from the cell membrane to the nucleus, controlling cell proliferation, differentiation, survival and motility.

EGFR has been associated with tumorigenic, proliferative, apoptotic, invasive and metastatic processes of epithelial origin. EGFR is involved in the pathogenesis of non-small-cell lung carcinoma (NSCLC), colorectal carcinomas, and oral squamous cell carcinoma (OSCC). The use of EGFR as a molecular biomarker in conjunction with molecules involved in signal transduction are ideal targets for OSCC therapies and useful for early diagnosis, prognosis and treatment of cancer.

In the last decade, the association of EGFR with carcinomas has increased the interest in its genomic evaluation. Molecular detection is performed through epithelial cell membranes using diverse techniques depending on the objectives of each study.

The aim of this review is to describe the alterations in EGFR and identify the methods most commonly used to detect it in oral cancer.
DYSREGULATION OF EGFR IN ORAL CANCER.

Dysregulation of EGFR in cancer has been extensively studied. Mechanisms involved in the dysregulation of EGFR are many, including:
1) Increased EGF ligand;
2) Overexpression of EGFR and EGFR CNG;
3) EGFR mutations;
4) Failure in the downregulation of EGFR;
5) EGFR crosstalk.

Increased EGF ligand
Activation or inhibition of EGFR is determined by the binding of its ligands. Molecules that bind to EGFR include EGF, amphiregulin, epigen, transforming growth factor alpha (TGF-α), betacellulin, heparin-binding EGF, epiregulin, neuregulin or heregulin and insulin-type growth factor. However, the main ligand of EGFR is EGF. EGF is a single polypeptide chain comprising 53 amino acids. Increased synthesis of EGF has been associated with a number of tumors, including head and neck cancer (HNC). EGF contributes to the growth of malignant tumors by stimulating cell proliferation and migration. It also participates in the dysregulation of autophagic activity and tumor metastasis through metalloproteinases.

Some therapies with monoclonal antibodies have had an anti-proliferative effect on cancer cells expressing EGFR. Cetuximab (Erbitux), a chimeric monoclonal antibody, recognizes an epitope in the extracellular domain III of EGFR. It has been widely used in clinical studies due to its ability to inhibit EGFR by occupying the ligand-binding site, reducing the proliferation of cancer cells. However, it has been shown to have better anti-tumor effects when combined with chemotherapy or radiotherapy.

Overexpression and increase in copy number of the EGFR gene
Hyperactivation and dimerization of EGFR play an important role in the regulation of the PI3K/AKT/mTOR pathway. The activation of this pathway revert autophagy through inhibition of Beclin-1 tyrosine phosphorylation, which in turn prevents the formation of class III PIK3 complex, promoting epithelial neoplasm progression. Hyperactivation of the PI3K/AKT/mTOR pathway is associated with the development, growth and proliferation of up to 50% of cancers. EGFR overexpression has been observed in epithelial-origin tumors as well as in NSCLC, renal, ovarian, breast, prostate cancer and colorectal carcinomas.

In HNC, 70-90% of neoplasms present overexpression of EGFR. In OSCC, there has been observed an increase in EGF expression in the cell plasma membrane of oral keratinocytes, resulting in a poor prognosis, high recurrence and lower survival rate.

EGFR mutations
EGFR mutations can be classified according to the specific region of the receptor they affect: extracellular, intracellular or TK domain. Deletion type mutations affecting the sequence encoding the N-terminal region, deletions of exons 2-7, 12-13, 14-15, 25-27, 25-28; duplications of exons 2-7, 18-21, and point mutations have been described. As a result, small deletions and insertions alter codon sequence producing proteins with aberrant function. These aberrant proteins may have a decreased activity or maintain a constitutive activation.

The mutated variant, known as EGFR variant III (EGFRvIII), encoded by EGFRvIII, has a deletion of exons 2-7, which encode the extracellular domain of ligand binding. The altered protein is constitutively active with slow degradation, allowing more time to interact with its ligand. It is the most common EGFR mutation and the best described in relation to various malignancies. EGFRvIII has been associated with increased tumor cell proliferation in mouse model and it has been observed that its presence is associated with a lower response to treatment with radiation therapy. McItyre et al. studied the expression of EGFRvIII in OSCC, noting that this is overexpressed in 2% of patients. Melchers et al. analyzed 531 cases of HNC and found no difference in the prevalence of the mutation compared to healthy controls. Khattri et al. found that only 2 (0.31%) out of 638 cases...
had the $\text{EGFRvIII}$ mutation. Therefore, this type of mutation is extremely rare in HNC and OSCC.\textsuperscript{3,28,31}

Mutations in exons 18, 19, 20 and 21 correspond to rare amino acid variations of the TK intracellular domain. About 90\% of $\text{EGFR}$ mutations are deletions located in exon 19 and the L858R point mutation of exon 21.\textsuperscript{29} Mutations in exon 20 encode proteins that normally are located after the C-helix of TK domain. This occurs in 4\% of all $\text{EGFR}$ mutations, with T790M substitution being the most prevalent, representing 50\% of all mutations in exon 20.\textsuperscript{32}

Hsieh et al.\textsuperscript{33} studied $\text{EGFR}$ in patients with OSCC who chewed betel nut, finding that 30.36\% had silent mutation at nucleotide 2607 in exon 20. This mutation does not alter the amino acid sequence and results in a mutation at codon 787 (Q787Q). They also identified two types of silent mutations in exon 21 which corresponded to 1.79\% of the cases; however, they found no mutations in exons 18 and 19. Furthermore, Nagalakshmi et al.\textsuperscript{17} studied $\text{EGFR}$ mutations in OSCC, finding that the samples studied showed mutations in exons 18 (nucleotides G2155C, G2176A), 19 (nucleotide C2188G) and 21 (nucleotide G2471A), with frequencies of 44.96\%, 32.55\% and 65.11\%, respectively.

**Defects in $\text{EGFR}$ downregulation**

In normal conditions, after ligand binding, cytoplasmic tyrosine residues from $\text{EGFR}$ are autophosphorylated, producing binding zones for various proteins. The recruitment of these proteins occurs in catalytic domains and/or scaffolds actively involved in cell signaling. An important pathway for deactivating TK receptors is downregulation. In this process, the activated receptor is internalized by the plasma membrane by means of endocytosis. Then, it is ubiquitinated and transported to the lysosomes where it is degraded by acid hydrolases.

When the TK domain is not deactivated appropriately, a failure in normal activity and operation of the receptor can occur.\textsuperscript{34,35} Yang et al.\textsuperscript{36} suggested that the ability of mutated $\text{EGFR}$ to escape downregulation may be due to lack of: ubiquitin binding, dysregulation of kinase associated with cyclin-G, and reduced levels of CD82 (metastasis suppressor).

Zhen et al.\textsuperscript{34} studied the effect of curcumin on cultivate cells from patients diagnosed with OSCC. The study concluded that curcumin revert growth of tumor cells by inhibiting $\text{EGFR}$ phosphorylation. Curcumin is known for inhibiting the growth, invasion and metastasis of malignant cells and for inducing apoptosis in breast cancer.

Another molecule that has been studied in conjunction with $\text{EGFR}$, is $\text{E-cadherin}$. This molecule is responsible for preserving integrity and cell morphology. Wang et al.\textsuperscript{37} observed that *in vitro* reduction of $\text{E-cadherin}$ increases the upregulation of $\text{EGFR}$ transcription. This suggests that loss of $\text{E-cadherin}$ can induce proliferation of HNC by activating $\text{EGFR}$ and its signaling pathways to the nucleus. It is essential to determine if the increase in $\text{E-cadherin}$ plays a role in the downregulation of $\text{EGFR}$.

**$\text{EGFR}$ crosstalk**

Cytoplasmic and nuclear signaling pathways can be activated by proteins acting at similar levels and conditions. This feature is known as crosstalk and is a form of evolutionary compensation to avoid a receptor being activated by a single ligand. Crosstalk between $\text{EGFR}$ and other members of the ErbB family, cytokine receptors, ion channels, G protein-coupled receptors and various cell adhesion molecules has been described in the literature.\textsuperscript{38,39} The integrin family phosphorylates the TK domain increasing the receptor activity.\textsuperscript{40} Zein et al.\textsuperscript{38} studied the relationship that existed between the EGF-$\text{EGFR}$ complexes and nerve growth factor with its receptor, finding that there is a bidirectional crosstalk between ligands and their receptors.

In HNC it has been observed that some of the ligands that bind to G protein-coupled receptors activate EGFR pathway, contributing to carcinogenesis.\textsuperscript{39} It is suggested that stimulation of gastrin-releasing peptide receptor activates $\text{EGFR}$ and modulates the growth and invasion of HNC.\textsuperscript{41} Egloff et al.\textsuperscript{19} characterized the expression and signaling of estrogen receptors (Era and Erb) in HNC in relation to the EGF-$\text{EGFR}$ complex. At the level of signal transduction and transcription, they found that Era and Erb receptors were expressed and stimulated in HNC.
Table 1. Laboratory technique by author according to EGFR alteration.

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<thead>
<tr>
<th>Type of Alteration</th>
<th>Laboratory Technique</th>
<th>Authors</th>
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<tbody>
<tr>
<td>Increased EGF ligand</td>
<td>ELISA</td>
<td>Zhang et al.45 2014</td>
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<td></td>
<td>IHC</td>
<td>Naik et al.48 2011</td>
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<tr>
<td>Overexpression of EGFR</td>
<td>IHC</td>
<td>Aquino et al.44 2012</td>
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<tr>
<td></td>
<td>WB</td>
<td>Zhang et al.45 2014</td>
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<tr>
<td>Copy Number Gain of EGFR gene</td>
<td>RT-PCR</td>
<td>Wang et al.37 2011</td>
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<td></td>
<td>FISH</td>
<td>Huang et al.46 2012</td>
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<td></td>
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<td>Aquino et al.44 2012</td>
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<td>Szabó et al.9 2011</td>
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<td></td>
<td>CISH</td>
<td>Bernardes et al.47</td>
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<td></td>
<td>Real Time-PCR</td>
<td>Bagan et al.26 2012</td>
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<td>EGFR mutations</td>
<td>PCR</td>
<td>McIntyre et al.3 2012</td>
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<td>Nagalakshmi et al.17 2014</td>
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<td>HRM</td>
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<td></td>
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<td>Melchers et al.31 2014</td>
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<tr>
<td>Defects in EGFR downregulation</td>
<td>MMF</td>
<td>Capuani et al.35 2015</td>
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<td></td>
<td>WB</td>
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<td>EGFR crosstalk</td>
<td>WB</td>
<td>Egloff et al.19 2009</td>
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<td>Thomas et al.39 2006</td>
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<td></td>
<td>ELISA</td>
<td>Zein et al.38 2010</td>
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DETECTION METHODS OF EGFR IN ORAL CANCER.

Clinical significance of EGFR detection in oral mucosa lies in its role as a biomarker or indicator of malignant transformation, diagnosis, progression and prognosis of OSCC. The National Cancer Institute defines a biomarker as any molecule found in fluids or tissues that is a sign of a physiological or pathological process.42

Identification of EGFR as an indicator of malignant transformation is based on its overexpression in potentially malignant samples as leukoplakia and oral epithelial dysplasia (OED).16 The expression of EGFR varies according to the degree of OED; expression is greater with increasing malignancy. Consequently, EGFR can be considered as a marker of cell epithelial proliferation, of OED, and as the onset of progression from dysplasia to OSCC.43 Bagan et al.26 reported that EGFR CNG is a potential marker for predicting malignant transformation of OED. The authors noted that EGFR CNG was significantly higher in malignant lesions and in non-homogeneous leukoplakia compared to homogeneous leukoplakia. Regarding OSCC, Aquino et al.44 evaluated overexpression of EGFR protein and EGFR CNG by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH), respectively. They found high expression of EGFR and EGFR CNG (14%), confirming the important role...
Desregulación y métodos de detección del EGFR en cáncer oral. Revisión narrativa.

Resumen: El receptor del factor de crecimiento epidérmico (EGFR) es una glicoproteína transmembrana, con un dominio intracelular y función tirosina quinasa (TK) que participa en la proliferación celular. Las fallas en las vías de señalización del EGFR se han asociado con la formación de tumores malignos orales como el carcinoma oral de células escamosas (COCE). El incorrecto funcionamiento del EGFR puede producirse por: aumento del ligando EGF; sobreexpresión del EGFR y ganancia en el número de copias del gen EGFR (GNC EGFR); mutaciones del EGFR; falla en la regulación negativa del EGFR; y diafonía del EGFR. De las alteraciones mencionadas, la sobreexpresión de EGFR es por lejos la disfunción más común. La EGFR, sin embargo, puede ser útil como marcador predictivo, pero es esencial desarrollar un método más accesible y no invasivo.

Dysregulation and detection methods of EGFR in oral cancer. A narrative review.

The most studied EGFR dysregulations in OSCC are receptor overexpression and mutations by means of IHC, FISH and PCR. These techniques are costly and complex, so it is crucial to develop a low-cost, non-invasive and easy to use method.
actuar como un biomarcador de diagnóstico y pronóstico para COCE. En la actualidad existen diversos métodos para detectar el EGFR. La inmunohistoquímica (IHC), la hibridación fluorescente in situ (FISH) y la reacción en cadena de la polimerasa (PCR), son técnicas utilizadas para identificar la sobreexpresión del EGFR, GNC EGFR y mutaciones del EGFR, respectivamente. La necesidad de detección de estas alteraciones se debe a la transcendencia del EGFR como biomarcador de transformación maligna. Lo anterior, hace necesario implementar un método de diagnóstico precoz para COCE que sea no invasivo y de bajo costo para la práctica clínica.

**Palabras clave:** Receptor del Factor de Crecimiento Epidermico, Neoplasias de la Boca, Mutación.

**REFERENCES.**


