Review Article

A Review of Salivary Biomarker: A Tool for Early Oral Cancer Diagnosis

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Abstract

The oral squamous cell carcinoma (OSCC) is one of the most common epithelial malignancies with significant morbidity and mortality. Recent observations indicate that the clinical and histological appearance of oral mucosa may not truly depict the damage occurring at the genetic level. This phenotypic and genotypic disparity may account in part for the failure to establish effective screening and surveillance protocols, based on the traditional clinical and microscopic examination. The tumor markers are playing an increasingly important role in cancer detection and management. These laboratory-based tests are potentially useful in screening for early malignancy, aiding in cancer diagnosis, determining prognosis, surveillance following curative surgery for cancer, up-front predicting drug response or resistance, and monitoring therapy in advanced disease. A systematic review of the literature was performed based on the English titles listed in the PubMed, EBSCO, Cochrane, Science Direct, ISI web Science, and SciELO databases using the keywords. Abstracts and full-text articles were assessed. This article may help to identify the potential biomarkers for screening and the molecular pathology analysis in the high-risk patients with the OSCC.

Keywords: *DNA marker, oral squamous cell carcinoma, protein marker, RNA marker, saliva*

Introduction

The head and neck cancers are one of the most common causes of cancer death worldwide with incidence rate varying in different regions in the Southeast Asia and Africa, the head and neck cancers accounts for approximately 8–10% of all cancers.[1] The primary anatomic sites of the oral squamous cell carcinoma (OSCC) are buccal mucosa, lip, alveolar ridge, retromolar trigone, hard palate, floor of the mouth, the ventral two-thirds of the tongue, and oropharynx.[2]

The key challenge to reduce the mortality and morbidity of this disease is to develop strategies to identify and detect the OSCC when it is at a very early stage, which will enable effective intervention and therapy. Detection of the OSCC is currently based on the expert clinical examination and histological analysis of suspicious areas, but it may be undetectable in hidden sites. Therefore, sensitive and specific biomarkers for OSCC may be helpful in screening high-risk patients. The biomarkers for early cancer detection must meet the following criteria: (a) the altered can be objectively measured; (b) must be measurable in small specimens; (c) must be altered in the high-risk tissues, but not in the normal tissues; and (d) must be altered in the early stages of cancer development. Unlike the other deep cancers, the OSCC occurring in the oral cavity is much easier to be monitored, specimens are easier to be collected for diagnosis, and the treatment is easier to be applied.[3]

Tumor cells inhabit or produce biochemical substances which are referred to as tumor markers. These can be normal endogenous products that are produced at a greater rate in the cancer cells or the products of newly switched on genes that remain quiescent in the normal cells.[4] The tumor markers may be present as intracellular substances in tissues or as released substances in the circulating body fluids such as serum, urine, cerebrospinal fluid, and saliva. Examples of using body fluids for tumor detection include sputum for the lung cancer diagnosis,[5] urine for the urologic tumors,[6] saliva for the OSCC,[7] breast fluid,[8] as well as serum or plasma for almost all types of cancers. With the recent diagnostic technological advances, however, the role of saliva as a tool for diagnosis has advanced exponentially.

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The Tumor Markers

Definition

Lehto and Pontén have defined tumor markers as "specific, novel, or structurally altered cellular macromolecules or temporarily, spatially, or quantitatively altered normal molecules that are associated with malignant (and in some cases benign) neoplastic cells."[9]

The tumor markers may be unique genes or their products are formed only in the tumor cells or they may be genes or gene products that are found in the normal cells, but are aberrantly expressed in the unique locations in the tumor cells.[10]

The Potential Uses of Tumor Markers

Chan and Sell have summarized the potential uses of tumor markers [Table 1].[11]

An Ideal Tumor Marker

A biological marker should have certain characteristics that are applicable in all situations. Kaplan and Pesce have suggested the following criteria for an ideal tumor marker:[12]

- Be easy and inexpensive to measure in readily available body fluids
- Be specific to the tumor being studied and commonly associated with it
- Have a stoichiometric relationship between plasma levels of the marker and the associated tumor mass
- Have an abnormal plasma level, urine level, or both in the presence of micro-metastases, that is, at a stage when no clinical or presently available diagnostic methods reveal their presence
- Have plasma levels, urine levels, or both that are stable and not subjected to wild fluctuations
- They should prognosticate a higher or lower risk for eventual development of recurrence
- They should change as the current status of the tumor changes over time
- They should precede and predict the recurrences, before they are clinically detectable.

Table 1: Potential uses of tumor markers

Screening in general population
Differential diagnosis in the symptomatic patients
Clinical staging of cancer
Estimating tumor volume
Prognostic indicator for disease progression
Evaluating the success of treatment
Detecting the recurrence of cancer
Monitoring responses to therapy
Radioimmunolocalization of tumor masses
Determining direction for immunotherapy

The Saliva as a Perfect Diagnostic Medium

The blood and saliva are the most widely studied body fluids that may contain reliable biomarkers for cancer detection. The saliva is an informative body fluid containing an array of analytes (protein, mRNA, and DNA) that is used as biomarkers for translation and clinical applications.[13] The saliva has many advantages as a clinical tool over the serum and tissues, including simplicity of collection, storing and shipping, cost-effectiveness, easy availability of large sample volume for analysis, and repeated sampling for monitoring over time.[14]

Thus, the saliva-based analysis, a noninvasive alternative to serum analysis, is an effective modality for diagnosis and prognostication of cancer as well as for monitoring post-treatment therapeutic response of the patients. Hence, the development of salivary diagnostic tools is of paramount importance, especially in the identification of high-risk group, patients with premalignant lesions and patients with a previous history of cancer.[15]

Among all the malignancies, oral cancer is one such malignancy, where the saliva examination for detection shows the greatest benefit because of its direct contact with oral cancer lesions. The most important point for selecting saliva as a diagnostic tool is that it also contains the fallen cells in oral cavity which allow saliva to be the first choice of screening and identification of potential biomarkers in the oral cancer.[3]

The Appearance of the Genotypic and Phenotypic Tumor Markers in the Saliva

Although the exact mechanism for the presence of these tumor markers in saliva is unknown, they may be either derived from the serum or are produced locally.[16]

Figure 1 summarizes the possible mechanism that leads to the presence of biomarkers in the saliva.[17]

The Salivary Tumor Markers in Various Malignancies

Clinical significance of the salivary biomarkers in various malignancies is studied by several investigators [Table 2]. Streckfus and Dubinsky discovered the presence of the

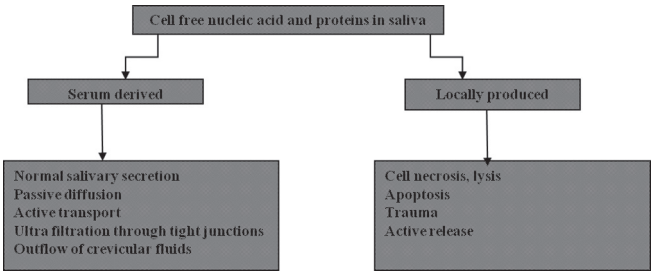

Figure 1: The possible leading mechanism for the presence of molecular markers in the saliva

Table 2: The salivary tumor markers in various malignancies

Malignancies	Salivary tumor markers	Researchers
Breast cancer	Estrogen receptor α	Bretschneider *et al*. 2008[20]
	CA 15-3, HER2/neu, p53	Streckfus and Dubinsky, 2007[18]
		Streckfus *et al*., 2000[19]
Ovarian cancer	CA 125	Chen *et al*., 1990[21]
Salivary gland tumors	Salivary leptin	Schapher *et al*., 2009[22]
Hepatocellular carcinoma	Alpha-fetoprotein	You *et al*., 1993[23]
Pancreatic cancer	ACRV1; DMX like 2, DMXL2 and, catalytic subunit, DPM1	Wong *et al*., 2009[24]
Adenocarcinoma pancreas	CA 19-9	Lodhi *et al*., 2006[25]

ACRV1: Acrosomal vesicle protein 1, DPM1: Dolichyl phosphate mannosyltransferase polypeptide 1, HER2: Human epidermal growth factor receptor 2, CA: Cancer antigen

salivary proteomics and genomics signatures in the patients with breast cancer. The authors reported human epidermal growth factor receptor 2 (Her2)/neu as the first salivary biomarker for the breast cancer and also documented raised levels of cancer antigen (CA 15-3) and Her2/neu as well as low levels of p53 in the patients with breast cancer.[18,19]

The Salivary Tumor Markers in Oral Cancer

The salivary tumor markers in oral cancer include genomic markers, transcriptome markers, protein markers and microbiota. These have been summarized in Table 3.

The salivary genomic markers

Tumor-specific genomic markers consisting of DNA and RNA markers which are identified in the saliva for the detection of oral cancer considering that the initiation and progression of malignant tumors is driven by the accumulation of specific genetic alterations. DNA shows tumor-specific characteristics such as somatic mutations in tumor suppressor genes and p53, microsatellite alteration, abnormal promoter methylation, mitochondrial DNA mutations, and presence of the tumor-related viral DNA.

Loss of heterozygosity (LOH) is defined as a loss of genomic material in one of the chromosomal pairs. Studies

Table 3: Summary of the tumor markers in the diagnosis of oral carcinoma

Salivary genomic markers	Salivary transcriptome markers	Salivary protein markers	Salivary microbiota
Somatic mutations in tumor suppressor genes (p53)	IL-8	Elevated levels of defensin-1	Significant increase in the levels of *Porphyromonas gingivalis, Tannerella Forsythia* and *Candida albicans*
Loss of heterozygosity in chromosome 3p, 9q, 13q and 17p	H3F3A	Elevated CD44	Significantly elevated levels of *Bacteroides melaninogenica* and *Streptococcus mitis*
Promoter hypermethylation of genes (p16, MGMT, or DAP-K)	IL1β	Elevated IL-8	Presence of HPV and EBV
Cyclin D1 gene amplification	S100P	SCC-Ag	
Decrease in 8-oxoguanine DNA glycosylase, phosphorylated-Src and mammary serine protease inhibitor (Maspin)	DUSP1	Calcyclin, Rho GDP dissociation inhibitor	
Microsatellite alterations of DNA	OAZ1	CEA, carcinoantigen (CA19-9), CA128	
	SAT (spermidine/ spermine N1-acetyltransferase)	Intermediate filament protein (Cyfra 21-1)	
		RNS	
		8-OHdG DNA damage marker	
		LDH)	

H3F3A: H3 histone, family 3A, DUSP1: Dual specificity phosphatase 1, SCC-Ag: Squamous cell carcinoma antigen 2, IL: Interleukin, OAZ1: Ornithine decarboxylase antizyme 1, CEA: Carcino-embryonic antigen, RNS: Reactive nitrogen species, LDH: Lactate dehydrogenase, HPV: Human papilloma virus, EBV: Epstein–Barr Virus, CA: Cancer antigen

have shown that LOH in regions that contain a known human suppressor gene is an early predictor of malignant transformation of precancerous lesion.[26] Studies have demonstrated frequent LOH in chromosomes 3p, 9q, 13q, and 17p as an early event in oral carcinogenesis.[27-30] Mitochondrial DNA mutations have also been useful to detect the exfoliated OSCC cells in saliva. Such mutations have been identified in 67% of the saliva samples from the OSCC patients by direct sequencing.[31]

P53 gene is located on chromosome 17p and its main functional activity is cell cycle arrest and initiation of apoptosis in response to the DNA damage. Liao *et al.* have observed the mutation of p53 in the DNA extracted from the saliva of the OSCC patients, suggesting a potential use as biomarker for the oral cancer detection. The study concentrated on p53 exon 4 codon 63 mutations which was significantly higher.[32] Other genes such as p16, p27, p63, p73 related to p53, and cell cycle are altered in varying degrees in oral cancer.[33]

Promoter hypermethylation of several genes has been reported in the head and neck cancer. Rosas *et al.* identified aberrant methylation of, at least, one of the three genes (p16, MGMT, or DAP-K) in the OSCC.[34]

Detection of telomerase activity in the saliva of the OSCC was performed by Zhong *et al.*, and they detected telomerase positivity in 75% of the cases, suggesting that telomerase detection could be used as an assistant marker in the OSCC.[35]

Cyclin D1 gene amplification is associated with poor prognosis in the OSCC.[36] In another study, Ki67 marker was increased, while 8-oxoguanine DNA glycosylase, phosphorylated-Src, and mammary serine protease inhibitor (Maspin) were decreased in the saliva of patients with the OSCC.[37] Microsatellite alterations of DNA were also observed in the saliva of patients with the small cell lung cancer.[38]

The salivary transcriptome markers

Salivary transcriptome diagnostics constitutes a novel clinical approach where a large panel of human RNAs is readily detected in the saliva. A speculation is that salivary mRNA is contained in apoptotic bodies or actively released in exosomes or microvesicles. Recently, microRNAs and small RNA molecules, 18–24 molecules in length, that seem to regulate transcription were also discovered in the existing saliva samples.[39]

Li *et al.* by using the microarray analysis of the salivary transcriptome showed that seven markers displayed a significant elevation in the saliva of the OSCC patients. The validated seven genes could be classified in three ranks by the magnitude of increase into highly upregulated mRNA: interleukin-8 (IL-8), moderately upregulated mRNA: H3F3A (H3 histone, family 3A), IL-1-β, S100P (S100 calcium binding protein P) and low upregulated mRNA: DUSP1 (dual specificity phosphatase 1), OAZ1 (ornithine decarboxylase antizyme 1), and SAT (spermidine/spermine N1-acetyltransferase).[40]

The salivary protein markers

The proteome represents the complete set of proteins encoded by genome and proteomics which is the study of the proteome that investigates the cellular levels of all the isoforms and post-translational modifications of proteins that are encoded by the genome of the cell under a given set of circumstances. While a genome is more or less static, the protein levels in a cell can change dramatically as genes get turned on and off during the cells response to its environment.[41]

The protein biomarkers in the saliva are being analyzed both individually and as a panel of markers to aid in the early detection of the oral cancer and in implementing appropriate therapeutic regime. A 3-year effort by the human salivary proteome project, a consortium of three research groups including the Scripps Research Institute/University of Rochester, the University of California-San Francisco, and the University of California-Los Angeles/University of Southern California, has led to the identification of over 1100 nonredundant proteins in the human parotid and submandibular/sublingual secretions.[42] With the successful compilation of the saliva proteome, a next step would be to identify potential diagnostic and/or prognostic biomarkers that could be used in a clinical context for disease detection and monitoring the saliva.

Hu *et al.* by using the in-depth analysis of the human salivary proteome revealed several salivary proteins (such as Mac-2 binding protein, myeloid related protein 14, CD59, profilin 1, and catalase) at differential levels between the oral cancer patients.[43] Several salivary protein markers in the OSCC have been investigated in various studies and have shown relatively moderate sensitivity and specificity values relative to prognosis prediction. For example, defensins are peptides which possess antimicrobial and cytotoxic properties.[44] Elevated levels of salivary defensin-1 have indication for the detection of OSCC, since higher concentrations of salivary defensin-1 were detected in the patients with OSCC compared with the healthy controls.[45] In another study, soluble CD44 was elevated in the majority of patients with OSCC and distinguished cancer from benign disease with a high specificity.[46]

St John *et al.* investigated whether IL-6 and/or IL-8 could serve as informative biomarkers for the OSCC in the saliva. Elevation of IL-6 has been shown to promote immune unresponsiveness and induction of wasting, cachexia, and hypercalcemia, all of which are observed in patients with OSCC who have a poor prognosis. IL-8 plays an important role in the stimulation of angiogenesis, proliferation, and

chemotaxis of granulocytes and macrophages, which are prominent constituents in the stroma of OSCCs. In their study, the IL-6 levels in serum and IL-8 levels in the saliva of patients with OSCC were all higher than the determined cutoff value. It is known that salivary and serum IL-6 and IL-8 levels may be increased as a result of various oral cavity inflammatory conditions. However, the results were significant for IL-8 in saliva and not for IL-6 which suggest that the OSCC contribution to the elevation of IL-8 in saliva outweighs any potential background contribution by the host's potential inflammatory conditions.[47] Many proteins at the elevated levels in OSCC patients' saliva have been previously associated with the human cancers (e.g., squamous cell carcinoma antigen 2 [SCC-Ag 2], calcyclin, Rho GDP dissociation inhibitor, heat shock 70-kDa protein 1, Annexin I, cathepsin G, peroxiredoxin II, thioredoxin, short palate, and lung and nasal epithelium carcinoma-associated protein). Apart from the potential clinical applications, these target proteins may contribute to an understanding of the molecular mechanism of the disease.[43]

Furthermore, some of the salivary proteins are underexpressed in the OSCC. For instance, clusterin is present in normal controls, but absent in the OSCC by subtractive proteomic analysis. This secretory protein is involved in programmed cell death, and downregulation of clusterin in esophageal squamous cell carcinoma and prostate cancer has been reported in previous studies.[43]

Other salivary biomarkers which are significantly altered in the OSCC patients as compared with the healthy controls are inhibitors of apoptosis, SCC-Ag, carcino-embryonic antigen, carcinoantigen (CA19-9), CA128, CA125, intermediate filament protein (Cyfra 21-1), tissue polypeptide-specific antigen, reactive nitrogen species and 8-OHdG DNA damage marker, lactate dehydrogenase and immunoglobulin, s-IgA, insulin growth factor, metalloproteinase MMP-2 and MMP-11.[48]

The salivary microbiota

Certain alterations in the diet, medications, habits, and host immune status may lead to the overgrowth of minor components of the oral microflora which predispose the site to disease. Kang *et al.* demonstrated a significant increase in the levels of *Porphyromonas gingivalis, Tannerella forsythia,* and *Candida albicans* in the cancer group than in the normal controls.[49] Mager *et al.* found significantly elevated levels of *P. gingivalis, P. melaninogenica,* and *Streptococcus mitis* in the saliva of OSCC patients, thereby suggesting the role of salivary microbiota as a diagnostic indicator in OSCC.[50]

Studies have found increased candidal carriage in the salivary samples of the OSCC group than in the normal controls.[49] This indicates that the salivary analysis of candida species might be useful as a diagnostic and prognostic indicator of the oral precancer and cancer.

The presence of Human Papilloma virus and Epstein–Barr virus genomic sequences has been identified.[17]

Conclusion

Salivary screening can be the best choice as the primary screening test for the high-risk cases of OSCC, since the collection procedure is noninvasive and low cost. In addition, the specimen is with low background and inhibitory substances and less complex than blood. Not only the proteins, but also the saliva contains cell which may fall from the cancerous tissue in the oral cavity.

With the advances in nanotechnologies applied in proteomics and genomics, many biomarkers for the OSCC have been identified extensively. It is now required further studies to confirm its specificity in a large sample size, although to dissect the extraordinary complex genetic or proteomic expression profile and to find the "true" biomarker remain a challenge. Since, more and more bioinformatic computation platforms are generated, the systematic analysis will facilitate the identification of sensitive and specific biomarkers for the OSCC as well as for the other cancers.

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Conflicts of interest

There are no conflicts of interest.

References

1. Santarelli A, Lo Russo L, Bambini F, Campisi G, Lo Muzio L. New perspectives in medical approach to therapy of head and neck squamous cell carcinoma. Minerva Stomatol 2009;58:445-52.
2. Pathak KA, Al Hajjaj H, Viallet NV, Sutherland DS, Kerr PD, Nason RW. Squamous cell carcinoma of the oropharynx: Influence of site of primary tumor. Oral Abstr 2009; Suppl 3 (1):73.
3. Wu JY, Yi C, Chung HR, Wang DJ, Chang WC, Lee SY, *et al.* Potential biomarkers in saliva for oral squamous cell carcinoma. Oral Oncol 2010;46:226-31.
4. Malati T. Tumour markers: An overview. Indian J Clin Biochem 2007;22:17-31.
5. Palmisano WA, Divine KK, Saccomanno G, Gilliland FD, Baylin SB, Herman JG, *et al.* Predicting lung cancer by detecting aberrant promoter methylation in sputum. Cancer Res 2000;60:5954-8.
6. Hoque MO, Begum S, Topaloglu O, Jeronimo C, Mambo E, Westra WH, *et al.* Quantitative detection of promoter hypermethylation of multiple genes in the tumor, urine, and serum DNA of patients with renal cancer. Cancer Res 2004;64:5511-7.
7. Nunes DN, Kowalski LP, Simpson AJ. Detection of oral and oropharyngeal cancer by microsatellite analysis in mouth washes and lesion brushings. Oral Oncol 2000;36:525-8.
8. Lee A, Kim Y, Han K, Kang CS, Jeon HM, Shim SI. Detection of tumor markers including carcinoembryonic antigen, APC, and cyclin D2 in fine-needle aspiration fluid of breast. Arch Pathol Lab Med 2004;128:1251-6.

9. Lehto VP, Pontén J. Tumor markers in human biopsy material. Acta Oncol 1989;28:743-62.
10. Rassekh CH, Johnson JT, Eibling DE. Circulating markers in squamous cell carcinoma of the head and neck: A review. Eur J Cancer B Oral Oncol 1994;30B: 23-8.
11. Chan DW, Sell S. Tumor markers. In: Burtis CA, Ashwood ER, editors. Tietz's Textbook of Clinical Chemistry. 2nd ed. Philadelphia: WB, Saunders; 1994. p. 897-925.
12. William B, Ronald BH. Characteristics of the Ideal Tumor Marker in Hollandfrei Cancer Medicine. 6th ed. Hamilton, (ON): BC, Decker; 2003.
13. Jiang J, Park NJ, Hu S, Wong DT. A universal pre-analytic solution for concurrent stabilization of salivary proteins, RNA and DNA at ambient temperature. Arch Oral Biol 2009;54:268-73.
14. Wong DT. Salivary diagnostics powered by nanotechnologies, proteomics and genomics. J Am Dent Assoc 2006;137:313-21.
15. Shah FD, Begum R, Vajaria BN, Patel KR, Patel JB, Shukla SN, *et al.* A review on salivary genomics and proteomics biomarkers in oral cancer. Indian J Clin Biochem 2011;26:326-34.
16. Baum BJ. Principles of saliva secretion. Ann N Y Acad Sci 1993;694:17-23.
17. Krishna Prasad RB, Sharma A, Babu HM. An insight into salivary markers in oral cancer. Dent Res J (Isfahan) 2013;10:287-95.
18. Streckfus CF, Dubinsky WP. Proteomic analysis of saliva for cancer diagnosis. Expert Rev Proteomics 2007;4:329-32.
19. Streckfus C, Bigler L, Tucci M, Thigpen JT. A preliminary study of CA15-3, c-erbB-2, epidermal growth factor receptor, cathepsin-D, and p53 in saliva among women with breast carcinoma. Cancer Invest 2000;18:101-9.
20. Bretschneider N, Brand H, Miller N, Lowery AJ, Kerin MJ, Gannon F, *et al.* Estrogen induces repression of the breast cancer and salivary gland expression gene in an estrogen receptor alpha-dependent manner. Cancer Res 2008;68:106-14.
21. Chen DX, Schwartz PE, Li FQ. Saliva and serum CA 125 assays for detecting malignant ovarian tumors. Obstet Gynecol 1990;75:701-4.
22. Schapher M, Wendler O, Gröschl M, Schäfer R, Iro H, Zenk J. Salivary leptin as a candidate diagnostic marker in salivary gland tumors. Clin Chem 2009;55:914-22.
23. You XY, Jiang J, Yin FZ. Preliminary observation on human saliva alpha-fetoprotein in patients with hepatocellular carcinoma. Chin Med J (Engl) 1993;106:179-82.
24. Wong DT, Zhang L, Farrell J, Zhou F, Elashoff D, Gao K, *et al.* Salivary biomarkers for pancreatic cancer detection [abstract]. J Clin Oncol 2009;27 Suppl 15S: 4630.
25. Lodhi FB, Ali M, Iftikhar, Hussain R. Serum tumor markers. Prof Med J 2006;13:110.
26. Zhang L, Rosin MP. Loss of heterozygosity: A potential tool in management of oral premalignant lesions? J Oral Pathol Med 2001;30:513-20.
27. Califano J, van der Riet P, Westra W, Nawroz H, Clayman G, Piantadosi S, *et al.* Genetic progression model for head and neck cancer: Implications for field cancerization. Cancer Res 1996;56:2488-92.
28. Lee JJ, Hong WK, Hittelman WN, Mao L, Lotan R, Shin DM, *et al.* Predicting cancer development in oral leukoplakia: Ten years of translational research. Clin Cancer Res 2000;6:1702-10.
29. Partridge M, Pateromichelakis S, Phillips E, Emilion GG, A'Hern RP, Langdon JD. A case-control study confirms that microsatellite assay can identify patients at risk of developing oral squamous cell carcinoma within a field of cancerization. Cancer Res 2000;60:3893-8.
30. Rosin MP, Cheng X, Poh C, Lam WL, Huang Y, Lovas J, *et al.* Use of allelic loss to predict malignant risk for low-grade oral epithelial dysplasia. Clin Cancer Res 2000;6:357-62.
31. Fliss MS, Usadel H, Caballero OL, Wu L, Buta MR, Eleff SM, *et al.* Facile detection of mitochondrial DNA mutations in tumors and bodily fluids. Science 2000;287:2017-9.
32. Liao PH, Chang YC, Huang MF, Tai KW, Chou MY. Mutation of p53 gene codon 63 in saliva as a molecular marker for oral squamous cell carcinomas. Oral Oncol 2000;36:272-6.
33. Tilakaratne WM. Oral cavity and major and minor salivary glands. In: The Cancer Handbook. 2nd ed. United States: John Wiley and Sons Ltd.; 2007. p. 1-15.
34. Rosas SL, Koch W, da Costa Carvalho MG, Wu L, Califano J, Westra W, *et al.* Promoter hypermethylation patterns of p16, O6-methylguanine-DNA-methyltransferase, and death-associated protein kinase in tumors and saliva of head and neck cancer patients. Cancer Res 2001;61:939-42.
35. Zhong LP, Chen GF, Xu ZF, Zhang X, Ping FY, Zhao SF. Detection of telomerase activity in saliva from oral squamous cell carcinoma patients. Int J Oral Maxillofac Surg 2005;34:566-70.
36. Vielba R, Bilbao J, Ispizua A, Zabalza I, Alfaro J, Rezola R, *et al.* p53 and cyclin D1 as prognostic factors in squamous cell carcinoma of the larynx. Laryngoscope 2003;113:167-72.
37. Shpitzer T, Hamzany Y, Bahar G, Feinmesser R, Savulescu D, Borovoi I, *et al.* Salivary analysis of oral cancer biomarkers. Br J Cancer 2009;101:1194-8.
38. Chen XQ, Stroun M, Magnenat JL, Nicod LP, Kurt AM, Lyautey J, *et al.* Microsatellite alterations in plasma DNA of small cell lung cancer patients. Nat Med 1996;2:1033-5.
39. Park NJ, Zhou H, Elashoff D, Henson BS, Kastratovic DA, Abemayor E, *et al.* Salivary microRNA: Discovery, characterization, and clinical utility for oral cancer detection. Clin Cancer Res 2009;15:5473-7.
40. Li Y, St John MA, Zhou X, Kim Y, Sinha U, Jordan RC, *et al.* Salivary transcriptome diagnostics for oral cancer detection. Clin Cancer Res 2004;10:8442-50.
41. Crocker J, Murray PG, editors. Molecular Biology in Cellular Pathology. Chichester: John Wiley and Sons Ltd.; 2003. p. 352.
42. Denny P, Hagen FK, Hardt M, Liao L, Yan W, Arellanno M, *et al.* The proteomes of human parotid and submandibular/sublingual gland saliva collected as the ductal secretions. J Proteome Res 2008;7:1994-2006.
43. Hu S, Arellano M, Boontheung P, Wang J, Zhou H, Jiang J, *et al.* Salivary proteomics for oral cancer biomarker discovery. Clin Cancer Res 2008;14:6246-52.
44. Lehrer RI, Ganz T, Selsted ME. Defensins: Endogenous antibiotic peptides of animal cells. Cell 1991;64:229-30.
45. Mizukawa N, Sugiyama K, Fukunaga J, Ueno T, Mishima K, Takagi S, *et al.* Defensin-1, a peptide detected in the saliva of oral squamous cell carcinoma patients. Anticancer Res 1998;18:4645-9.
46. Franzmann EJ, Reategui EP, Pedroso F, Pernas FG, Karakullukcu BM, Carraway KL, *et al.* Soluble CD44 is a potential marker for the early detection of head and neck cancer. Cancer Epidemiol Biomarkers Prev 2007;16:1348-55.
47. St John MA, Li Y, Zhou X, Denny P, Ho CM, Montemagno C, *et al.* Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 2004;130:929-35.

48. Markopoulos AK, Michailidou EZ, Tzimagiorgis G. Salivary markers for oral cancer detection. Open Dent J 2010;4:172-8.
49. Kang MS, Oh JS, Kim HJ, Kim HN, Lee IK, Choi HR, *et al*. Prevalence of oral microbes in the saliva of oncological patients. J Bacteriol Virol 2009;39:277-85.
50. Mager DL, Haffajee AD, Devlin PM, Norris CM, Posner MR, Goodson JM. The salivary microbiota as a diagnostic indicator of oral cancer: A descriptive, non-randomized study of cancer-free and oral squamous cell carcinoma subjects. J Transl Med 2005;3:27.