TGF-ß alterations in oral squamous cell carcinoma.
Narrative review.

Abstract: The transforming growth factor beta (TGF-ß) is a cytokine that plays crucial roles in the regulation of angiogenesis, immune response, proliferation, migration and apoptosis of cells. In addition, it can inhibit cell progression and stimulate apoptosis in early stages of cancer. TGF-ß is a multifunctional homodimeric protein secreted by various cell lines, which have three different isoforms: TGF-ß1, TGF-ß2 and TGF-ß3. In normal conditions, TGF-ß1 activates some tumor suppressor cell signaling pathways that inhibit proliferation and are involved in cell migration, differentiation and apoptosis. However, in more advanced stages of cancer, when TGF-ß1 is altered, it acts as a promoter of tumorigenesis and may cause: 1) increased TGF-ß1, 2) overexpression of TGF-ß1 receptors (TßR), 3) TßR mutations, and 4) downregulation of TGF-beta receptor. In oral squamous cell carcinoma, the path is altered especially at the level of transmembrane receptors, with the TßR-II and TßR-III subtypes being the most affected. However, there is little information on the prognostic role it plays in the various types of cancers. It is important to study the signaling pathways of TGF-ß in order to develop techniques that may help detect their alterations and restore their normal operation. The objective of this review is to describe the alterations of TGF-ß in oral squamous cell carcinoma.

Keywords: Transforming Growth Factor Beta1, Smad4 protein, Squamous Cell Carcinoma, Oral Cancer.

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INTRODUCTION.

Oral cancer (OC) is a worldwide problem and the sixth most frequent among all cancers. Its most common histopathologic form is oral squamous cell carcinoma (OSCC), accounting for 90% of oral neoplasias. Although the quality of life of patients with OC has improved in recent decades due to new therapies, they continue to show low survival at 5 years, around 50%. Due to the above, the use of cellular biomarkers to diagnose the condition at an early stage and provide a prognosis prediction of malignant lesions is greatly encouraged.

The transforming growth factor beta (TGF-ß) has been defined as a potential biomarker for the diagnosis and prognosis of OSCC, because it regulates the tumor microenvironment. TGF-ß is a cytokine involved in the control of gene expression, in the immune system and in tissue repair. It also belongs to the superfamily of growth factors and is produced by different cell strains, such as: epithelial cells, fibroblasts, platelets, endothelial cells, lymphocytes, macrophages and even by malignant neoplastic cells.

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STRUCTURE OF TGF-ß.

The TGF-ß consists of 390 amino acids and comes...
from dimeric propeptides known as latency-associated proteins (LAPs). LAPs are cut proteolytically inside the cell by an endoprotease called furin at amino acid 278, resulting in the TGF-β ligand with biological activity and a second latent component.

There are several isoforms of TGF-β, designated as TGF-β1, TGF-β2, TGF-β3 in mammals. Isoform type I is encoded on human chromosome 19q13 and is 70% homologous to type II isoform. TGF-β isoforms are expressed in different cell types. Type I isoform is expressed in endothelial cells, hematopoietic cells and connective tissue cells; type II, in epithelial and neural cells; and type III, in mesenchymal cells. The different isoforms of TGF-β bind to transmembrane receptors TGF-β1 (TβR-I) and TGF-β2 (TβR-II) and these receptors have a short extracellular domain rich in cysteine, N-glycoside and an intracellular domain with serine-threonine kinase function. Unlike TβR-I and TβR-II receptors, the TGF-β3 receptor (TβR-III), also known as receptor betaglycan, does not have an intracellular signaling domain and its function is to facilitate the binding of TGF-β to TβR-II.

**Figure 1.** The binding of TGF-β to TβR-II allows phosphorylation of TβR-I. R-Smad proteins that were inactive become active once both receptors are phosphorylated. R-Smads form complexes with Co-Smad, which allows translocation into the nucleus and thus interact with other molecules and/or transcriptional factors. I-Smad can translocate into cytoplasm to antagonize signaling, either by binding to the type I receptor, preventing phosphorylation of R-Smad, or by directly binding to the R-Smad/Co-Smad complex.
MECHANISM OF ACTION OF TGF-ß.

Homodimeric ligands TGF-ß1 or TGF-ß3 first bind to TßR-II and then to TßR-I, allowing the formation of a pentameric complex. This complex consists of four serine-threonine kinase receptors (2 TßR-II and 2 TßR-I) and ligand TGF-ß. TßR-II activates TßR-I by phosphorylating its GS domain, which is rich in glycine and serine. The betaglycan receptor or TßR-III, binds to TGFß-2 and facilitates activation of TßRII, allowing the signaling pathway to continue.

Other molecules involved in the signaling pathways of TGF-ß are Smad proteins, which are a family of intracellular signaling molecules. Smad proteins are responsible for continuing the signaling cascade caused by TGF-ß from the cytoplasm to the target genes located in the nucleus. Smad proteins possess a highly conserved terminal portion consisting of a C-terminal (MH1) and N-terminal (MH2) connected by a proline-rich region.

These proteins are classified into 3 subfamilies involved in the signaling pathway of TGF-ß. The R-SMADs (SMAD2 and SMAD3) are directly activated by TßR-I, Co-Smads (SMAD4) are a common mediator and I-Smads (SMAD6 and SMAD7) have an inhibitory effect.

R-Smad proteins are found in the cytoplasm and are attached to chaperone proteins of Smad-anchor for receptor activation (SARA). Once TßR-I is activated, phosphorylation of the complex formed by SMAD2 and SMAD3 occurs. This results in the release of SARA proteins, allowing the binding of R-Smad to SMAD4, facilitating their translocation into the nucleus of the cell.

Inhibitory Smads act at the level of TGF-ß affecting its correct signaling. This mechanism occurs by binding of I-Smad to activated TßR-I and/or SMAD4 protein, resulting in an altered TGF-ß pathway (Figure 1). The strength and duration of TGF-ß signaling will depend on the amount of active Smad complexes and on the transcription rate generated in the nucleus. The latter process is critical to maintain proper biological control. High concentrations of this gene produce negative feedback activating SMAD7, resulting in the formation of an inhibitory complex capable of degrading the ligand-receptor binding.

ROLE OF TGF-ß1.

TGF-ß1 is the most studied isoform and the most commonly associated with cancer pathogenesis. Because of this, we believe it is important to discuss the various roles this molecule plays in the body.

TGF-ß1 can be considered a multifunctional cytokine, due to its effects on different cellular targets. What distinguishes TGF-ß1 from other cytokines is its great ability to inhibit cell proliferation, besides participating in cell differentiation, migration and apoptosis. Its involvement in the differentiation of multipotent progenitor cells, for example, in chondrogenesis from stem cells, is highly relevant.

TGF-ß1 also helps in stabilizing neoformed capillaries, intervening in the processes for the formation of the extracellular matrix, which is why it relates to processes of angiogenesis and vascular repair. In addition, TGF-ß1 inhibits the activation of T cells and antibody secretion by B cells.

The change in the concentration of TGF-ß1 in different organs and lymphoid tissues causes a deregulation of the immune system. This deregulation leads to inappropriate activation of cellular immune response, causing uncontrolled inflammation and promoting the formation of various types of cancers, such as lymphoma and leukemia.

TGF-ß, TßR AND ORAL CANCER.

TGF-ß pathway is considered a pleiotropic regulator of human cancer and its deregulation is associated with oral carcinogenesis. In the early stages of cancer, TGF-ß has suppressive effects by inhibiting cell cycle progression and promoting apoptosis. However, in later stages, TGF-ß has promoting effects, increasing tumor invasiveness and metastasis. Alterations involving TGF-ß, TßR and Smad molecules are multiple, including:

1) decrease or overexpression of the ligand, receptor and Smad molecules.
2) mutations of the genes,
3) failure in downregulation pathways and
4) Crosstalk of the molecules involved in the signaling
pathway of TGF-β. Deregulation of TGF-β pathways has been associated with the development and progression of oral squamous cell carcinoma. However, its type 1 isoform is the most reported in studies and experiments related to OSCC.

**Decreased or overexpression of TGF-β, TβR and Smad molecules**

It has been observed that some of the early changes in malignancy of keratinocytes is a decrease of TGF-β1 ligand. Deregulation of TGF-β1 in head and neck squamous cell carcinomas (HNSCC) has been already studied. Pring et al. concluded that TGF-β1 deregulation induces Smad activity in most HNSCC cell lines. At oral level, Mincione et al. studied by immunohistochemistry (IHC) and Western blot (WB) the expression of TGF-β1 in OSCC. They concluded that the decreased expression of these proteins is a common event and is significantly related to the progressive loss of cell differentiation in OSCC. Another study comparing by IHC and WB the presence of SMAD4 in normal mucosa and OSCC concluded that the alteration in TGF-β pathways following the decrease or absence of expression of SMAD4 promotes carcinogenesis of OSCC.

Furthermore, in OSCC loss of TβR expression is described as the most common alteration in the signaling pathway, as evidenced by the research conducted by Meng et al., the first systematic study of the importance of TβR-II and -III TβR in the development of carcinogenesis. The researchers reported that as the lesion progresses from normal tissue to OSCC, expression of TβR-II and TβR-III decreased by 35.3% and 52.9%, respectively. This decrease in the expression of TβR-II and TβR-III correlated as the disease progressed. Similar results were obtained by Andl et al., who showed that inhibiting the formation of E-cadherin (CDH1) and TβR-II may be sufficient for the development of OSCC, indicating a worse prognosis for the patient. However, Li et al. reported that overexpression of TβR-III restores sensitivity of TGF-β1 in cells CAL-27, providing a possible target for the treatment of OSCC.

**Mutations of TGF-β, TβR and SMAD molecules**

Followed by decreased expression of TGF-β1, gene polymorphism is the most frequent alteration associated with an increased risk of developing HNSCC compared to its normal genetic form. Single-nucleotide mutations (SNPs) in the TGF-β genes can affect the expression or function of signaling. Various SNPs have implications for the predisposition to cancer, being mutated codon 10 (T869>C) and codon 25 (915G>C) of exon 25 the most documented polymorphisms of TGF-β1. Polymorphism T869>C is located in the promoter region of the TGF-β1 gene, and it has been observed that it increases the risk of HNSCC. The presence of that specific polymorphism in OSCC has also been studied, being the most frequent in the group of patients with oral cancer. This would increase the secretion of TGF-β which suppresses anti-tumor immune responses altering the risk of developing OSCC. On the other hand, Hsu et al. were the first researchers to determine the association between the presence of TGF-β1 polymorphism of codon 25 and oral cancer. For the analysis of polymorphism 915G>C, they obtained cellular DNA through blood samples. DNA was analyzed by the method of polymerase chain reaction (PCR). Their results showed that the oral mucosa was the most affected area with histological diagnosis of OSCC. They concluded that if polymorphism of TGF-β1 is present, there is a risk of 11.09 times greater of developing oral cancer in comparison with controls.

Few studies have tried to identify mutations at TβR level. Park et al. studied the presence of a new mutation with a gain of function of TβRII receptor in OSCC. TβRII mutant expression improves TGF-β signaling which leads to more invasive phenotypic changes in the tumor. Moreover, TβRIII mutation is considered a SNP of codon 191 of exon 4, which induces a structural change of an amino acid with altered hydrophobicity. In addition, its high concentration in oral cancer suggests the possibility of considering it a predisposing factor for the development of OSCC.

Mutations of Smad molecules have been studied in
HNSCC associating deregulation of TGF-β pathways with cancer development. Alterations in TGF-β signaling and Smad were evaluated by Sivadas et al. by reverse transcriptase PCR in OSCC, showing the existence of genetic alterations at the level of TßRII and SMAD3. These results suggest that transcription levels of TßRII can be used as prognostic biomarkers of oral cancer.

**Failure in downregulation pathways of TGF-β, TßR and SMAD molecules.**

It is known that increased levels of expression of TGF-β during tumor progression stimulates the downregulation of TßR in the tumor and stroma. This results in a reduction of the apoptosis mediated by TßR. It has been determined that the functional loss of TßRII and the absence of TßRIII signaling can contribute to apoptosis evasion facilitating tumorigenesis. Because of this, it is believed that the restoration of the expression levels of TßRII and TßRIII in malignant tissues of the oral mucosa can serve as a new target in the treatment of OSCC. Observations by Mincione et al. suggest the possibility that the downregulation of TGF-ß1 is involved in an increase in the progression of OSCC and its detection can be a useful tool for the diagnosis or prognosis of oral cancer.

**Crosstalk of the molecules involved in the signaling pathway of TGF-β**

Information regarding the crosstalk of Smads and other molecules is scarce. The existing crosstalk between TGF-β signaling and Nuclear Factor-kB (NF-kB) pathway has been studied in HNSCC. TGF-ß1 molecules of activated kinase 1 (TAK1) and SMAD7 are those that are deregulated in the tumors. Normally the small interfering RNA (siRNA) of TAK1 inhibits cell migration-proliferation and invasion. This makes TAK1 a key molecule in aberrant NF-kB activation and promotion of a malignant phenotype in HNSCC. Moreover, NF-kB can contribute to the decrease in the downregulation of TGF-ß through increased expression of SMAD7. Freudlsperger et al. suggest Celastrol as a potential therapy for deregulation of the signaling pathway of TGF-ß and -NF-kB.

SMAD7 contributes to the process of invasion through the epithelial-mesenchymal transition (EMT) specifically in OSCC. A recent study by Ge et al. concludes that dysfunction of protein ubiquitination plays a critical role in carcinogenesis of OSCC. So the removal of CYLD (desubiquitinase) in OSCC may promote cancer invasion mediated by SMAD7.

**Epithelial-mesenchymal transition in oral squamous cell carcinoma.**

It has been observed that OSCC with similar clinical pathological characteristics may differ dramatically in their clinical evolution, suggesting that EMT has an important role during the invasion and metastasis of cancer. The mechanism that allows the invasion of neoplastic epithelial cells into adjacent connective tissue has been associated with the expression of cytokines, such TGF-ß28,45. Hwang et al. suggested that TGF-ß1 synthesized by stromal fibroblasts promotes expression of Podoplanin, which participates in the separation of tumor cells. This facilitates neoplastic cellular invasion with a more aggressive growth phenotype.

In oral cancer, TGF-ß1 deregulation promotes EMT. This is why studies focused on EMT and its relationship in carcinogenesis have increased recently. A study by Qiao et al. about Snail and Slug molecules, which play a evolutionary role in mesoderm formation in vertebrates, and TGF-ß1 concludes that they all together regulate EMT. It has been observed that functions of Snail as a molecular mediator regulated by TGF-ß1 increase the expression of metalloproteinases subtype 9 and contribute to the development of oral cancer. Moreover, Richter et al. reported that EMT in OSCC was mediated by multiple growth factors, including EGF and TGF-ß1.

Stimulation of osteoclastogenesis is another mechanism by which TGF-ß1 contributes to the progress of OSCC through the tissues. It is assumed that this mechanism could occur because TGF-ß1 promotes the differentiation of stromal cells to osteoclast cells. This suggests that TGF-ß1 induces EMT not only to increase the capacity of invasion of OSCC, but it could also promote factors that prolong the survival of osteoclasts.
CONCLUSION

The study of the cell line of OSCC and histopathologic samples or body fluids of patients diagnosed with OSCC has helped to identify alterations in TGF-β contributing to the development and invasion of OSCC. However, the importance of studying this cytokine in OSCC is to develop drug targets that increase the synthesis of TGF-β1, the expression of TBR-II and TBR-III, or partial inhibition of the action of SMAD7 or devices for diagnosis or prognosis biomarkers in order to increase the life expectancy of the patient. However, it is important to evaluate the points of convergence of TGF-β with other oncogenic signaling pathways and factors in the tumor microenvironment, with the aim of improving the understanding of carcinogenesis and providing better cancer therapies. This will allow to develop personalized treatments according to the specific alterations of each tumor, through more effective drugs with fewer adverse effects that significantly impair the quality of life of patients.

Alteraciones de TGF-β en el carcinoma oral de células escamosas. Revisión narrativa.

Resumen: El factor de crecimiento transformante beta (TGF-β) es una citocina que cumple funciones fundamentales en la regulación de la angiogénesis, respuesta inmune, proliferación, migración y apoptosis celular. Además, puede inhibir la progresión celular y estimular la apoptosis en etapas tempranas del cáncer. El TGF-β es una proteína homodimérica multifuncional secretada por diversas líneas celulares, que presentan 3 isofomas: TGF-β1, TGF-β2 y TGF-β3. En condiciones normales TGF-β1 activa a algunas vías de señalización celular supresoras de tumores que inhiben la proliferación, y participan en la migración, diferenciación y apoptosis. Sin embargo, cuando esta se ve alterada, en etapas más avanzadas del cáncer actúa como promotor de la tumorogénesis, pudiendo producir: 1) aumento del TGF-β1, 2) sobre expresión de los receptores del TGF-β1 (TβR), 3) mutaciones de TβR, y 4) falla en la regulación negativa de TβR. En el carcinoma oral de células escamosas, la vía se ve alterada especialmente a nivel de sus receptores transmembranales, siendo los subtipos TβR-II y TβR-III los más afectados. Sin embargo, es escasa la información sobre el rol pronóstico que juega en los diversos tipos de cánceres. Es importante estudiar las vías de señalización de TGF-β para desarrollar técnicas que detecten sus alteraciones y restauren el funcionamiento del sistema. El objetivo de esta revisión es describir las alteraciones de TGF-β en carcinoma oral de células escamosas.

Palabras clave: Factor de crecimiento transformante beta 1, Proteína Smad4, Carcinoma Epidermoide, Cáncer Oral.

REFERENCES.


