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REVIEW ARTICLE

Early Diagnosis of Oral Squamous Cell Carcinoma by Salivary microRNAs

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SUMMARY

Background: **Oral squamous cell carcinoma (OSCC), the most common type of oral cancer, and represents more than 90% of malignancies of the oral cavity. Worldwide, each year about 275,000 are newly diagnosed. If detected at an early stage, OSCC has a survival rate of up to 80% compared to the detection in later stages (T_3-T_4) when a survival rate of 20 - 30% is present.**
Methods: **Because OSCC presents these survival rates, there is an urgent need to introduce new non-invasive molecular biomarkers for the early detection of OSCC from saliva, which will contribute to an increased long term survival rate for these patients.**
Results: **MicroRNAs represent small, non-coding RNAs that have important roles in biochemical mechanisms, carcinogenesis, cell proliferation, embryogenesis, and other mechanisms involved at the molecular level in the functioning of the human body.**
Conclusions: **In the last decade, due to the fact that forensic genetics developed significantly, salivary microRNAs were increasingly studied as non-invasive molecular biomarkers which could aid in early diagnosis, monitoring, and prognosis of oral cancers.**
This review will present the most important salivary microRNAs which are involved in oral carcinogenesis, especially those which could be used as potential biomarkers in early detection, monitoring, and prognosis of oral cancers by non-invasive techniques.
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KEY WORDS

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) represents more than 90% of malignant cancers of the oral cavity, including buccal mucosa, tongue, hard and soft palate [1, 2]. It is estimated that worldwide each year approximately 275,000 new cases are detected. Regarding the lifetime risk of developing OSCC in the European population, it has been demonstrated that about 1.85% of men are prone to develop this malignancy compared to 0.37% of women [3].

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Because OSSC has a complex etiology, it is hypothesized that many genetic and especially epigenetic factors (environmental factors) are involved in its development. In about 75% of the cases of OSCC, carcinogens such as smoking and alcohol consumption are correlated with their development [4-6]. It is estimated that the remaining 25% of OSCC are caused by papilloma viruses [7].

In recent decades a significant number of studies in the field of oncology research have demonstrated the usefulness of body fluids in early detection of tumor cells in different types of cancers. Body fluids (saliva, blood, urine, cerebrospinal fluid) can aid in early and non-invasive detection of different types of cancers.

Due to recent developments in the field of molecular genetics and in salivary studies, researchers have proposed a new concept to be introduced known as "salivaomics". Salivaomics consists of the analysis of proteomics, genomics, transcriptomics, and microRNAs. In 2012, Wong et al. created a salivaomics knowledge base (SKB) which includes new research studies and information related to saliva (http://www.hspp.ucla.edu/skb.swf) [8].

In OSCC detection, saliva represents the ideal body fluid because it is a new, simple, and non-invasive way in aiding early diagnostics of this disease. In their study regarding the use of molecular biomarkers in early diagnosis of oral cancers from saliva, Wong et al. compared the DNA based biomarkers from saliva of patients with oral tumors and in plasma of patients with cancers in other parts of the body. In saliva samples, DNA was found in 100% of patients with oral cancers, compared to 47% - 70% of patients presenting tumors in other parts of the body [9].

Previous studies have demonstrated that human molecules present an increased stability in saliva; thus, potential molecular biomarkers can be studied [10,11]. In a research study by Bahn et al., they compared salivary noncoding RNA (ncRNA) to those from other body fluids [12]. Using cell-free saliva (CFS) they confirmed in the study that miRNAs are stable and found in abundance in saliva and found within the exosomes, findings that confirmed results from studies made by Gallo et al. and Patel et al. [13,14]. In their studies they quantified and compared the microRNAs' expressions in healthy individuals observing an increased concordance between them. Also, they found similarities between microRNAs' expressions in saliva and other body fluids such as blood and cerebral spinal fluid. Other findings have been obtained from the study. The presence of piwi-interacting RNA (piRNA) [15-17] and the novel discovery of circular RNAs (circRNA) [18-20] present in abundance in saliva indicate that these types of molecules can serve as molecular biomarkers in early detection of OSCC by non-invasive methods.

In 2010, Weber et al. analyzed the types of microRNAs in 12 biological fluids, identifying 714 different species of microRNA. The results of the study highlight the existence of a significant range of microRNAs in biological fluids. In order to be used as tumoral biomarkers in oral cancer detection, microRNAs must have some characteristics, such as simple noninvasive detection technique, high accuracy, and low cost analysis [21].

Salivary microRNAs as biomarkers in oral cancer diagnosis

MicroRNAs (miR) are small, non-coding, single-stranded, 22 base nucleotide sequences. miRNAs bind to more than 60% of the genes [22-24]. Recent studies have suggested that over 2500 miRNAs are encoded in the human genome. They are expressed differently being involved in many different biological processes like embryogenesis, differentiation or carcinogenesis [25-27]. Also they are differently expressed in stages of development, cell types, or disease stages [28-30].

Cao et al. have studied the involvement of microRNAs in tooth development and in the process differentiation of oral stem cell epithelia [31].

Biogenesis of microRNAs

MicroRNA biogenesis begins with protein-coding gene transcriptions by RNA polymerase II inside the nucleus. Thus, the first species of microRNA which are obtained are known as pri-microRNA [49]. The obtained pri-microRNAs are then poly-adenylated by an enzyme called RNase III Drosha, resulting the pre-microRNAs. Pre-microRNAs are transported from inside the nucleus to the cytoplasm using exportin 5 (nucleo-cytoplasmic transporter). Further, pre-microRNAs are processed by RNase III Dicer enzyme, which cleaves it into a mature microRNA (double-stranded microRNA with approximately 19 to 23 nucleotides) and miRNA* (passenger strand) [50]. The mature microRNA is further embedded into RNA-induced silencing complex (RISC). The RISC complex contains the protein Argonaute (Ago) which can degrade miRNA* [51].

Oral premalignant lesions (leukoplakia)

Oral precancerous lesions known as leukoplakia are present at the beginning of oral carcinogenesis. Roy et al. compared the expression of miRNA-29a, miRNA-34b, and miRNA-423 from samples of patients with leukoplakia with controls, observing that there are differential expressions of mirRNAs between the two groups [32].

Another research study, conducted by Cervigne et al., observed that miRNA-21, miRNA-181b, and miRNA-345 were up-regulated in patients with leukoplakia, being associated with disease progression [33].

Oral squamous cell carcinoma (OSCC)

In one study including 50 salivary microRNAs, Park et al. [34] used whole saliva and saliva supernatant for their analysis in healthy control subjects and patients with OSCC diagnosis. In the study, they found that microRNA-125 and microRNA-200a were decreased in OSCC patients compared to the healthy subjects. Another study conducted by Langevin et al., studied

Table 1. Modifications of microRNAs in different types of oral cancers and leukoplakia.

Oral cancer pathology	Upregulated microRNAs	Downregulated microRNAs
Oral squamous cell carcinoma	miR-21, miR-7, miR-34b, miR-155, miR-182, miR-15b, miR-185, let-7, miR-363	miR-23b, miR-125, miR-145, miR-155, miR-146a, miR-99a, let-7i, miR-375, miR-127, miR-137, miR-200, miR-205, miR-15a, miR-375, miR-127-3p, miR-126
Tongue squamous cell carcinoma	miR-184, miR-21, miR-24	miR-100,miR-125, miR-133, miR-7, miR-138, miR-195, miR-222
Leukoplakia	miR-29a, miR-34b, miR-423, miR-21, miR-181b, miR-345, miR-31	

Table 2. Different types of cancers with microRNAs modifications.

Cancer type	Up-regulated microRNAs	Down-regulated microRNAs
Breast cancer	miR-155,miR-195,miR-10b, miR-34a,miR-29a, miR-21	-
Colorectal cancer	miR-17-3p, miR-92,miR-29a, miR-92a,miR-221,miR-29a	miR-34a
Non-small cell lung carcinoma (NSCLC)	miR-25, miR-223,miR-17-3p, miR-21, miR-106a, miR-146, miR-155, miR-191, miR-192, miR-203, miR-205, miR-210, miR-212, miR-214	miR-146b, miR-221, let-7a, miR-155
Acute myeloid/leukemia (AML), Acute lymphoblastic leukemia (ALL)	let-7b, miR-523	-
Prostate cancer	miR-141, miR-200b,miR-16, miR-34b, miR-92a, miR-92b, miR-103, miR-107, miR-197, miR-328,miR-485-3p, miR-486-5p, miR-574-3p	miR-145, miR-155, miR-24, miR-26b, miR-30c, miR-223
Diffuse large B-cell lymphoma	miR-21, miR-155, miR-210	-

salivary microRNAs from oral rinses in patients diagnosed with OSCC and found an association between the promoter methylation of microRNA-137 and gender and body mass of the patients [35].

Other studies on salivary microRNAs have focused on tumor samples from tongue and the floor of the mouth. In these cases, a significant number of microRNAs have been found upregulated, such as microRNA-21, microRNA-7, microRNA-34b, microRNA-155, microRNA-182, microRNA-15b, and microRNA-185. Other microRNAs were downregulated: microRNA-23b, microRNA-125a, and microRNA-125b [36,37].

Other research groups studied the involvement of microRNAs in the immunomodulatory functions and found microRNA-155 and microRNA-146a upregulated [38-41].

Ricieri et al. conducted a study of the microRNAs involved in OSCC staging and concluded that circulating miR-15 could be associated with OSCC staging [42]. In a recently published study, expressions of microRNA-1293, microRNA-31, microRNA-7 were found to be upregulated in gingivobuccal cancer samples, while microRNA-206, microRNA-204, and microRNA-133a were found to be downregulated [43].

Tongue squamous cell carcinoma

Recent studies have discovered a number of microRNAs which are upregulated and downregulated in tongue squamous cell carcinoma (TSCC). Upregulation of miR-184 and miR-21 and downregulation of miR-100, miR-125, and miR-133a have been found to play important roles in tongue squamous cell carcinoma [44]. In TSCC, upregulation of miR-24 has been demonstrated to be associated with reduced apoptosis [45]. In contrast, other microRNAs, such as miR-138 [46], miR-195 [47], and miR-70 [48], with a downregulated expression have been linked with cancer migration and invasion.

MicroRNAs as biomarkers in other diseases

Also, microRNAs are used as noninvasive biomarkers in early detection and monitoring of other diseases, such

as different types of cancers, cardiovascular diseases, diabetes mellitus, sepsis, and neurodegenerative disorders.

In 2008, Lawrie et al. mentioned the presence of microRNAs in serum of cancer patients for the first time. They discovered the association of microRNA-21 in serum of patients with diffuse large B cell lymphoma with relapse-free survival [52].

Yu et al., in 2008, discovered that using a panel of 5 miRNAs can aid in predicting the treatment outcome in NSCLC patients in non-small cell lung cancer (NSCLC), the most common type of lung cancer, using samples from 112 patients with this diagnosis [53].

For early detection in prostate cancer (PCa), microRNA-141 was found to be increased with a specificity of 100% and a sensitivity of 60% [54]. In blood it was demonstrated that microRNA-141 has an increased concentration compared to prostate-specific antigen (PSA) thus being a good biomarker for early PCa detection [55].

Due to the fact that cardiovascular diseases (CVD) represent the world's leading cause of mortality and morbidity, there is an urgent need for noninvasive biomarkers that could aid in establishing an early diagnosis and the initiation of an effective therapy, thus decreasing the rate of mortality [56,57].

In a recent study D'Alessandra et al. found that in myocardial infarction serum levels of microRNA-1, microRNA-133a, microRNA-133b, and microRNA-499 are increased suggesting that they could be used as biomarkers in MI [58]. Chang et al. found that microRNA-1 can be used as a new biomarker in MI prediction. They discovered that microRNA-1 levels increase in the first 6 hours following myocardial infarction and decrease to their normal values after 3 days [59].

Vickers et al. found that microRNA-223 is involved in dyslipidemia. They demonstrated that human HDL contains microRNA and transports it to the cells through the blood [60].

In type 2 diabetes mellitus (T2D), Zampetaki et al. discovered that microRNA-126 has decreased levels and it is involved in the development of insulin resistance through the inhibition of substrate 1 of insulin [61].

Regarding the association between smoking and the development of cardiac diseases, Badrynia et al. found that microRNA-223 could be used as a predicting biomarker [62].

CONCLUSION

Saliva presents numerous advantages such as simple, non-invasive methods for sample collection and storing compared to other body fluids. In the last decade, microRNAs started to be studied for their roles as potential biomarkers in early detection, prognosis, and management of oral cancers by non-invasive methods.

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Declaration of Interest:
The author declares there is no conflict of interest.

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