ORAL CANCER BIOCHEMICAL RESEARCH: CURRENT STATUS AND EMERGING FRONTIERS
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Abstract
Cancer represents one of the leading causes or mortality worldwide, oral cancer accounting for almost 9% of deaths, early diagnosis playing a crucial role. Salivary biomarkers analysis is proving to be an alternative diagnosis method. Oral cancer biomarkers can be compounds that play role in every aspect of malignancy from triggering factors to markers of progression, inflammation or invasiveness. There are numerous genomic markers, ranging from well known ones such as p16, p21, p27 and p53 genes, cyclin D1, EGFR gene (epidermal growth factor receptor), C-kit gene (KIT proto-oncogene, receptor tyrosine kinase), bcl-6(B-cell lymphoma 6 protein gene) to least studied ones such as OXSR1(oxidative stress-responsive kinase-1gene). Proteomic markers range from inflammatory factors such as interleukins IL-8 and IL-6, transcription factors such as FOXO3 (forkhead box O3) protein and S100B protein, matrix metalloproteinases (MMP) involved in extracellular matrix degradation and their inhibitors (TIMP - tissue inhibitor of metalloproteinases), specific proliferation markers such as Ki-67 protein and many more. Developing saliva based oral cancer screening and prognosis tests may lead to better treatment options.

Keywords: Oral cancer, biomarkers, receptors, signalling pathway

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Introduction
Cancer represents one of the leading causes or mortality worldwide, oral cancer accounting for almost 9% of deaths (1), oral squamous cell carcinoma, being the most common type with an incidence of nearly 90% (2).

Early diagnosis plays a crucial role in oral cancer therapy, clinical examination being still the most widely used screening method, early detection could greatly increase five-year survival rates(3). The need for cancer lesions to be clinically detectable means that very often these lesions are diagnosed in advanced stages making oral examination an inefficient screening method. The chances of early stage oral cancer detection could be highly increased by the existence of specific biomarkers based biochemical analysis. These biomarkers could be used in all stages of cancer development from screening to prognostic assessment and potential post treatment recurrence(4) and can be analyzed either in serum, saliva or tissue samples.

Serum analysis is an invasive method requiring trained medical personnel. Tissue
sample analysis require even more invasive methods and longer time periods than serum or saliva investigation methods and often are performed after the surgical removal of the tumor (5).

Salivary biomarkers analysis is proving to be an alternative diagnosis method for several diseases due to a noninvasive character (6) and because it does not require trained medical personnel or special medical devices for collection (7, 8). It contains almost all the compounds found in blood, oral cells and in oral cancer research another further advantage is the continuous contact with oral malignant or premalignant lesions (9).

**Oral cancer biomarkers**

Oral cancer biomarkers can be compounds that play role in every aspect of malignancy from triggering factors to markers of progression, inflammation or invasiveness. They can be classified in genomic, proteomic or metabolomic markers (10, 11).

Genetic modifications such as mutations, amplifications, silencing and epigenetic changes play a crucial role in normal oral cells transformation into cancer cells and are influenced by extrinsic factors (smoking, viral infections) and intrinsic factors (random errors in DNA replication) resulting in the triggering, progression and recurrence of oral cancer (12).

There are numerous genomic markers studied in different types of cancer including oral cancer, ranging from well-known markers such as p16, p21, p27 and p53 genes, cyclin D1, EGFR gene (epidermal growth factor receptor), C-kit gene (KIT proto-oncogene, receptor tyrosine kinase), bcl-6 (B-cell lymphoma 6 protein gene) to least studied ones such as OXSR1 (oxidative stress-responsive kinase-1 gene) (13-15).

OXSR1 gene encodes the protein OSR1 (oxidative stress-responsive kinase-1) that regulates NKCC1 (Na⁺/K⁺/2Cl⁻ Cotransporter-1), an ion carrier with crucial roles in ion homeostasis and cell-volume regulation (16, 17). Other roles are cell differentiation and proliferation and thus regulating apoptotic cell death, its expression and suppression being influenced by environmental factors such as oxidative stress (18). One study found that in oral cancer patients OXSR1 gene is downregulated, oxidative stress protection and other protective pathways in which OSR1 is involved being inhibited (19).

Due to the high content of proteins found in saliva, proteomic analysis in oral cancer can offer new ways for detection and progression assessment in oral malignancies (20). Proteomic markers range from inflammatory factors such as interleukins IL-8 and IL-6, transcription factors such as FOXO3 (forkhead box O3) protein and S100B protein, matrix metalloproteinases (MMP) involved in extracellular matrix degradation and their inhibitors (TIMP - tissue inhibitor of metalloproteinases), specific proliferation
markers such as Ki-67 protein and many more.

FOXO3 is coded by the FOXO3 gene and belongs to the O subclass of the forkhead family of transcription factors regulating gene expression. It is inactivated through phosphorylation by phosphatidylinositol-3-kinase (PI3Ks) pathways and Ras-Raf-MEK-extracellular signal regulated kinase (ERK) signaling pathways (21). PI3Ks and ERK pathways are under different growth factors control. FOXO3 protein controls apoptosis and cell cycle arrest mechanisms by regulating the expression of genes involved in those cellular processes. FOXO3 levels are associated with cancer development and tumoral progression (21). This marker has not been analyzed in human saliva.

S100B protein is a member of a protein family that regulates cellular responses by acting as intracellular Ca^{2+} sensors. They have two functional domains joined by a hinge portion. The functional domains bind Ca ions and thus exposing a hydrophobic center that attaches to the cellular target (22). One key role of S100B protein is to inhibit protein kinase C phosphorylation of different compounds such as p53 protein thus inhibiting cellular apoptosis. It is also responsible for regulating cell shape, energy metabolism intracellular signal transduction and cellular growth (23).

High levels of S100 proteins were detected in oral cancers patients (24). High levels of S100B protein were found in other types of cancer such as lung cancer (25) and melanoma (26).

Inflammation plays a crucial role in cancer development and progression. Molecular messengers such interleukins are produced either by normal cells as a response to malignancy or by cancer cells as a mechanism for influencing local tumoral environment.

There are several classes of interleukins with each with multiple roles. IL-6 and IL-8 are regarded as two of the most important due to their proinflammatory role. They are released mainly from activated inflammatory cells from the tumoral microenvironment. IL-8 acts on two receptors CRCX-1 and CRCX-2 (chemokine receptor 1 and 2) found on macrophages, neutrophils and cancer cells (27) and induces cellular proliferation and angiogenesis in the tumoral microenvironment (28).

IL-6 acts via mitogen-activated protein kinase pathway (MAPK or MAP kinase) also inducing cellular proliferation and angiogenesis (29). IL-8 and IL-6 combined effects are to induce and promote tumoral progression and metastasis. Both these biomarkers have elevated levels in oral cancer patients saliva (30).

Local invasiveness is another key mechanism in oral cancer progression. Matrix metalloproteinases (MMPs) are a group of zinc-dependent end proteases with roles in degradation and remodeling of extracellular matrix (ECM) and are secreted by both normal and tumoral cells.
Structurally all classes of MMPs share a common structure consisting of a propeptide domain, a catalytic metalloproteinase domain that contains Zn2+ atom, a hinge region and a hemopexin domain(31). They act on all types and collagen and elastin in the ECM, hemopexin domain conferring substrate specificity for different collagen types(32). The catalytic domain is responsible for their action on the noncollagen constituents of the ECM(33). MMPs expression is regulated at transcription level by cytokines such as IL-1, at proenzyme activation level by oxidative stress and by the TIMP concentration (34).

Tissue inhibitor of metalloproteinases are a group of proteins that block MMPs action. TIMPs N-terminal domain is structurally similar with MMPs substrate, their interaction inhibits the catalytic domain of MMPs. There are four classes of TIMP all inhibiting various MMPs(35). Recent studies linked TIMPs with other inhibitory roles on non-MMP metalloproteinase such as ADAMs and ADAMTSs metallopeptidases(36).

In normal tissues there is a balance between MMPs and TIMPs levels regulating ECM formation and degradation. In malignant tumors overexpression of MMPs and coupled with lower levels of TIMPs plays a crucial role in local invasiveness and tumoral progression and are associated with a higher metastatic rate and poor prognosis(37-39).

Cellular proliferation is a fundamental characteristic of cancer. There are several markers of proliferation one of them being Ki67 protein. It is encoded by Ki67 gene and it is expressed in all active stages of cell cycle (G1, S, G2 and mitosis) and absent in the resting phase (G0). This characteristic makes this marker a great tool for monitoring the growth rate of a cellular population both in tumoral and in potentially malignant lesions such as leukoplakia (40). High levels of this protein are associated with high rate tumoral growth, high invasiveness and poor prognostic and can also play a role in recurrence screening(41-43).

In our research department from Carol Davila Faculty of Dental Medicine salivary research is a topic of great interest. In several studies we proposed the possible usage of saliva as a diagnostic tool in several diseases such as periodontal disease, systemic lupus erythematosus and oral cancer(44, 45).

Salivary biomarkers in oral cancer and their correlations were studied in a research paper that focused on several key aspects of cancer development such as oxidative stress markers (TAC - total antioxidant capacity of saliva and salivary uric acid – UA), markers of cellular proliferation (Ki67), specific markers for oral squamous cell carcinoma (SCCAg - squamous cell carcinoma antigen). For progression and local invasiveness matrix metalloproteinases (MMP-9) and tissue inhibitor of metalloproteinases (TIMP-2) were investigate(46). Inflammation markers such as IL-6 were also analyzed in this research.
All biomarkers included in this study showed modified levels in oral cancer patients saliva compared to healthy volunteers. Low levels of TAC and UA were found indicating a high level of oxidative stress and a poor antioxidant defense in oral cancer that alters tissue homeostasis. A positive correlation between UA and TAC levels was found indicating UA as the main antioxidant in saliva.

Ki67 proliferation marker showed significant increased levels in our study and was positively correlated with MMP9 levels confirming the high invasiveness and rapid proliferation of oral cancers.

High levels of SCCAg and the absence of this biomarker in control group confirmed that oral squamous cell carcinoma is the main type of malignancy in oral cavity.

The high invasiveness and poor prognosis of oral cancers was confirmed in our study by elevated MMP-9 levels and lowered TIMP-2 levels. A negative correlation between these two parameters was also found indicating that a high rate of ECM degradation is needed for local and metastatic dissemination of oral tumors.

Conclusions

Salivary research of old and established biomarkers associated with new emerging ones can lead to better understanding of oral cancer processes and can lead to the usage of saliva as a quick and reliable screening and diagnostic tool for oral cancer.

Conflict of interest

The authors declare no conflict of interest.

References

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