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RESEARCH ARTICLE

MIRACLE OF SALIVA IN DIAGNOSIS OF ORAL CANCER

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ABSTRACT

Using saliva for disease diagnostics and health surveillance is a promising approach as collecting saliva is relatively easy and non-invasive. Over the past two decades, using salivary biomarkers specifically for early cancer detection has attracted much research interest, especially for cancers occurring in the oral cavity and oropharynx, for which the five-year survival rate (62%) is still one of the lowest among all major human cancers. More than 90% of oral cancers are oral squamous cell carcinoma (OSCC) and the standard method for detection is through a comprehensive clinical examination by oral healthcare professionals. Despite the fact that the oral cavity is easily accessible, most OSCCs are not diagnosed until an advanced stage, which is believed to be the major reason for the low survival rate, and points to the urgent need for clinical diagnostic aids for early detection of OSCC. Thus, much research effort has been dedicated to investigating potential salivary biomarkers for OSCC, and more than 100 such biomarkers have been reported in the literature. However, some important issues and challenges have emerged that require solutions and further research in order to find reliable OSCC salivary biomarkers for clinical use. This review article provides an up-to-date list of potential OSCC salivary biomarkers reported as of the fall of 2013, and discusses those emerging issues. By raising the awareness of these issues on the part of both researchers and clinicians, it is hoped that reliable, specific and sensitive salivary biomarkers may be found soon—and not only biomarkers for early OSCC detection but also for detecting other types of cancers or even for monitoring non-cancerous disease activity.

INTRODUCTION

Saliva is a complex fluid produced by the major and minor salivary glands and is a mixtureof several constituents of non-salivary origin such as gingival crevicular fluid, expectoratedbronchial and nasal secretions, serum and blood derivatives from oral wounds,microorganisms, desquamated epithelial cells, other cellular components and food debris(Kaufmann & Lamster, 2002). Saliva is considered a mirror of body health and is composed of variety of analytes fromsystemic sources that reach the oral cavity through various pathways. Because water is a major constituent, saliva plays a key role in the lubrication and repair of oral mucosa, formation and swallowing of food bolus, digestion of starch, facilitation of food tasting andcontrol of oropharyngeal microbial population (Lawrence, 2002).

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The role of saliva as a diagnostic tool has advanced exponentially over the past decade. Theability to measure a wide range of molecular components in saliva and compare them withserum coupled with the easy and non-invasive method of collection has made it feasible tostudy microbes, chemical and immunological markers. As a consequence these advances intechnology have helped to move saliva beyond measuring oral health characteristics towhere it may now be used to measure essential features of overall health (Streckfus & Bigler,2003). As a diagnostic fluid, saliva offers distinctive advantages over serum because it can be collected non-invasively byindividuals, even by patient. The ability to measure andmonitor a wide range of molecular components in salivaand compare them to serum components has made itfeasible to study microbes, chemicals and immunologicmarkers (Slavkin, 1998). Evolution of salivary proteomic, transcriptomic, genomic,and metabolomic research for oral cancer detection from saliva makes the diagnosis more reliable.

Table 1.

Non-organic compound	Na, Ca, F, and Mg
Peptide	Defensin-1
Proteins	P53 autoantibodyα-amylase IL-8 TNF-α IL-1 IL-6 Basic fibroblast growth factor Tissue polypeptide antigen (TPA) Cancer antigen 125 (CA125) Endothelin-1 IL-1β CD44 Total salivary protein Insulin growth factor 1 (IGF-1) MMP-2 MMP-9 CD59 Catalase Profilin S100A9/MRP14 M2BP Carcinoembryonic antigen (CEA) Carcinoma associated antigen CA-50 Salivary carbonyls Cyclin D1 Maspin 8-oxoguanine DNA glycosylase (OGG1) Phosphorylated-Src Ki-67 Lactate dehydrogenase Transferrin Zinc finger protein 501 peptide Hemopexin Haptoglobin Complement C3 Transthyretin α1-antitrypsin
DNAs	P53 gene codon Loss of heterozygosity in the combination of markers D3S1234, D9S156, and D17S799 Mitochondrial DNAs (cytochrome c oxidase I and cytochrome c oxidase II) Hypermethylation of promoters in tumor suppressor genes: DAPK, DCC, MINT-31, TIMP-31, TIMP-3, p16, MGMT, CCNA1
mRNAs	IL-8 IL-1β DUSP1 (dual specificity phosphatase 1) H3F3A (H3 histone family 3A) OAZ1 (ornithine decarboxylase antizyme 1) S100P (S100 calcium binding protein P) SAT (spermidine/spermine N1-acetyltransferase EST)
MicroRNAs	miR-125a miR-200a miR-31
Long non-coding RNAs	HOTAIR
Oxidative stress-related molecules	Reactive nitrogen species (RNS) such as nitric oxide (NO), nitrites (NO2) and nitrates (NO3) Peroxidase Glutathione S-transferase (GST) Superoxide dismutase (SOD) 8-hydroxy-2-deoxyguanosine (8-OHdG) Glutathione [30Malondialdehyde (MDA)
Glucocorticoid	Cortisol
Metabolomics	Cadaverine, alpha-aminobutyric acid, alanine, C5H14N5, piperidine, taurine piperideine, pipercolic acid, C4H9N, C8H9N, pyrroline hydroxycarboxylic acid, betaine, C6H6N2O2, leucine+isoleucine, tyrosine, histidine, tryptophan, beta-alanine, glutamic acid, threonine, serine, glutamine, choline, carnitine, C4H5N2O11P Lactic acid
Glycosylationrelated molecules	Sialic acid α-L-fucosidase
Other	Telomerase activity

Table 2.

Author/reographical region/year	OSCC		OLP		Chronic periodontitis		Controls	
	IL-6	IL-8	IL-6	IL-8	IL-6	IL-8	IL-6	IL-8
St. John et al./California, USA/2004 [23]		720						250
Rhodus et al./Minnesota, USA/2005 [24]	88.2± 43.2	3154.1±1023.2					1.4±0.9	1580± 789
Rhodus et al./Minnesota, USA/2005 [67]			371.35±205.52	2194.3± 496.7			47.46±18.74	703.8± 131.6
Katakura et al./Tokyo, Japan/2007 [34]	86.5	720					0	250
Zhang et al./Sichuan, China/2008 [69]			48.79±8.53	1737.49±1073.54			29.9±4.68	641.46±172.91
Saheb Jamee et al./Tehran, Iran/2008 [35]	40.9± 79.5	1093.7±1089.0					2.5±1.3	700.7± 1031.5
Arellano- Garcia et al./California, USA/2008 [70]		3347.7±2929						759.4± 563
Teles et al./Massachusetts, USA/2009 [68]						2268±111		1945±181
Sharma et al./Manipal, India/2011 [71]					311.35±11.51		17.15±8.44	
Ebersole et al./Kentucky, USA/2013 [72]					35.57±48.17		3.30±2.32	
Cheng et al./Texas, USA/2014 [38]	178.41±172.32	1525.33±1123.95	20.74±22.28	1328.37±731.80	5.85±4.02	738.79±394.00	4.92±8.77	890.83±563.22

The primary purpose of this review is tosummarize some important recent applications ofsaliva-based diagnostics in oral cancer (Wong, 2006).

Review

Whole saliva (oral fluid) is unique and complex, both inits sources and composition. It consists not only of secretionsfrom the three major salivary glands (parotid,submandibular and sublingual) and the minor glands,but also gingival crevicular fluid, oral mucosa transudate,secretions from nasal and pharyngeal mucosa, nonadherentbacteria, desquamated oral epithelial cells, keratin debris, blood cells, and perhaps food or medicationresiduals. The functions of saliva include lubrication, digestion, antimicrobial activity, facilitatingremineralization of the tooth enamel, and maintainingnormal taste sensation (Wong, 2006). These important functionsare achieved by the various chemical components of salivaincluding water, inorganic compounds (ions), organiccompounds (non-proteins and lipids), protein/polypeptides, and hormones.

Salivary proteins and polypeptidesconstitute a significant portion of the mix, and playan important role in carrying on the main functions ofsaliva. So far, more than 2300 proteins and peptides havebeen found in human saliva. The most abundantproteins are α-amylase, albumin, cystatins, hystatins,secretory-IgA, lactoferrin, mucins, lysozymes, prolinerich proteins, statherin and transferrin–which togetheraccount for more than 98% of the total salivary proteins.Most of the potential OSCC salivary biomarkersare also salivary proteins (see Table 1) (Castagnola *et al*., 2011). However, except for three, α-amylase, statherin, and transferrin, thoseproteins, as well as the non-protein OSCC salivarybiomarker candidates, are present in a very low concentration in saliva and require methods/instruments with highsensitivity for detectionsalivary biomarkers for oral cancerdetection.

Apart from oral cancer detection saliva can also be used for detection of many oral diseases (see Table 2). The research methodology involved so far in investigatingthese potential OSCC salivary biomarkers can begrouped according to the types of biomarker, as follows:

- Non-organic compound biomarkersFlame photometry, atomic absorption, and spectrophotometry (Wu *et al*.,2010)
- Peptide or protein biomarkersHigh performance liquid chromatography (HPLC)
- Enzyme-linked immunosorbent assay (ELISA)
- Radio-immunoassay
- Two-dimensional gel electrophoresis (2DE), followed by mass spectrometry (MS)
- 2DE and reverse-phase liquid chromatography (LC), followed by LC-tandem MS
- Matrix-assisted laser desorption/ionization time-off lightmass spectrometry (MALDI-TOF MS)
- 2DE followed by MALDI-TOF MS
- DNA, mRNA or microRNA biomarkers, Polymerase chain reaction (PCR), Quantitative PCR (qPCR), Microarrays followed by qPCR
- Metabolomic biomarkers, Capillary electrophoresis TOF MS, HPLC with quadrupole/TOF MS
- Miscellaneous biomarkers (chemical and enzymeactivity), HPLC, Colorimetric (mostly commercially available) assays (Thomas *et al*., 2009)

Most OSCC salivary biomarker research has involvedinvestigating the constituents of the whole saliva in anunstimulated state, although two studies did investigatethe stimulated saliva samples (Kim *et al*., 2012). After a saliva sampleis collected, a centrifugation processing procedure isoften performed to remove the solid constituents (desquamatedoral epithelial cells, keratin debris, blood cells,bacteria and food residuals, if any), but some studies appearto have analyzed the whole saliva content withoutcentrifugation (Jehmlich *et al*., 2012).

After separating outthose solid constituents, samples were often stored in afrozen state until further analysis (Fraser *et al*., 2004). Most salivary biomarkerresearch studies have investigated only thesupernatant (cell-free) portion of the saliva samples, while other studies investigated only the pellet portionof the saliva or both the supernatant and the pellet portions after centrifugation (Denver *et al*., 2000).

ADVANTAGES OF SALIVA AS A DIAGNOSTIC FLUID

- Noninvasive diagnosis of disease and monitoring of general health.
- Painless, patient suffers no discomfort and little anxiety in the collection process.
- Simple in collection with a modest trained assistant and applicable in remote areas.
- Relatively cheap technology as compared to other tests.
- Cost effective applicability for screening large population.
- Can be used to study special population where blood sampling is a problem e.g children, anxious /handicap/ elderly patients.
- Convenient for multisampling.
- Safer for health professionals than blood tests.
- Compared to blood and urine, saliva is also cheaper to store and ship.
- In addition saliva does not clot and can be manipulated more easily than blood (Lam *et al*., 2004).

LIMITATIONS

- Levels of certain markers in saliva are not always a reliable reflection of the levels of these markers in serum.
- Salivary composition can be influenced by the method of collection and degree of stimulation of salivary flow (Streckfus *et al*., 2000).
- Changes in salivary flow rate may affect the concentration of salivary markers and also their availability due to changes in salivary pH.

Variability in salivary flow rate is expected between individuals and in the same individual under different conditions (Streckfus *et al*., 2000). In addition, many serum markers can reach whole saliva in an unpredictable way (*i.e.* gingival crevicular fluid flow and through oral wounds). These parameters will affect the diagnostic usefulness of many salivary constituents (Zhang *et al*., 2012).

Furthermore, certain systemic disorders, numerous medications and radiation may affect salivary gland function and consequently the quantity and composition of saliva (Agha-Hosseini *et al*., 2009). Whole saliva also contains proteolytic enzymes derived from the host and from oral microorganisms. These enzymes can affect the stability of certain diagnostic markers. Some molecules are also degraded during intracellular diffusion into saliva (Agha-Hosseini *et al*., 2009).

Conclusion

Salivary biomarkers represent a promising non-invasiveapproach for oral cancer detection, and an area of strongresearch interest. However, some issues/challenges havearisen that need to be resolved in order to establish thisapproach as a reliable, highly sensitive and specificmethod for clinical use. These issues include a lack ofstandardization for saliva sample collection, processing,and storage; wide variability in the levels of potentialOSCC salivary biomarkers in both non-cancerous individualsand OSCC patients; and a need for further validationof OSCC salivary biomarkers with individuals whohave either a chronic oral inflammatory disease or othertypes of cancers, but do not have OSCC. These issuescall for convening a panel of researchers in this field toaim for eventual standardization, plus further research,especially concerning biological variance and physiologicalchanges affecting the potential oral cancer salivarybiomarkers. The experience gained in OSCC salivarybiomarker research also can serve as an important referencein salivary diagnostics, including identifying, validating, and applying salivary biomarkers for other typesof cancer detection and for monitoring non-cancerous disease activity

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