Biochemical markers in saliva of patients with oral squamous cell carcinoma

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PEER REVIEW

ABSTRACT

Oral squamous cell carcinoma (OSCC) is consider to be one of the common type of head and neck cancer. Only 50% of advanced oral cancer would survive for 5 years, as this rate has been constant over the last two decades. In order to decrease mortality rate, a new tools are require for early stage diagnosis. Saliva is of great importance for diagnosis of several systemic diseases, and its use for diagnosis of OSCC has been used extensively. Many salivary enzymes along with DNA, RNA and protein obtained from saliva, cancerous cells and inflammatory cells of oral cavity. Extensive studies carried out from genomic and proteomic perspective to identify the potential biomarkers in body fluid as well as saliva and blood for diagnosis and prognosis of OSCC.

This article reviewed the recently identified biomarkers from saliva for OSCC. In addition, the biomarkers which have been correlated with OSCC tumor malignancy by molecular pathology analysis are also described. Finally, the potential biomarkers that have been demonstrated to associate with the malignant OSCC may be used for salivary screening for high−risk patients are discussed. This review article may help to identify the potential biomarkers for screening and the molecular pathology analysis for high−risk patients of OSCC.

1. Introduction

Saliva as a biological fluid has growing interest as a diagnostic tool over the past decade. Saliva contain a wide spectrum of proteins/peptides, nucleic acids, electrolytes, and hormones that originate from multiple local and systemic sources. In our earlier investigation some of salivary biomarkers in oral cancer patients found to have significant correlation with pathogenesis of oral squamous cell carcinoma (OSCC)[1]. Even though saliva consider as a reflection of body's health but limitation of using saliva as

a diagnostic tool is because of lack of information regarding the biomarker's present in saliva and their correlation with disease etiology and above all the absence of high−sensetive method for detection of these biomarkers. Diurnal/ circadian variations of some biomarkers available in saliva and not being the actual reflection of concentration in serum is consider to be disadvantage of using saliva as a diagnostic medium. Method implied for collection and degree of stimulation affect the composition of saliva[2,3]. Concentration of some of the biomarkers in saliva are 300 to 3 000−fold less than those in blood[4]. Accurate and sensitive

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detection methods are require for utilization of saliva as potential diagnostic medium. In this review, we explore the diagnostic potential of saliva with regard to oral cancer that is prevalent in India.

Oral cancer is sixth leading cancer worldwide[5]. Tobacco and alcohol consumption are known to be an important risk factors. These two factors have a complementary effect, apart from that infection with human papilloma viruse is considered in head and neck squamous cell carcinoma (HNSCC), particularly those of the oropharynx[6].

OSCC is characterized by multiple genetic alteration that result in clinically known malignant neoplasm. The accumulation of damaged genetic material leads oral keratinocytes in an uncontrolled division of mutant cells[7].

OSCC is a multiple disease, and variety of epigenetic and genetic changes have been correlated to malignant transformation of potentially malignant oral lesions[8]. Several genetic alteration have been identified related to OSCC that can used as a diagnostic markers[9,10]. Many of these genetic alterations have been identified in oral epithelial cells, saliva, and serum of OSCC's patients[11–15]. However, identification of salivary markers that could replace a reliable pathological identification is not yet fully understood. The most knowledge on the pathogenesis of HNSCC has been acquired from the studies of oral cancer, likely because oral cancer is the most commonly diagnosed HNSCC. In addition, oral premalignant lesions are the most frequently diagnosed pathology. Oral leukoplakias are visible precursor lesions that are macroscopically recognizable[16,17]. However, there are several studies addressing that many precursor changes in the oral mucosa are not clinically visible. The term 'field cancersization' has been introduced to explain the high propensity of HNSCC to develop local recurrences after treatment, and the high likelihood that multiple independent tumors can develop in the head and neck region[18].

In general, cancers including HNSCC develop from the accumulation of genetic and epigenetic variation and abnormalities in cancer–associated signaling pathways, causing the formation of cancer–related phenotypes that have previously been summarized by Hanahan and Weinberg[19]. This includes high replicative potential of tumors, self–sufficiency in growth signals, insensitivity to anti–growth signals, ability to evade apoptosis, increased angiogenesis, and invasion and metastasis. However, cancers are complex tissues and is the cause of multiple factors. They contain tumor cells and surrounding stroma, which is constructed by various types of mesenchymal cells and the extracellular matrix. Collectively, this tissue is referred to as the tumor microenvironment (TME). Therefore, when the cell become cancerous, it does not have a cell–centric view because as the cancer develop, the surrounding

microenvironment will be affected and turn to be an state through continuous tumor–stromal interactions. For these reasons, six hallmarks of cancer delineated by Hanahan and Weinberg are provided by various stromal components[20]. Around the world, the 5–years mortality rate of oral cancer is about 50%, which has not changed significantly in recent 50 years despite of the advances in surgery, radiotherapy, and chemotherapy. This could be as a result of diagnosis at the late stage and no reliable early diagnostic marker is available. In addition, OSCC has a very high recurrence rate, the early identification of recurrence or second primary tumors remains a challenge[21].

Histopathological analysis and clinical examination are the most reliable sources for diagnosis of OSCC but even in very early stage, could be undetectable. Therefore, a most reliable biomarkers require for detection of OSCC. Appearance of oral leukoplakias that are visible precursor lesions that can be recognized macroscopically. Leukoplakia in the tongue and oral cavity shown in Figure 1[22]. Cancer has been consider as a disease that causes transformation of cell that result in hyper–proliferation and metastasis and long term survival capacities. Accordingly, therapeutic form of anticancer have been focused on restricting a cancer in local area of development. Emerging evidence indicates that to effectively control cancer, we need to consider tumorigenesis and tumor progression not as a cell autonomous, cancer cell–centered condition, but rather as a disease involving complex heterotypic multicellular interactions within a newly formed tissue, the cancerous tissue. In fact a solid tumor is a tissue disease and a systemic disease rather than a cell disease. Hence, the concept of TME as an integrated and essential part of the cancer tissue was proposed. Recent evidence emerging from the study of TME is forcing the cancer research community to revise basic concepts of cancer biology[23].

Figure 1. Appearance of leukoplakia in oral cavity.

TME contains many distinct cell types, including fibroblasts, carcinoma–associated fibroblasts, myofibroblasts, smooth muscle cells, endothelial cells and their precursors, pericytes, neutrophils, eosinophils, basophils, mast cells, T and B lymphocytes, natural kill cells, and antigen presenting cells such as macrophages and dendritic cells. Numerous data have demonstrated a role for these individual components, in particular carcinoma–associated fibroblasts, macrophages and endothelial cells,

in promoting tumor growth and progression. While most cellular components of the immune system are capable of rejecting tumors, basically, they are enslaved by cancer cells to promote tumor growth and invasion. For these reasons, knowledge and control of TME is becoming as essential as the knowledge and control of the cancer cells for better understanding of cancer biology and for devising novel therapeutic approaches[24,25]. As mentioned above, six acquired, hallmark capabilities of cancer are thought to be required for tumorigenesis. The orders by which these hallmark capabilities are acquired vary across cancer types.

However, recently several studies suggested the essential role of tumor stroma in acquisition of hallmark capabilities. The stroma provides support with growth factors and cytokines and promotes angiogenesis, tissue invasion, and metastasis. In addition, it has become evident that the stroma provides a chemo resistant capability to the tumor, preventing chemotherapeutics from reaching their targets[20,24,26].

The first evidence that non–cancerous tissue elements might affect tumor formation and growth came from the field of inflammation. A link between inflammation and cancer has been recognized since 1863 when Rudolf Virchow, demonstrated the presence of leukocytes in tumor tissues. Based on his observation he proposed the hypothesis that cancer originates at sites of chronic inflammation[20]. The presence of leukocyte in the site of tumors is an indication of a failed attempt of the immune system to reject the tumor. However, this observation remained largely neglected for over a century until it was shown that innate immune cells, in particular phagocytes, play an active role in promoting the tumorigenesis. In addition to leukocyte infiltration, angiogenesis is now being recognized as another stromal reaction promoting cancer progression. Therefore, chronic inflammatory and neovascularization are critical, if not essential, for cancer progression[27,28].

2. Saliva as a diagnostic biofluid

Approximately, healthy adults produce 500–1 500 mL of saliva per day and the rate of production is 0.5 mL/min[29], but several physiological and pathological conditions can alter saliva production quantitatively and qualitatively. Smell and taste stimulate saliva production and secretion, as do chewing, psychological and hormonal status, drugs, age, hereditary influences, oral hygiene, and physical exercise[30].

Blood, exfoliated cells, saliva and urine, comprises a non–invasive, easy, and rapid to collect, and yet, a cost–effective specimen[31]. Analytes are present in low concentrations in saliva, and they are transferred from blood through the capillary walls, interstitial space, and the acini or duct cells into the lumen of the salivary gland duct[32]. Several protocols for collection of saliva have been proposed[33]. Although secretion of saliva could be stimulated by many ways, collection of unstimulated whole saliva between 9 a.m. and 11 a.m. has been considered the most informative way of performance. Whole saliva (oral biofluid) is a mixture of major and minor salivary gland secretions, gingival cervicular (sulcular) fluid, bronchial and nasal secretions, serum and blood derivatives from oral wounds, microorganisms, leukocytes, desquamated epithelial cells, and food debris[32].

The use of saliva for diagnosis of disease has been sought centuries ago. However, the introduction of molecular laboratory techniques in 1980s has drawn more attention to saliva as a tool for detection of disease and for health monitoring in general[34]. Oral fluid analysis has been proved useful for detection of Sjögren's syndrome, diabetes mellitus, infection with *Helicobacter pylori*, Cushing syndrome, HIV, and hepatitis C virus[35–44]. It is now being routinely used for the detection of abused as well as therapeutic drugs[32]. Saliva has also been suggested for breast cancer screening programs by detecting Her2/neu[35,45]. Withal, diseases of the oral cavity have been the most attractive ones for saliva diagnostics researchers, and the use of saliva for detection of dental caries and periodontal diseases has been suggested[46–48]. Salivary diagnostics for OSCC, if becoming a routine, would also comprise a suitable tool for population screening, monitoring of patients at risk of recurrent tumor, and consequently for improving the survival rate of patients with this disease[34,49]

3. Saliva for OSCC detection

Although many investigators have dealt with saliva of OSCC patients and have tried to find out the differences when compared to normal controls, the research in this field is still in its infancy and needs large–scale studies optimizing for many confounding factors introduced by the complexity of the oral environment.

Both the genomic and proteomic approaches have been used to investigate salivary biomarkers for OSCC detection. Antibodies against mutated P53, mutations of TP53, levels of hyaluronic acid, SCC antigen, Cyfra 21–1, CD44 and myosin and actin have been suggested as possible markers for early diagnosis of OSCC[50–55]. In addition, many other potential salivary markers have been reported[56]. However, the results of all these studies were lacking either proper samples size or the biological significance of the marker in carcinogenesis.

The potential biomarkers identified from saliva in patients with OSCC are listed in Table 1.

Table 1

Potential biomarkers of OSCC in saliva.

Salivary molecule	References
Albumin	[57]
Cancer antigen 125 (CA125)	[53]
Catalase	[58]
CD44	[54]
CD59	[58]
Cofilin–1	[59]
Endothelin–1	[60]
Glutathione	[61]
Interleukin 1a(IL–1a)	[62]
Interleukin 1b(IL–1b)	[63]
Interleukin 6 (IL–6)	[62–64]
Interleukin 8 (IL–8)	[62–65]
Immunoglobulin heavy chain constant region gamma	[59]
Mac–2 binding protein (M2BP)	[58]
MRP14	[58]
p53 antibodies	[66]
Profilin	[58]
S100 calcium binding protein	[62]
Telomerase	[67]
TNF–a	[62]
Tissue polypeptide antigen (TPA)	[53,68]
Transferrin	[59]
Transthyretin	[59]
a–Amylase	[69]
bFibrin	[59]
Bacteria	[70]
CD44	[54]
DNA (promoter hypermethylation)	[70]
DUSP1	[71]
HA3	[71]
IL–1β	[71,72]
IL–8	[71,72]
OAZ	[72]
S100P	[71]
SAT	[71,72]

The oral epithelium is in a continues process of turnover. Thin non–keratinized epithelia such as those of the epithelium of the floor of the mouth and of the ventral surface of the tongue turn over more rapidly than do the thick keratinized epithelia, such as those of the hard palate and the gingiva. This is due to the speed of proliferation of basal keratinocytes is higher in non–keratinized epithelium than keratinized epithelium. Basal keratinocytes have more potential of transforming into a cancerous status that lead to higher rate of cell division, hence the greater risk in non–keratinized epithelium[73,74].

Proliferation of cells occurs in the basal and parabasal layers of the oral epithelium which are referred to as the progenitor cell compartment. This progenitor cell compartment comprises two functionally distinct populations of cells: a smaller population of tissue–specific stem cells, and a larger population of transient–amplifying cells[73,75]. The tissue–specific stem cells occupy a specialized niche in relation to their neighbouring cells. The stem cells contain the genomic information of the oral epithelium, are undifferentiated but have the capacity to differentiate. They divide infrequently, have the capacity for unlimited self–

renewal, maintain active expression of telomerase and do not readily undergo apoptosis[73,74,76,77].

The mitotic division of a tissue–specific stem cell gives rise either to two daughter stem cells which remain in the stem cell niche or to one daughter stem cell which remains in the stem cell niche and to a second daughter transit–amplifying cell that leaves the stem cell niche, but remains in the progenitor compartment. Subsequently, the transit–amplifying cells undergo mitosis and each gives rise either to two daughter transit–amplifying cells which remain in the progenitor compartment, or to two daughter cells which begin to differentiate. Those daughter cells which exit the progenitor compartment and differentiate into keratinocytes, begin the process of maturation and gradually rise through the morphologically distinct cell layers of the epithelium, to the surface where they are shed[74,76,78].

Studies have shown that certain type of oral bacteria are increased on or in oral and esophageal cancer lesions and their associated lymph nodes[79,80]. Although increased colonization of facultative oral streptococci have been reported[81,82] most often anaerobic *Prevotella*, *Veillonella*, *Porphyromonas* and *Capnocytophaga* species were also elevated[80,83,84]. Presently, studies are analyzing the probability of whether bacteria are causally associated with oral cancer. Extensive research is require to determine the usage and feasibility of salivary biomarker as a potential early diagnostic tool for oral cancer.

The reason for involvement of bacteria for cancer lesions is unclear. Studies have shown that oral bacteria have specific tropism toward numerous biological surfaces in the oral cavity such as the teeth, mucosa, and other bacteria[85,86]. The non–shedding surfaces of the teeth offer a far different habitat than the continually shedding surfaces of the oral mucosa. Due to the repeated shedding of epithelial cells, there is less time for a complex biofilm to develop on soft tissue surfaces; thus, a premium is placed on potent mechanisms of adhesion. The differences in bacterial tropisms for specific oral sites suggest that different intra–oral surfaces and bacterial species have different receptors and adhesion molecules that dictate the colonization of different oral surfaces.

It is now known that bacteria bind to and colonize mucosal surfaces in a highly selective manner. Adhesins on bacteria bind specifically to complementary receptors on the mucosal surfaces of the host. These adhesins differ from species to species leading to specificity in attachment to different surfaces.

Many studies have reported that even within genera, colonization patterns of individual species may differ markedly[85–87]. For instance, streptococcus salivarius, preferentially colonized the oral soft tissues and saliva compared to the teeth, while the opposite was true for *Streptococcus sanguis*.

The OSCC precursor cell originate by malignant transformation of a single cell which by clonal expansion gives rise to a monoclonal cancer cell population. Unlimited

self–renewal and decrease rate of apoptosis with the result of gaining ability to sustain the growth of the cancerous tissue are the capacity of precursor cancer cells[88].

The origin of the precursor cell which gives rise to OSCC is uncertain. It is likely that it arises, as is the case in other cancers, from a tissue–specific stem cell or its progenitor cell, which has acquired epigenetic and/or genetic alterations. However, it is also possible that the OSCC precursor cell may have arisen from a stem cell which has acquired a precancerous phenotype during embryogenesis and has then differentiated into a tissue–specific cancer stem cell[89,90]. Another possibility is that the OSCC precursor cell originates from a mature keratinocyte which has undergone cytogenetic alterations resulting in its dedifferentiation into the analogue of an immature progenitor/stem cell which can express the dysregulated intracellular pathways and transcription factors of a tissue–specific cancer stem cell phenotype[91,92].

Cancer precursor cells, regardless of how they may have arisen possess the capacity for self–renewal, and hence are capable of initiating and sustaining growth of a cancer. It is likely that the OSCC comprises a heterogeneous population of cancer stem cells, cancerous transit–amplifying cells and post–mitotic cancerous cells at different stages of abnormal differentiation[91,92]. The cancer stem cells constantly provide new cancerous transit–amplifying cells which have a high proliferative rate, thus perpetuating the growth of the carcinoma. The transit–amplifying cancerous cells exhibit uncontrolled cell proliferation and prolonged survival and are the force behind tissue invasion and destruction; but owing to their limited capacity of cell renewal, they cannot alone sustain tumour growth. The post–mitotic cancerous keratinocytes at different stages of differentiation have no proliferative capacity[93,94].

Thus the overall growth of OSCC is brought about by the multiplication of cells with a cancer stem cell phenotype and by the uncontrolled proliferation of monoclonal cancerous transit–amplifying cells.

4. Saliva collection methods

Accurate measures of salivary flow rate and composition are essential for many clinical, experimental, and diagnostic protocols. Saliva can be collected under unstimulated (resting) or stimulated conditions, as described in detail by Navazesh[95]. In brief, whole–mouth resting saliva can be collected by the draining/drooling method, the spitting method, the swabbing method, and the suction method. Stimulated saliva is collected by either having the patient chew a piece of paraffin and/or by applying 0.1–0.2 mol/L (approximately 1 drop) citric acid to the tongue. In addition, saliva can be probed from individual glands by using cannulation of the glandular ducts or by the application of specific collecting devices to the emergence area of the glandular ducts[95]. However, these procedures are complex, slow, and invasive, and require skilled personnel.

5. Future perspective on saliva diagnostics

Saliva consider to be a potentially important diagnostic fluid. However, the growth of diagnostic opportunities for saliva has been slowed mainly due to limitations in sensitive detection technologies and lack of understanding of saliva biology, in particular the lack of correlation between biomolecules in blood with saliva and the circadian variations of biomolecules in saliva.

The wide spectrum of compounds present in saliva may provide information for clinical diagnostic applications. Saliva is a good medium because its collection is noninvasive and the donation process is relatively stress free, so that multiple collections can be performed without imposing too much discomfort on the donor. Saliva is easy to collect, store, and transport; it does not require highly trained personnel; and it is safer for hospital staff to handle compared to blood and other body fluids. In addition, saliva is a "real–time" fluid because the salivary glands are exocrine glands that produce protein profiles indicative of an individual's health and well–being status at the moment of collection. These characteristics make it possible to monitor several biomarkers in infants, children, elderly individuals, and uncooperative patients, as well as circumstances in which blood and urine sampling are not available.

Both basic and clinical research on the development of methods to assay saliva are increasing. Whole saliva is most frequently used for diagnosis of systemic diseases, because it can be readily collected and, more importantly, it contains serum constituents. Some systemic diseases affect salivary glands directly or indirectly and may influence the quantity and composition of saliva. Human saliva proteins also have diagnostic value for systemic diseases[96]. Although proteomic constituents are a logical first choice as salivary diagnostic analytes, genomic targets have emerged as highly informative and discriminatory biomarkers. The future for saliva diagnostics relies on combinations of biomarker panels used as screening tools to improve on diagnostic accuracy and specificity. One biomarker alone may not suffice as a reliable source to enable investigators to define the pathogenesis of the underlying disease. The use of combinations of biomarkers may provide additive and powerful diagnostic information.

Thus far we have focused mainly on collating information on salivary biomarkers and their potential utility in diagnosis, prognosis, of OSCC.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Saliva as a biological fluid has growing interest as a diagnostic tool over the past decade. Saliva contain a wide spectrum of proteins/peptides, nucleic acids, electrolytes, and hormones that originate from multiple local and systemic sources.

Research frontiers

This article reviewed the recently identified biomarkers from saliva for OSCC. In addition, the biomarkers which have been correlated with OSCC tumor malignancy by molecular pathology analysis are also described.

Applications

This review article may help to identify the potential biomarkers for screening and the molecular pathology analysis for high–risk patients of OSCC.

Peer review

In this review article recenlty identified biomarkers from saliva for OSCC have been extensively covered. And among which shown correlating positively with (OSCC) tumor malignancy by molecular pathology have also been extensively described. Over all this is a good review article with respect to salivary markers in OSCC.

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