

Biomarkers profile of oral squamous cell carcinoma

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ABSTRACT

More than 95% of oral cancers are represented by squamous cell carcinomas (OSCC). Despite the serious progress concerning the oral cancer treatment made in the last decade, the OSCC prognosis remains poor. Currently, the OSCC detection and diagnosis is mainly based on clinical oral examination combined with biopsy for a histopathological examination if a suspect area is detected. Our study has included 20 patients (12 females and 8 males) diagnosed with OSCC. TAC, OXSR1 and MMP-9 were measured in the tumoral cells lysates by ELISA. Our results revealed that the MMP-9 levels were significantly and negatively correlated with OXSR1 levels. Also, our study results illustrated a significant positive correlation of TAC with OXSR1. In conclusion, corroborating our findings with literature reports, we can outline that OXSR1 and MMP-9, together, possibly, with TAC, could be regarded as new steps in order to elaborate diagnostic tools for early detection OSCC.

Keywords: oxidative stress, biomarker, carcinoma squamous cell, oral cavity, OXSR1, MMP-9

INTRODUCTION

More than 95% of oral cancers are represented by squamous cell carcinomas (OSCC) [1]. Despite the serious progress concerning the oral cancer treatment made in the last decade, the OSCC prognosis remains poor. Although the oral cavity is quite accessible for direct visual examinations, most patients are diag-

nosed with OSCC in advanced stages. This is believed to be one of the major reasons for the low survival rate (of about 50 to 63%) which characterizes OSCC [2]. However, the early diagnosed oral cancers have less morbidity and are often highly curable [3]. These statistic data highlight the importance of early and accurate diagnosis of OSCC.

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The laboratory testing provides important and accurate information in order to sustain the diagnosis and prognosis of human diseases, and especially of cancer. Chronic diseases, like cancer, may have progressed to intermediate or advanced stages by the time of diagnosis, which will trigger a poorer prognosis. Consequently, an earlier diagnosis is an important, but challenging, goal. Clinical early diagnosis may be built on a foundation represented by the developing of accurate laboratory testing methods.

More than 100 potential OSCC biomarkers have been suggested in the current literature [4, 5]. Some of these promising potential biomarkers are: 1) the oxidative stress biomarkers, oxidative stress responsive protein 1 (OXSR1) and the total antioxidant capacity (TAC) and 2) the metalloproteinase-9 (MMP-9).

MMP-9 is a protease that has been closely associated with cancer pathogenesis and progression, as being involved in the extracellular matrix degradation. Significant differences have been reported in salivary MMP-9 levels between the OSCC patients' group and the respective control group [6, 7].

OXSR1 is a 58-kD protein of 527 amino acids encoded by the OXSR1 gene. It is widely expressed in most of the tissues [8]. OXSR1 belongs to the GCK VI subfamily and is composed of an N-terminal catalytic domain and two regulatory regions [9]. An increasing number of studies have reported that OXSR1 is deeply involved in vital cell events: apoptosis, migration, autophagy [10, 11]. Recent experimental data have suggested that OXSR1 may play an important role in malignant progression [12]. However, the prognostic value and function of OXSR1 in cancer are still unclear.

The purpose of our study was to analyze the existence of some correlations between oxidative stress biomarkers (OXSR1 and TAC) and MMP-9 in OSCC tumors.

MATERIAL AND METHODS

Our study has included 20 patients (12 females and 8 males) diagnosed with OSCC. The mean age was 55.0±10.9 years (mean±SD).

Our prospective study was initiated after the ethics committee of „Carol Davila“ UMF have approved it. The informed written consent has been obtained from all individual participants included in the study.

Tumoral tissue samples have been collected from the included participants during surgery. Tumoral cells lysates have been obtained according to the assay kits manufacturer's recommendations.

TAC, OXSR1 and MMP-9 were measured in the tumoral cells lysates by ELISA, using assay kits from Elabscience, USA (for TAC and MMP-9) and Abbexa, United Kingdom (for OXSR1).

The statistical analysis has been made by IBM SPSS Statistics 25, Microsoft Office Excel/Word 2013 and the Shapiro-Wilk distribution test. For the correlations analysis we have used the correlation coefficient Spearman's Rho.

RESULTS

Our results presented in Table 1 revealed a non-parametric distribution, according to Shapiro-Wilk test ($p < 0.05$), for TAC and OXSR1 levels. Also, TAC has been significantly and positively correlated with OXSR1, ($p < 0.001$, $R = 0.892$) (Figure 1).

TABLE 1. TAC and OXSR-1 correlation

Correlation	p*
TAC ($p = 0.004^{**}$) x OXSR-1 ($p = 0.012^{**}$)	< 0.001, R = 0.892

*Spearman's rho correlation coefficient, **Shapiro-Wilk Test

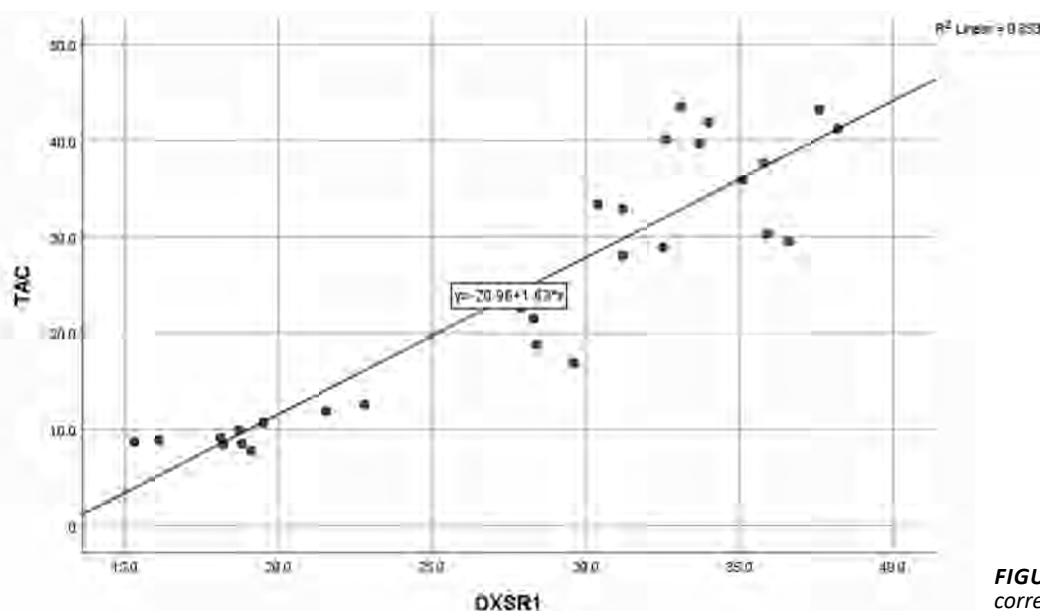


FIGURE 1. TAC and OXSR-1 correlation

Moreover, our experimental data have shown a non-parametric distribution, according to Shapiro-Wilk test ($p < 0.05$), for MMP-9 and OXSR1 levels (Table 2). We also have found that the MMP-9 levels were significantly and negatively correlated with OXSR1 levels ($p = 0.013$, $R = -0.462$) (Figure 2).

TABLE 2. MMP-9 and OXSR-1 correlation

Correlation	p*
MMP-9 ($p < 0.001^{**}$) x OXSR-1 ($p = 0.012^{**}$)	0.013, $R = -0.462$

*Spearman’s rho correlation coefficient, **Shapiro-Wilk Test

DISCUSSION

Currently, the OSCC detection and diagnosis is mainly based on clinical oral examination combined with biopsy for a histopathological examination if a suspect area is detected [1,2]. Development cancer is characterized by the consequence of multiple molecular events, going through three phases: initiation, promotion, and progression [13]. Reactive oxygen species (ROS) are involved in all these phases. Oxidative stress (OS) effect at a certain stage of carcinogenesis has been shown to be directly proportional to the type and the reactivity of radicals involved [13].

Initiation of cancer by OS is sustained by the presence of oxidative DNA modifications in cancer tissues [13]. The promotion phase is represented by induction of cell proliferation and/or inhibition of apoptosis and by the clonal expansion of initiated cells. OS seems to play a key role during this phase. Normally, the redox status should be regarded as signals translator triggering pro-survival actions. However, experimental data suggested that ROS can stimulate mutated cell clones’ expansion by temporarily modulating the genes

involved in cell proliferation and apoptosis [14]. This genes’ modulation is based on the regulating activity of certain transcription factors such as nuclear factor- κ B (NF κ B), Nrf2, HIF, and p53, main actors on the cell growth and oncogenesis stage [15]. Karin et al. suggested that a redox imbalance can lead to NF κ B activation, with subsequent induction of genes encoding for apoptosis inhibitors [16]. OS, as consequence of a redox imbalance, can activate the PI3K/AKT pathway. This molecular event could trigger proapoptotic proteins’ inactivation and the up regulation of antiapoptotic genes [16,17]. On the other hand, the redox imbalance caused by reduced levels of ROS, may stimulate cell division during promotion stage, and thus triggering tumor growth [18-20]. This highlights the fact that, at least this phase, ROS production represents the main mechanism of ROS-related tumor promotion [18-20].

ROS are also deeply involved in the molecular landscape of the last phase of carcinogenesis - progression. In this stage, high levels of ROS may play important roles in anti-proteases’ inhibition and could upregulate matrix-metalloproteinases (MMPs), causing injuries of neighboring tissues.

Moreover, increased levels of oxidatively modified DNA bases may induce genetic instability, enhancing the metastatic potential of tumor cells, in the fully developed cancer [21-23]. Maulik et al. have reported that ROS seemed to be important for angiogenic responses, crucial events in cancer metastasis [24].

All these presented above suggest that ROS are involved in all phases of carcinogenesis and determined us to elaborate this study, in order to highlight the possible significant correlations between OS biomarkers and MMP-9 levels in the OSCC context. As OS biomark-

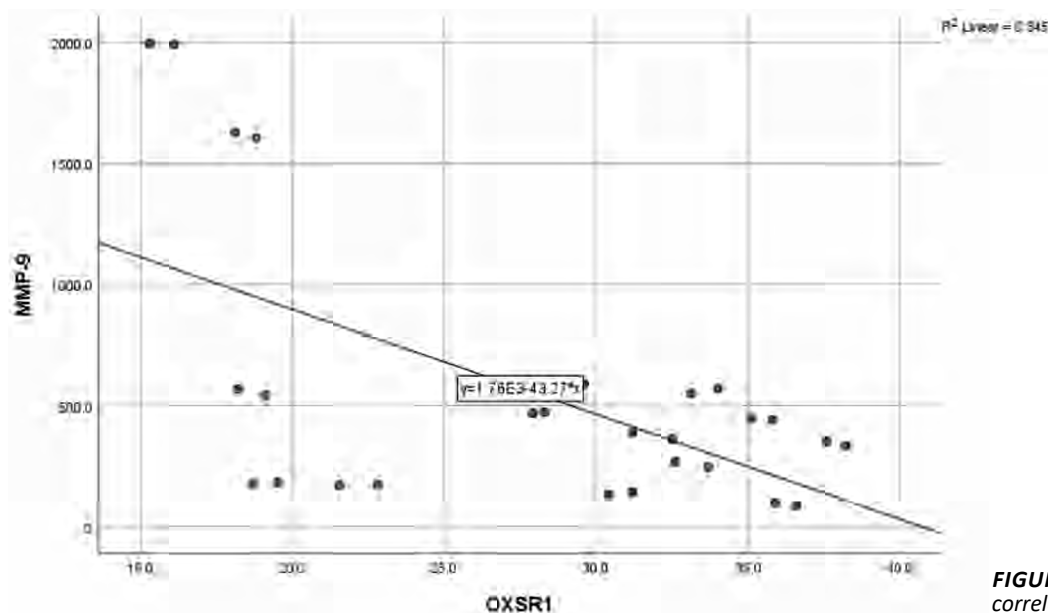


FIGURE 2. MMP-9 and OXSR-1 correlation

ers we thought to dwell on TAC and protein OXSR1. In our opinion, TAC represents a good illustrator of the redox balance. OXSR1 is activated in response to OS [25] and, also, plays important roles in angiogenesis by activation of WNK 1 (With no lysine (K)) protein [10]. Rauch et al. reported that the OXSR1 gene's hyper-methylation of the promoter region led to the inhibition of gene transcription, in lung squamous cell carcinoma [26]. Moreover, it has been noticed that OXSR1 is a main actor in molecular pathways associated with anti-tumoral protection mechanisms. Cusik et al. showed that OSR1 induced the phosphorylation of the tumor necrosis factor receptor RELT (Receptor expressed in lymphoid tissues) [27]. Tumor necrosis factors are crucial in tumor cell death, proliferation, apoptosis, inflammation, and stress response [28]. Consequently, OXSR1 mediated activation of RELT should be considered an important mechanism for the apoptosis induction and tumor development inhibition. We have also considered of great interest the level of MMP-9 in the OSCC tumoral cells MMP-9, considering that it is regarded as important player in numerous pathological processes, as they specifically degrade type IV collagen, a major component of the basal lamina, elastin and fibronectin [7, 29]. Nagase et al. have reported a high expression of MMP-9 in stromal cells surrounding the invading front of metastasizing tumors [7, 29].

Our results revealed that the MMP-9 levels were significantly and negatively correlated with OXSR1 levels ($p = 0.013$, $R = -0.462$) (Figure 2). Also, our study results illustrated a significant positive correlation of TAC with OXSR1, ($p < 0.001$, $R = 0.892$) (Figure 1).

According to our knowledge, there are no data in the literature on OXSR1 levels' correlation with MMP-9 or TAC, in OSCC tumoral cells. However, experimental data showed that OXSR1 expression was abnormally elevated in hepatocellular carcinoma [30]. Furthermore, Jianhui Chena et al. highlighted that upregulated OXSR1 should be regarded as an independent prognostic factor to predict the worse clinical outcome of hepatocellular carcinoma patients [30].

Moreover, the study of Li Y showed that OXSR1 announced a poor clinical outcome and lymph node metastasis in breast cancer context [31].

Regarding MMP-9, Chen L et al. reported enhanced levels of this protease in melanoma tumor endothelium [32], leading to the conclusion that MMP-9 may play a significant role in angiogenesis. Thomas et al. revealed that in vitro MMP-9 expression was controlled by integrin alpha v beta 6, expressed only in OSCC and not in the normal oral epithelium cells [33]. Shpitzer et al. reported that MMP-9 increased in OSCC patients compared to controls by 35% to 39% [34].

CONCLUSION

Corroborating our findings with all these literature reports, we can outline that OXSR1 and MMP-9, together, possibly, with TAC, could be regarded as new steps in order to elaborate diagnostic tools for early detection OSCC. Therefore, improved and more sensitive diagnostic protocols able to identify patients with high risk for tumor relapse are acutely needed.

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