Research Paper

A Review on Body Fluids Proteomics of Oral Cancer

Biotechnology

Nishant Sharma	Asst. Proff., DEPARTMENT OF BIOTECHNOLOGY, COLLEGE OF LIFE SCIENCES CANCER HOSPITAL AND RESEARCH INSTITUTE GWALIOR 474009, MP INDIA
Dr. Archana Shrivastav	COLLEGE OF LIFE SCIENCES, GWALIOR 474009, MP

ABSTRACT
The evolving of novel technologies allows researchers to assist the comprehensive research in cancer. Proteomic study of human body fluid (saliva and serum) holds promise as a non-invasive method to identify biomarkers for human oral cancer. The statistics are fairly shocking with 14 deaths per hour due to oral cancer in India which inhabits 40% of world smokers. This review provides a brief overview of body fluids proteome and their applications in admiration to oral cancer proteomics. In this way we characterize several promising biomarkers for detection of oral cancer. However, IL-8 and MMP-1 and MMP-3 showed good sensitivity, specificity and both are tested in saliva. Due to its proximity to oral cavity and non-invasive collection procedure, salivary screening can be the finest option as primary screening test for oral cancer.

Introduction

Proteomics is a foremost approach for comprehensive study of the structure and function of all proteins expressed in a biological system. it is a powerful approach to discover protein biomarkers in human diseases and cancer for clinical and diagnostic applications (Wilkins *et.al.,* 1995).

Biomarkers are quantifiable biological parameters that can serve as indicates for health-related assessments, such as disease diagnosis (Srinivas *et.al.,* 2001). a approach for proteomic analysis of human tumors is described by (Emmert-Buck *et. al.,*2000)

Oral cancer is a diverse group of cancers arising from different parts of the oral cavity that includes cancers of the lip, tongue, gingiva, oral mucosa, oropharynx, and hypopharynx (Miller, 1988). It is reported that frequency of this cancer is 6th in men and 14th in women (Sankaranarayan *et.al.,* 1998). The statistics are fairly shocking with 14 deaths per hour due to oral cancer in India which inhabits 40% of world smokers. (Ferlay *et.al.,* 2010). In India, the age consistent incidence rate of oral cancer is 12.6 per 100,000 populations and a sharp increase in the incidence rate of this cancer have been reported in recent years (Misra *et.al.,* 2009).

The high incidence of oral cancer in India has also been linked with habits of tobacco chewing and smoking (Patel *et.al.,* 2009).

Body fluids and oral cancer
Human body fluid such as saliva, plasma, tissue fluid contains proteins that can be instructive for disease detection and surveillance of oral health. At the molecular level, the development of oral cancer is a multistep process accompanied by genetic mutations and expression changes of many genes that lead to uncontrolled cellular growth (Lippman *et.al.* 2005 & Chen *et.al.,* 2004). Due to this abnormality some of the proteins may be secreted and may be found in the body fluid that helps in protein biomarker discovery.

Serum proteome

Serum is a complex body fluid, containing More than 10000 different proteins and many of them are secreted or shed by cells during different physiology or pathology processes (Anderson *et.al.* 2002). Low molecular weight human serum proteins, peptides, and other small components have been associated with pathological conditions such as cancer (Petricoin *et.al.,* 2002). Serum is attractive approach due to testing is simple, safe and minimally invasive (Hu *et.al.* 2006).

Saliva proteome

Human whole saliva has a protein content of about 0.5 to 3 mg/mL the protein concentration is fairly stable and independent from the flow rate (Burgen *et.al.,* 1961). The number of distinct salivary proteins is roughly between 100 and 140 (Wilmarth *et.al.,* 2004 & Yao *et.al.,* 2003) . From which roughly 30.40 % are produced by the salivary glands, whereas other proteins are originate from serum, from mucosal and/or immune cells, or from microorganisms (Wilmarth *et.al.,* 2004).

Saliva is easily collected in the clinic in non-invasive relatively large, easily stored quantities, making it an optimal bodily fluid for clinical diagnostics (Hofman, 2001 & Lawrence, 2002).

Proteomics

Various Proteomic techniques such as 2D-liquid chromatography/mass spectrometry (2D-LC/MS) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF/MS) have been used in saliva/serum studies. Based on those techniques, potential salivary biomarkers for diseases such as Sjögren syndrome (Baldini *et.al.,* 2011), diabetes mellitus (Caseiro *et.al.,* 2012) and some different cancers (Xiao et.al., 2012).

This article provides a brief overview of body fluids proteome and their selection in admiration to oral cancer proteomics.

Proteomics and Oral Cancer
In evaluation with gene expression profiling of oral cancer, the studies on proteomic profiling of oral cancer are limiting. The first proteomic examination of oral tongue carcinoma was reported by (Qing-Yu *et.al.,*2004) Protein expression profiles of 10 tumors and their matched normal mucosa resection margins were analyzed by matrix-assisted laser desorption / ionization-time of flight mass spectrometry (MALDI-MS). Several tumor associated proteins including HSP60, HSP27, alpha-B-crystalline, ATP synthase beta, calgranulin B, myosin, tropomyosin and galectin 1 were found to be significantly altered in tongue carcinoma tissues. Using the same technique, also provided the proteomic signatures of buccal squamous cell carcinoma (Chen *et.al.,* 2004). These authors observed multiple cellular pathways are involved in oral tumorigenesis.

In this way also 52 salivary proteins were found to be present in oral squamous cell carcinoma but are absent in the healthy control subjects, whereas 29 proteins were found only in the healthy subjects but absent in oral squamous cell carcinoma (Shen *et.al.,*2008).

In other hand discovered five potential protein biomarkers (calgranulin B, Mac-2 binding protein, CD-59, catalase, and profilin) in saliva. The combination of these applicant biomarkers yielded a sensitivity of 90% and a specificity of 83% in detecting oral squamous cell carcinoma (Shen *et.al.*2010).

Previous studies of in vitro human cell lines as well as oral cancer tumors have demonstrated that concentration of certain proinflammatory, proangiogenic cytokines such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1, IL-6, and IL-8 are

increased that they have roles in cell growth, invasion, interruption of tumor suppression (Rhodus *et.al.,* 2005).

MMPs (Metalloproteinases) are proteolytic enzymes that cause degradation of extracellular matrix (ECM) and basement membranes thus may play a key role in cancer development and allowing for tumor cell migration (Rodrigo *et.al.,* 2005). Several MMPs, including MMP-1, the gelatinases MMP-2 and MMP-9, and the stromelysins MMP-3 and MMP-10, have been implicated in cancer cell invasion and metastasis (Duffy *et.al.,* 1998 & Marcos *et.al.,* 2009). High levels of Salivary concentrations of MMP1 and MMP3 in OSCC patients (33 stage I/II, 26 stage III/IV) were 6.2 times (95% CI: 3.32–11.73) and 14.8 times (95% CI: 6.75–32.56) higher, respectively, than in controls, and displayed an increasing trend with higher stage disease (Marni *et.al.,* 2011).

(Ranuncolo *et.al.,*2002) detected enhanced MMP-9 activity using euglobulin plasma fraction demonstrating 80% positive rates with 10 cases of stage I disease of oral cancer.Interleukin (IL)-6 and IL-8. In two studies (Li *et.al.,* 2004 & St.John *et.al.,* 2004) encompassing 32 patients with primary T1 or T2 squamous cell carcinoma of the oral cavity or oropharynx (OSCC), IL-8 was detected at higher concentrations in the saliva of patients with OSCC and IL-6 was detected at higher concentrations in the serum of patients with OSCC, indicating that IL-8 in saliva and IL-6 in serum hold promise as biomarkers for OSCC. These cytokines have also been linked with increased tumor growth and metastasis (Zimmermann *et al.,* 2007). Cytokeratin 17 (CK-17) is over expressed in OSCC compared to normal mucosa (Toyoshima *et.al.,*2008). Prominent levels of salivary soluble CD44 were shown in the majority of OSCC and could distinguish cancer from benign disease with high specificity (Franzmann *et.al.,* 2007). Three tumor markers, cytokeratin 19 fragment Cyfra21- 1, tissue polypeptide antigen, and cancer antigen 125, were found significantly elevated in the saliva of OSCC patients (Nagler *et.al.,* 2006). the five candidate markers (M2BP, MRP14, CD59, catalase, and profilin), collectively, provide a sensitivity of 90% and a specificity of 83% for OSCC detection (Shen *et.al.,* 2008).

CD59 (protectin) is one of the complement restriction factors that are over expressed on tumor cells, and they enable tumor cells to escape from complement-dependent and antibody-mediated killing (Ravindranath *et.al.,* 2007). so that different over expressed proteins are involved in molecular alteration of oral cancer. We summarized all these proteins in a **Table**.

Conclusion

Saliva and serum research field is rapidly evolving and advancing due to the use of novel approaches including proteomics and bioinformatics. We reviewed several promising biomarkers or over expressed proteins of body fluids for early detection of oral cancer. However, IL-8 and MMP-1, MMP-3 showed good sensitivity, specificity and both are tested in saliva.

Author Reference	Year	Specimen	Marker
Ranuncolo SM et al.(94)			MMP-9
Li Y et al.(10), MA John (130)	2002	Plasma	IL-8 IL-6
Li et al.(13)	2004	Saliva Serum	IL-8,IL-1B DUSP,HA3
He et al.(13)	2004	Saliva	HSP-60,HSP-27,αβ-CrystalineATP
	2004	Tissue fluid	Synthease, β calgranulin B, myosin, tropomyosin and Galectin 1
Chem et al.(30)	2004	Tissue fluid	Glycolytic enzyme, Heat Shock proteins.
Nagler R et al.(12)	2006	Saliva	cytokeratin 19 fragment Cyfra21- 1, tissue polypeptide antigen, and cancer antigen 125
Franzmann EJ et al.(16)	2007	Saliva	
Jamee M et al.(13)	2008	Saliva	CD44
Toyoshima et al.(48)	2008	Tissue fluid	IL-8
	2008	Saliva	CK-17
Hu et al.(14)	2009	Tissue fluid	MRP14, CD59, profilin 1, and catalase.
Yen CY et al.(14)	2010	Saliva	MMP-1,MMP-10
De jong et al.(60)	2011	Saliva	Actin and Myosin
Stott-Miller et al.(12)			MMP-1 and MMP-3

Table: Summary of over-expressed proteins in oral cancer.

IL: Interleukin, MMP: Metalloproteinase's, DUSP: Dual specific phosphate, HA: hemagglutinin, CK: Cytokeratin.

Research Paper

REFERENCE

1 Anderson N. L. & Anderson N. G: The human plasma proteome: history, character, and diagnostic prospects. Molecular and Cellular Proteomics, 2002; 1(11):pp. 845–867. | 2 Baldini C, Giusti L, Ciregia F, Da Valle Y, Giacomelli C, Donadio E, Ferro F, Galimberti S, Donati V, Bazzichi L, Bombardieri S, Lucacchini . A Correspondence between salivary proteomic pattern and clinical course in primary Sjögren syndrome and non- Hodgkin's lymphoma: a case report. J Transl Med, 2011; 9: 188. | 3 Burgen ASV, Emmelin NG. Physiology of the Salivary Glands. Edward Arnold LTD, London 1961; pp. 140-194. | 4 Caseiro A, Vitorino R, Barros AS, Ferreira R, Calheiros-Lobo MJ, Carvalho D, Duarte JA & Amado F : Salivary peptidome in type 1 diabetes mellitus. Biomed Chromatogr, 2012; 26: 571-82. | 5 Chen J, He QY, Yuen AP & Chiu JF. Proteomics of buccal squamous cell carcinoma: the involvement of multiple pathways in tumorigenesis. Proteomics, 2004 4: 2465-75. | 6 Chen J, He QY, Yuen AP & Chiu JF . Proteomics of buccal squamous cell carcinoma: the involvement of multiple pathways in tumorigenesis. Proteomics, 2004;4(8):2465-75. | 7 Duffy MJ, McCarthy K: Matrix metalloproteinases in cancer: Prognostic markers and targets for therapy. Int J Oncol, 2005; 12: 1343-1348, 1998. | 8 Emmert-Buck MR, Gillespie JW & Paweletz CP. An approach to proteomic analysis of human tumors. Molecular Carcinogenesis, 2000; 27: 158-165. | 9 Ferlay J, Shin HR, Bray F, Forman D, Mathers C & Parkin DM Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer, 2010; 127:2893–917. | 10 Franzmann EJ, Reategui EP & Pedroso F . Soluble CD44 is a potential marker for the early detection of head and neck cancer. Cancer Epidemiol Biomarkers Prev, 2007; 16:1348-55. | 11 Hofman, L. F: Human saliva as a diagnostic specimen. J. Nutr, 2001;131:1621S–1625S. | 12 Hu, S., Loo, J.A.& Wong, D.T. Proteomics, 2006; 6: 6326. | 13 Lawrence, H. P: Salivary markers of systemic disease: noninvasive diagnosis of disease and monitoring of general health. J. Can. Dent. Assoc, 2002; 68:170– 174. | 14 Li Y, StJohn MA, Zhou X, Kim Y, Sinha U, Jordan RC, Eisele D, Abemayor E,Elashoff D, Park NH & Wong DT. Salivary transcriptome diagnostics for oral cancer detection. Clin Cancer Res, 2004; 10: 8442-8450. | 15 Lippman SM, Sudbo J, Hong WK: Oral cancer prevention and the evolution of molecular-targeted drug development. J Clin Oncol, 2005; 23:346-56. | 16 Marcos CÁ, Martínez DAK, delosToyos JR, DomínguezIglesias F, Hermsen M,Guervós M A & Pendás JLL. The usefulness of new serum tumor markers in head and neck squamous cell carcinoma. Otolaryngol Head Neck Surg, 2009; 140:375-380. | 17 Marni Stott-Miller, John R. Houck1, Pawadee Lohavanichbutr, Eduardo Méndez, Melissa P. Upton. Tumor and Salivary Matrix Metalloproteinase Levels Are Strong Diagnostic Markers of Oral Squamous Cell Carcinoma. Cancer Epidemiol Biomarkers, 2011; 20(12): 2628–36. | 18 Miller BA. Cancer/gov [homepage on the net]. Racial/Ethnic Patterns of Cancer in the United States 49, 1988. | 19 Misra C, Majumder M, Bajaj S, Ghosh S, Roy B & Roychoudhury S Polymorphism at p53, p73, and MDM2 loci modulate the risk of tobacco associated leukoplakia and oral cancer. Mol Carcinogen, 2009; 48:790–800. | 20 Nagler R,BaharG & ShpitzerT . Concomitant analysis of salivary tumor markers new diagnostic tool for oral cancer. Clin Cancer Res, 2006; 12:3979-84. | 21 Patel JB, Shah FD, Shukla SN, Shah PM & Patel PM. Role of nitric oxide and antioxidant enzymes in the pathogenesis of oral cancer. J Cancer Res Ther, 2009; 5:247–253. | 22 Petricoin, E. F, Ardekani, A. M , Hitt, B. A, Levine, P. J, Fusaro, V. A & Steinberg, S. Use of proteomic patterns in serum to identify ovarian cancer. Lancet, 2002; 359:572– 577. | 23 Qing-Yu He, Jia Chen, Hsiang-Fu Kung, Anthony Po-Wing Yuen & Jen-Fu Chiu. Identification of tumor-associated proteins in oral tongue squamous cell carcinoma by proteomics, 2004; 4(1):271-8. | 24 Ranuncolo SM, Matos E, Loria D, Vilensky M, Rojo R, Bal de Kier Joffe E, Ines Puricelli L . Circulating 92-kiloDalton matrix metalloproteinase (MMP-9) activity is enhanced in the euglobulin plasma fraction of head and neck squamous cell carcinoma. Cancer, 2002; 94: 1483-1491. | 25 Ravindranath NM & Shuler C. Cell-surface density of complement restriction factors (CD46, CD55, and CD59): oral squamous cell carcinoma versus other solid tumors. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2007; 103:231- 9. | 26 Rhodus NL, Ho V, Miller CS, Myers S & Ondrey F. NF-kappab dependent cytokine levels in saliva of patients with oral preneoplastic lesions and oral squamous cell carcinoma. Cancer Detect Prev, 2005; 29(1):42-5. | 27 Rodrigo JP, Ferlito A, Suarez C, Shaha AR, Silver CE & Devaney KO. A New molecular diagnostic method in head and neck cancer. Head Neck 27: 995-1003,. | 28 Sankaranarayan R, Masuyer E, Swaminathan R, Ferley J & Whelan S. Head and neck cancer: a global perspective on epidemiology and prognosis. Anticancer Res, 1998; 18:4779-86. | 29 Shen Hu, Martha Arellano & Pinmanee Boontheung . Salivary Proteomics for Oral Cancer Biomarker Discovery. Clin Cancer Res, 2008; 14:6246-6252. | 30 Shen Hu, Jiang Jiang, & David T. Wong. Proteomic Analysis of Saliva: 2D Gel Electrophoresis, LC-MS/MS, and Western Blotting. Methods Mol Biol, 2010; 666: 31–41. | 31 Shen Hu,Martha Arellano, Pinmanee Boontheung, Jianghua Wang & Hui Zhou. Salivary Proteomics for Oral Cancer Biomarker Discovery. American Association for Cancer Research, 2008; 10: CCR-07-5037. | 32 Srinivas P.R, Kramer B.S & Srivastava S: Trends in biomarker research for cancer detection. Lancet Oncol, 2001; 2:698-704. | 33 St.John MA, Li Y, Zhou X, Denny P, Ho CM, Montemagno C & Shi W. Interleukin-6 and interleukin-8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. Arch Otolaryngol Head Neck Surg, 2004; 130: 929-935. | 34 Toyoshima T, Vairaktaris E, Nkenke E, Schlegel KA, Neukam FW & Ries J.Cytokeratin-17 mRNA expression has potential for diagnostic marker of oral squamous cell carcinoma. J Cancer Res Clin Oncol, 2008; 134: 515-521. | 35 Wilkins M. R., Sanchez J.C. & Goosey A: A Progress with proteome projects: why all proteins expressed by a genome should be identified and how to do it, Biotechnology and Genetic Engineering Reviews, 1995; vol. 13:pp. 19–50. | 36 Wilmarth PA, Riviere MA, Rustvold DL, Lauten JD, Madden TE & David LL. Two-dimensional liquid chromatography of the human whole saliva proteome. J. Proteome, 2004; Res. 3:1017-1023. | 37 Xiao H, Zhang L, Zhou H, Lee JM, Garon EB & Wong DT . Proteomic analysis of human saliva from lung cancer patients using two-dimensional difference gel electrophoresis and mass spectrometry. Mol Cell Proteomics, 2012; 11: M111.012112. | 38 Yao Y, Berg EA, Costello CE, Troxler RF & Oppenheim FG. Identification of protein components in human acquired enamel pellicle and whole saliva using novel proteomics approaches. J.Biol. Chem, 2003; 278:5300-5308. | 39 Zimmermann BG, Park NJ & Wong DT. Genomic targets in saliva. Ann N Y Acad Sci, 2007; 1098: 184-191. | |