13

The Use of Saliva Protein Profiling as a Biometric Tool to Determine the Presence of Carcinoma among Women

Charles F. Streckfus and Cynthia Guajardo-Edwards
University of Texas Dental Branch at Houston,
United States of America

1. Introduction

1.1 Background information

Biometrics is the science and technology of measuring and analyzing biological data. It also refers to technologies that measures and analyzes human body characteristics for identification purposes. In the context of this book chapter, identification will refer to the recognition of those individuals in a disease state *i.e.*, carcinoma of the breast. Using "start-of-the-art" mass spectrometry protein analysis, the author will demonstrate the use of salivary protein profiles to recognize individuals at risk for carcinoma of the breast.

Proteomic analyses of varying body fluids are propelling the field of medical research forward at unprecedented rates due to its consistent ability to identify proteins that are at the fentomole level in concentration. These advancements have also benefited biometric research to the point where saliva is currently recognized as an excellent diagnostic medium for biometric authentication of human body characteristics. The saliva microbiome, for example, is reputed to be biometrically as accurate as a fingerprint. Collectively, these efforts are in the area of biological verification; however, biometric can be applied to identify the biological characteristics of a diseased individual.

1.2 Why Saliva as a diagnostic media?

1.2.1 Analytical advantages of Saliva

Saliva as a diagnostic fluid has significant biochemical and logistical advantages when compared to blood. Bio-chemically, saliva is a clear liquid with an average protein concentration of 1.5 to 2.0 mg/ml. As a consequence of this low protein concentration, it was once assumed that this was a major drawback for using saliva as a diagnostic fluid; however, current ultra sensitive analyte detection techniques have eliminated this barrier. Saliva specimen preparation is simple involving centrifugation prior to storage and the addition of a cocktail of protease inhibitors to reduce protein degradation for long-term storage.

Blood is a far more complex medium. A decision has to be made as to whether to use serum or plasma. Serum has a total protein concentration of approximately 60-80 mg/ml. Since serum possesses more proteins than saliva, assaying trace amounts of "factors" (*e.g.*, oncogenes, etc.), may result in a greater risk of non-specific interference and a greater chance

for hydrostatic (and other) interactions between the factors and the abundant serum proteins. Serum also possesses numerous carrier proteins, *e.g*., albumin, which must either be removed or treated prior to being assayed for protein content. Additionally, it has been demonstrated that clotting removes many background proteins, which may be altered in the presence of disease. It has been demonstrated that enzymatic activity continues during this process, which may cleave proteins from many relevant pathways (Koomen et al., 2005).
It would be ideal if all enzymatic activity in serum would cease at the time of collection; however, proteomic analyses of serum has shown that this is not the case. As a consequence, plasma is also being explored as a diagnostic fluid. The main consideration in using plasma is the selection of a proper anticoagulant (Koomen et al., 2005; Teisner et al., 1983). Heparin for example can be used as an anti-clotting agent; however, current research has found that heparin has a relatively short half life (3 to 4 hours) and can produce products of coagulation which are abundantly comparable to those assayed in serum. Based on these observations, it is recommended that blood specimens be collected with ethylenediamine tetraacetic acid (EDTA).

1.2.2 Collection advantages of Saliva

From a logistical perspective, the collection of saliva is safe (*e.g*., no needle punctures), non-invasive and relatively simple, and may be collected repeatedly without discomfort to the patient [4]. Consequently it may be possible to develop a simplified method for "home-testing", testing in a "health fair" setting or in dental clinics where individuals are available for periodic oral examinations. This diagnostic potential could reach many individuals who for personal, logistical or economical reasons lack access to preventive care.
Blood is a more complicated medium to collect. It requires highly trained personnel to collect it and if collected incorrectly, can lead to misinterpretations which can result in patient mismanagement (Ernest & Balance, 2006). Blood specimens need to be collected in a specific sequence and under-filling tubes with additives may possibly alter protein analyses. Additionally, if specimens are collected during hospital or clinical settings, there may be a lapse of time before being processed.

1.2.3 Saliva collection

The oral cavity receives secretions from three pairs of major salivary glands and numerous minor salivary glands that are located on the oral buccal mucosa, palate, and tongue each producing a unique type of secretion with varying protein constituents (Birkhed & Heintze, 1989). For example the parotid and Von Ebner glands (located on the tongue) produce serous secretions while the minor salivary glands produce mucinous secretions. The submandibular and sublingual glands, however, produce mixed secretions which are both serous and mucinous. As a consequence, composite or "whole" saliva is preferred as it enhances the chances of finding a biomarker due to the variety of sources from which it derives and because of its simplicity to collect.
There are basically two types of saliva to collect. One type is "resting" or unstimulated whole saliva and the other is stimulated whole saliva. There are several methods for collecting unstimulated whole saliva. These include the draining or drool method, spitting method, suction method and the swab method. These methods will yield 0.47, 0.47, 0.54 and 0.52 ml/minute of saliva respectively. Of the four methods, the most reliable is the suction method with a reliability coefficient of $r = 0.93$. It also revealed a within subject variance of

0.14. This is a very reliable method; however, a vacuum pump is required to collect the specimens (Birkhed & Heintze, 1989).

There are several drawbacks when using unstimulated saliva. The major problem is the small amount of saliva derived from collection. The 0.47-0.54 ml/minute is the range for healthy individuals (0.25–0.35 ml/minute normal range) under ideal conditions using those aforementioned collection methodologies. If the subject is taking medications that decrease flow rates (*e.g*., anti-hypertensive medications) the amount collected will be significantly reduced. Additionally, if the subject has autoimmune disorders (*e.g*., Sjögren's syndrome), has under gone head and neck radiation, or is very elderly, it will be difficult to obtain 0.5 ml over a five minute period. Unstimulated saliva flow rates are also influenced by circadian and circannual rhythms. Therefore, for consistency, individuals will need to be serially assessed at approximately the same time of day that the baseline specimen was collected. All other participants will need to be collected at approximately the same time in order to reduce inter-variability among the participating subjects. In conclusion, due to the small quantity of specimen obtained from these techniques and the large within subject variance, one can conclude that using unstimulated saliva is not the ideal medium for cancer biomarker discover.

The alternative to using unstimulated whole saliva is obviously to use stimulated whole saliva. Stimulated secretions produce about three times the volume of unstimulated secretions and are not subjected to the effects of circadian rhythm. Additionally, you will be able to collect sufficient quantities of saliva despite health status and medication usage. The flow rate range is 1 – 3 ml/minute for healthy individuals (Birkhed & Heintze, 1989; Gu et al., 2004).

There are two methods for collecting stimulated whole saliva. One method of collection is the gustatory method and the other is the reflexive or "masticatory" technique. The gustatory technique requires the use of an oral based secretory stimulant. Citric acid is the most widely used stimulant. Five drops of a 1-6% citric acid solution is applied to the dorsum of the tongue every 30 seconds. The saliva accumulates in the mouth and is expectorated intermittently for a period of five minutes. This technique produces copious amounts of saliva; however, the reliability is only r = 0.76 and has a within subject variance of 0.49.

The reflexive method is based on the reflex response occurring during the mastication of a bolus of food. Usually, a standardized bolus (1 gram) of paraffin or a gum base (Wrigley Co., Peoria, IL) is given to the test subject and they chew the substance at a regular rate. The subject expectorates intermittently during the collection period for duration of five minutes. This is an accurate technique as it has a reliability coefficient of r = 0.95 and a within subject variability of 0.11. The authors recommend this salivary collection method for biomarker discovery.

The procedure for collecting Stimulated Whole Salivary Gland Secretions is as follows: A standard piece of unflavored gum base (1.0 - 1.5 g.) is placed in the subject's mouth. The armamentarium used for this procedure is illustrated in Figure 1. The patient is asked to swallow any accumulated saliva and then instructed to chew the gum at a regular rate (using a metronome). The subject, upon sufficient accumulation of saliva in the oral cavity, expectorates periodically into a preweighed disposable plastic cup. This procedure is continued for a period of five minutes. The cup with the saliva specimen is reweighed and the flow rate determined gravimetrically. The volume and flow rate is then recorded along with a brief description of the specimen's physical appearance (Gu et al., 2004).

1.2.4. Long-term Saliva specimen banking

Roughly 2 - 5 ml of whole saliva will be obtained from the individual. In order to minimize the degradation of the proteins, protease inhibitor cocktail (Sigma, 1 mg/ml whole saliva) and 1 mM of sodium orthovanadate are added immediately after sample collection (Shevchenko et al., 2002). All samples are kept on ice during the process. The specimen is next divided into 0.5 ml aliquots, placed into bar code labeled cryotubes, and frozen (-80°C).
To assess specimen degradation, ten healthy subjects were serially sampled for saliva over a five-year period. We used c-*erb*B-2 to test for specimen stability as this is a large 185-kDa protein, which would be susceptible to degradation by proteases and other biochemical activity. The results are shown in Figure 1 and illustrate protein stability when frozen at -80°C.These results are consistent with Wu et al, 1993 where they assayed serially sampled salivary specimens which were collected over a ten year period for total protein, lactoferrin (77 kDa) and histidine rich proteins concentrations. In their study, they found no concentration differences due to specimen aging.

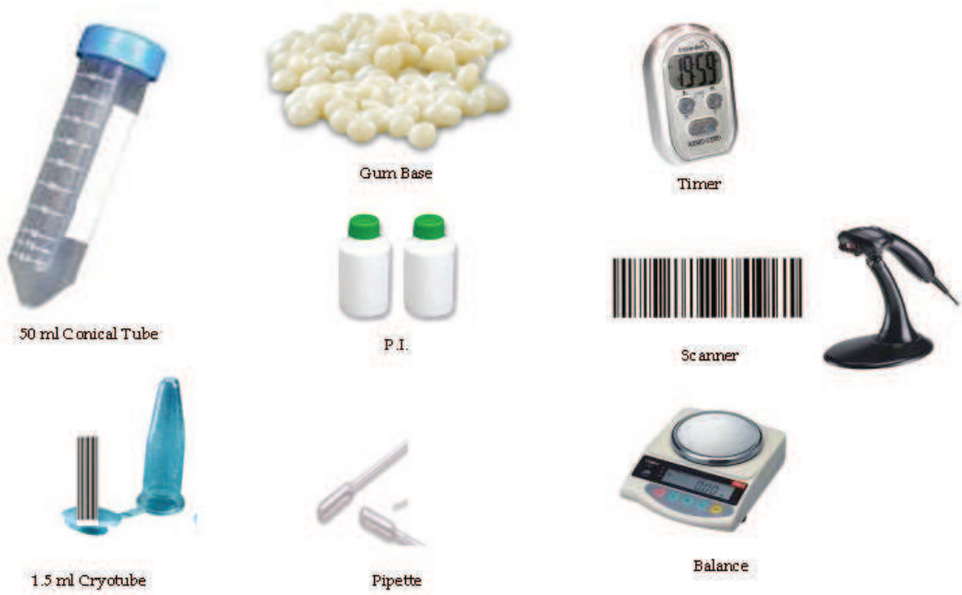


Fig. 1. Armamentarium for the collection and storage of stimulated whole saliva

1.3 Studies using Saliva protein profiling for disease state detection

The majority of the literature concerning human saliva biometrics is associated with the oral cavity and its associated maladies. An example of this statement is demonstrated in a manuscript assessing salivary proteins associated with burning mouth syndrome (Moura et al., 2007). The principle objective the present study was to analyze the characteristics of salivary production and its composition in individuals with burning mouth syndrome. The investigators compared salivary flow rates, potassium, iron, chloride, thiocyanate, magnesium, calcium, phosphorus, glucose, total protein and urea concentrations, as well as the expression profile of salivary proteins by SDS-PAGE among healthy individuals and those diagnosed with burning mouth syndrome. The results of the study showed that mean salivary flow rates among control patients were lower than that of burning mouth syndrome patients. Chloride, phosphorus and potassium levels were elevated in patients with burning

mouth syndrome ($p = 0.041$, 0.001 and 0.034, respectively). Total salivary protein concentrations were reduced in individuals with burning mouth syndrome ($p = 0.223$). Additionally, the analysis of the expression of salivary proteins by Coomassie blue SDS-PAGE revealed a lower expression of low molecular weight proteins in individuals with burning mouth syndrome compared to healthy controls. The results suggested that the identification and characterization of low molecular weight salivary proteins in burning mouth syndrome may be important in understanding BMS pathogenesis, thus contributing to its diagnosis and treatment.

Another study using salivary protein profiles investigated the modification of the salivary proteome occurring in type 1 diabetes and to highlight potential biomarkers of the disorder. High-resolution two-dimensional gel electrophoresis and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry was combined to perform a large scale analysis of the salivary specimens. The proteomic comparison of saliva samples from healthy subjects and poorly controlled type-1 diabetes patients revealed a modulation of 23 proteins. Fourteen isoforms of α-amylase, one prolactin inducible protein, three isoforms of salivary acidic protein-1, and three isoforms of salivary cystatins SA-1 were detected as under expressed proteins, whereas two isoforms of serotransferrin were over expressed secondary to type-1 diabetes. The proteins under expressed were all known to be implicated in the oral anti-inflammatory process, suggesting that the pathology induced a decrease of non-immunological defense of oral cavity. As only particular isoforms of proteins were modulated, type-1 diabetes seemed to differentially affect posttranslational modification of the proteins (Hirtz et al., 2006).

An additional study (Delaleu et al., 2008) investigated the involvement of 87 proteins measured in serum and 75 proteins analyzed in saliva in spontaneous experimental Sjögren's syndrome. In addition, they intended to compute a model of the immunological situation representing the overt disease stage of Sjögren's syndrome. In this animal study, they used non-diabetic, non-obese diabetic mice for salivary gland dysfunction. The mice aged 21 weeks and were evaluated for salivary gland function, salivary gland inflammation and extra-glandular disease manifestations. The analytes, comprising chemokines, cytokines, growth factors, autoantibodies and other biomarkers, were quantified using multi-analyte profile technology and fluorescence-activated cell sorting. Age-matched and sex-matched Balb/c mice served as a reference. The investigators found non-diabetic, non-obese diabetic mice tended to exhibit impaired salivary flow, glandular inflammation and increased secretory SSB (anti-La) levels. Thirty-eight biomarkers in serum and 34 in saliva obtained from non-diabetic, non-obese diabetic mice were significantly different from those in Balb/c mice. Eighteen biomarkers in serum and three chemokines measured in saliva could predict strain membership with 80% to 100% accuracy. Factor analyses identified principal components mostly correlating with one clinical aspect of Sjögren's syndrome and having distinct associations with components extracted from other families of proteins. They concluded that the autoimmune manifestations of Sjögren's syndrome are greatly independent and associated with various immunological processes; however, CD40, CD40 ligand, IL-18, granulocyte chemotactic protein-2 and anti-muscarinic M3 receptor IgG3 may connect the different aspects of Sjögren's syndrome. Processes related to the adaptive immune system appear to promote Sjögren's syndrome with a strong involvement of T-helper-2 related proteins in hyposalivation. This approach further established saliva as an attractive biofluid for biomarker analyses in Sjögren's syndrome and provides a basis for the comparison and selection of potential drug targets and diagnostic markers (Delaleu et al., 2008).

2. Current research in Salivary protein profiling for cancer detection

2.1 Methods

2.1.1 Study design

The purpose of this study was to determine if individuals could be protein profiled and potentially classified as having cancer. The investigator also wanted to ascertain if there were alterations of the protein profiles due to the primary tissue site and the varying degree of tumor staging. In order to achieve this objective, the investigators collected saliva from women that were healthy and from those diagnosed with carcinoma breast in the following stages: Stage 0, Stage I, Stage IIa and Stage IIb. All the tumors were adenocarcinomas. Additionally, specimens were collected from women diagnosed with varying gynecological carcinomas. These included women diagnosed with moderate cervical dysplasia, severe cervical dysplasia and cervical carcinoma in situ. These tumors were all squamous cell carcinomas. Women diagnosed with ovarian and endometrial carcinomas were also included in the study. These malignancies were identified as adenocarcinomas. The final group consisted of women diagnosed with head and neck squamous cell carcinomas ten women with varying stages of development. Due to the difficulty in obtaining early stage tumors for ovarian, endometrial and head/neck carcinomas, a composite of varying staged patients formed these saliva pools.

This study was performed under the UTHSC IRB approved protocol number HSC-DB-05-0394. All procedures were in accordance with the ethical standards of the UTHSC IRB and with the Helsinki Declaration of 1975, as revised in 1983. The specimens were banked at the University of Texas Dental Branch Saliva repository, which stores the specimens at -80°C. Ten saliva specimens were pooled for each type of carcinoma. The saliva samples were pooled by combining equal volumes of cleared stimulated whole saliva from a set of archived healthy and cancer subjects. The subjects were matched for age and race and were non-tobacco users. Previous studies by the investigator have demonstrated that properly prepared specimens can remain in storage for a long period of time.

2.1.2 Saliva collection and sample preparation

Stimulated whole salivary gland secretion is based on the reflex response occurring during the mastication of a bolus of food. Usually, a standardized bolus (1 gram) of paraffin or a gum base (generously provided by the Wrigley Co., Peoria, IL) is given to the subject to chew at a regular rate. The individual, upon sufficient accumulation of saliva in the oral cavity, expectorates periodically into a preweighed disposable plastic cup. This procedure is continued for a period of five minutes. The volume and flow rate is then recorded along with a brief description of the specimen's physical appearance (Navazesh &Christensen, 1982). The cup with the saliva specimen is reweighed and the flow rate determined gravimetrically. The authors recommend this salivary collection method with the following modifications for consistent protein analyses. A protease inhibitor from Sigma Co (St. Louis, MI, USA) is added along with enough orthovanadate from a 100mM stock solution to bring its concentration to 1mM. The treated samples were centrifuged for 10 minutes at top speed in a table top centrifuge. The supernatant was divided into 1 ml aliquots and frozen at -80°C.

2.1.3 LC-MS/MS mass spectroscopy with isotopic labeling

Recent advances in mass spectrometry, liquid chromatography, analytical software and bioinformatics have enabled the researchers to analyze complex peptide mixtures with the

ability to detect proteins differing in abundance by over 8 orders of magnitude (Wilmarth et al., 2004). One current method is isotopic labeling coupled with liquid chromatography tandem mass spectrometry (IL-LC-MS/MS) to characterize the salivary proteome (Gu et al., 2004). The main approach for discovery is a mass spectroscopy based method that uses isotope coding of complex protein mixtures such as tissue extracts, blood, urine or saliva to identify differentially expressed proteins (18). The approach readily identifies changes in the level of expression, thus permitting the analysis of putative regulatory pathways providing information regarding the pathological disturbances in addition to potential biomarkers of disease. The analysis was performed on a tandem QqTOF QStar XL mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with a LC Packings (Sunnyvale, CA, USA) HPLC for capillary chromatography. The HPLC is coupled to the mass spectrometer by a nanospray ESI head (Protana, Odense, Denmark) for maximal sensitivity (Shevchenko et al., 2002). The advantage of tandem mass spectrometry combined with LC is enhanced sensitivity and the peptide separations afforded by chromatography. Thus even in complex protein mixtures MS/MS data can be used to sequence and identify peptides by sequence analysis with a high degree of confidence (Birkhed et al., 1989; Gu et al., 2004; Shevchenko et al., 2002; Wilmarth et al., 2004).

Isotopic labeling of protein mixtures has proven to be a useful technique for the analysis of relative expression levels of proteins in complex protein mixtures such as plasma, saliva urine or cell extracts. There are numerous methods that are based on isotopically labeled protein modifying reagents to label or tag proteins to determine relative or absolute concentrations in complex mixtures. The higher resolution offered by the tandem Qq-TOF mass spectrometer is ideally suited to isotopically labeled applications (Gu et al., 2004; Koomen et al 2004; Ward et al., 1990).

Applied Biosystems recently introduced iTRAQ reagents (Gu et al., 2004; Koomen et al 2004; Ward et al., 1990), which are amino reactive compounds that are used to label peptides in a total protein digest of a fluid such as saliva. The real advantage is that the tag remains intact through TOF-MS analysis; however, it is revealed during collision induced dissociation by MSMS analysis. Thus in the MSMS spectrum for each peptide there is a fingerprint indicating the amount of that peptide from each of the different protein pools. Since virtually all of the peptides in a mixture are labeled by the reaction, numerous proteins in complex mixtures are identified and can be compared for their relative concentrations in each mixture. Thus even in complex mixtures there is a high degree of confidence in the identification.

2.1.4 Salivary protein analyses with iTRAQ

Briefly, the saliva samples were thawed and immediately centrifuged to remove insoluble materials. The supernatant was assayed for protein using the Bio-Rad protein assay (Hercules, CA, USA) and an aliquot containing 100 µg of each specimen was precipitated with 6 volumes of -20ºC acetone. The precipitate was resuspended and treated according to the manufacturers instructions. Protein digestion and reaction with iTRAQ labels was carried out as previously described and according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). Briefly, the acetone precipitable protein was centrifuged in a table top centrifuge at 15,000 x g for 20 minutes. The acetone supernatant was removed and the pellet resuspended in 20 µl dissolution buffer. The soluble fraction was denatured and disulfides reduced by incubation in the presence of 0.1% SDS and 5 mM TCEP (tris-(2-carboxyethyl)phosphine)) at 60ºC for one hour. Cysteine residues were

blocked by incubation at room temperature for 10 minutes with MMTS (methyl methane-thiosulfonate). Trypsin was added to the mixture to a protein:trypsin ratio of 10:1. The mixture was incubated overnight at 37°C.

The protein digests were labeled by mixing with the appropriate iTRAQ reagent and incubating at room temperature for one hour. On completion of the labeling reaction, the four separate iTRAQ reaction mixtures were combined. Since there are a number of components that can interfere with the LC-MS/MS analysis, the labeled peptides are partially purified by a combination of strong cation exchange followed by reverse phase chromatography on preparative columns. The combined peptide mixture is diluted 10 fold with loading buffer (10 mM KH_2PO_4 in 25% acetonitrile at pH 3.0) and applied by syringe to an ICAT Cartridge-Cation Exchange column (Applied Biosystems, Foster City, CA) column that has been equilibrated with the same buffer. The column is washed with 1 ml loading buffer to remove contaminants.

To improve the resolution of peptides during LCMSMS analysis, the peptide mixture is partially purified by elution from the cation exchange column in 3 fractions. Stepwise elution from the column is achieved with sequential 0.5 ml aliquots of 10 mM KH2PO4 at pH 3.0 in 25% acetonitrile containing 116 mM, 233 mM and 350 mM KCl respectively. The fractions are evaporated by Speed Vacuum to about 30% of their volume to remove the acetonitrile and then slowly applied to an Opti-Lynx Trap C18 100 ul reverse phase column (Alltech, Deerfield, IL) with a syringe. The column was washed with 1 ml of 2% acetonitrile in 0.1% formic acid and eluted in one fraction with 0.3 ml of 30% acetonitrile in 0.1% formic acid. The fractions were dried by lyophilization and resuspended in 10 ul 0.1% formic acid in 20% acetonitrile. Each of the three fractions was analyzed by reverse phase LCMSMS. The analytical strategy is illustrated in Figure 2.

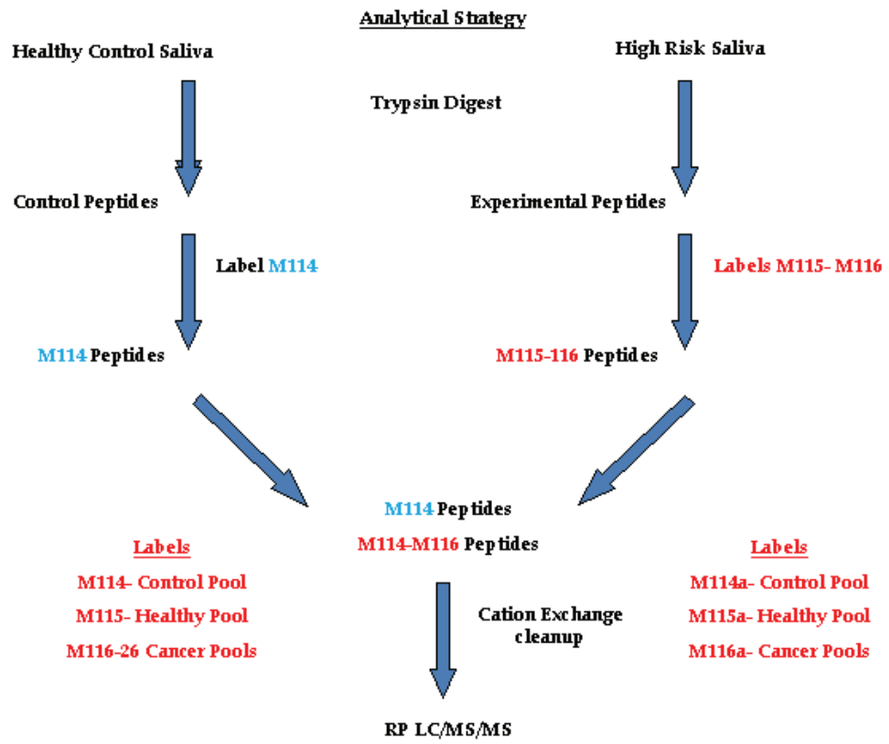


Fig. 2. Analytical strategy for quantifying peptides using iTRAQ tagging

2.1.5 Reverse phase LCMSMS

The desalted and concentrated peptide mixtures were quantified and identified by nano-LC-MS/MS on an API QSTAR XL mass spectrometer (ABS Sciex Instruments) operating in positive ion mode. The chromatographic system consists of an UltiMate nano-HPLC and FAMOS auto-sampler (Dionex LC Packings). Peptides were loaded on a 75cm x 10 cm, 3cm fused silica C18 capillary column, followed by mobile phase elution: buffer (A) 0.1% formic acid in 2% acetonitrile/98% Milli-Q water and buffer (B): 0.1% formic acid in 98% acetonitrile/2% Milli-Q water. The peptides were eluted from 2% buffer B to 30% buffer B over 180 minutes at a flow rate 220 nL/min. The LC eluent was directed to a NanoES source for ESI/MS/MS analysis. Using information-dependent acquisition, peptides were selected for collision induced dissociation (CID) by alternating between an MS (1 sec) survey scan and MS/MS (3 sec) scans. The mass spectrometer automatically chooses the top two ions for fragmentation with a 60 second dynamic exclusion time. The IDA collision energy parameters were optimized based upon the charge state and mass value of the precursor ions. Each saliva sample set there are three separate LCMSMS analyses.

The accumulated MSMS spectra are analyzed by ProQuant and ProGroup software packages (Applied Biosystems) using the SwissProt database for protein identification. The ProQuant analysis was carried out with a 75% confidence cutoff with a mass deviation of 0.15 Da for the precursor and 0.1 Da for the fragment ions. The ProGroup reports were generated with a 95% confidence level for protein identification.

2.1.6 Bioinformatics

The Swiss-Prot database was employed for protein identification while the PathwayStudio® bioinformatics software package was used to determine Venn diagrams were also constructed using the NIH software program (http://ncrr.pnl.gov). Graphic comparisons with log conversions and error bars for protein expression were produced using the ProQuant® software.

2.1.7 Western blot analysis for marker validation

2.1.7.1 Preparation of samples

We selected the protein profilin-1 for validating the presence of these proteins in saliva. The profilin-1 antibody was a rabbit polyclonal from the Abcam Co. #Ab10608 diluted 1:1000. The saliva samples from a healthy individuals, benign tumor subjects and individuals diagnosed with Stage IIa her2/neu receptor positive breast cancer subjects and Stage IIa her2/neu receptor negative breast cancer subjects were pooled by combining equal volumes of cleared stimulated whole saliva from a set of archived specimens.

The pooled saliva was mixed with loading buffer (Laemmli buffer containing BME) in 1:1 ratio. The sample was then incubated at 95°C for 5 minutes and was then loaded onto the 4-15% Tris-HCl polyacrylamide gel. Four-fifteen percent Tris-HCl polyacrylamide gels were loaded with molecular weight markers, controls, and the pooled saliva samples. Electrophoresis was run at 200 Volts, 30 minutes in 1X TGS buffer. The gels were equilibrated and extra thick blot paper and PVDF membranes were soaked in 1X TGS buffer for 15 minutes prior to running Western Transfer. Semi-dry transfer apparatus was used. Transfer conditions were 0.52mA constant, 17 volts, 19 minutes. Polyvinylidene fluoride (PVDF) membranes were air dried for minimum of 1 hour. Dry PVDF membranes were activated in methanol for about 10 seconds then transferred to soak in 1X PBS-T for 3 washes

of 5 minutes each. The membranes were then incubated for 1 hour in 5% NFDM in PBS-T. Afterwards the membranes were washed 3 times for 5 minutes in PBS-T. The membranes were incubated overnight with a primary antibody in PBS-T. The membranes were washed 3 times for 5 minutes in PBS-T and were incubated for 4 hours with secondary antibody (HRP conjugate) in PBS-T. Again, the membranes were washed 3 times for 5 minutes in PBS-T. Finally, the membranes were treated with ECL plus detection reagents and photographed with exposure of 800 seconds.

3. Experimental results in salivary protein profiling for cancer detection

3.1 Mass spectrometry analysis

Tables 1-5 summarize the results of the proteomic analysis and illustrates protein comparisons between breast (Stage 0 – IIb), cervical (moderate, severe dysplasia and in situ)

Proteins		Breast				Ovarian	Endo.	Cervical			H & N
		Staging									
Gene ID	Accession	Stage 0	Stage I	Stage IIa	Stage IIb	Variable	Variable	Mod.	Severe	Stage 0	Variable
A1AT	P01009										4.32
ANXA3	P12429										0.74
CO3	P01024										2.16
HPTR	P00739										3.57
K1C16	P08779									5.37	
COBA1	P12107							0.57			
LUZP1	Q86V48							0.62			
ZN248	Q8NDW4							0.84			
CYTC	P01034							0.77			
KAC	P01834					1.42					
K2C6C	P48666	3.40									
K1CJ	P13645		0.47		0.26						
SCOT2	Q9BYC2			0.83	0.50						
VEGP	P31025	1.36		0.47							
PROF1	P07737	0.74									
NGAL	P80188	0.90									
NUCB2	P80303		1.28								
HEMO	P02790	0.74									
CYTD	P28325		0.82								
CRIS3	P54108	0.65									
ACBP	P07108	1.42									
KLK	P06870	0.80	1.46	0.86							
PIP	P12273	0.88	0.80								
PERL	P22079		1.31	0.88							
PPIB	P23284		1.32								

Table 1. The table shows which proteins are unique to each type and stage of carcinoma

Protein Status	Breast				Ovarian	Endo.	Cervical			H & N
	Staging									
	Stage 0	Stage I	Stage IIa	Stage IIb	Variable	Variable	Mod.	Severe	Stage 0	Variable
Unique Proteins										
Up Regulated	3	4	0	0	1	0	0	0	1	2
Down Regulated	6	3	4	2	0	0	4	0	0	2
Total Proteins	9	7	4	2	1	0	4	0	1	4
Common Proteins										
Up Regulated	16	7	2	7	17	21	20	13	13	15
Down Regulated	8	11	11	7	5	5	7	4	8	7
Total Proteins	24	18	13	14	22	26	27	17	21	22
Total Number Proteins										
Up Regulated	19	11	2	7	18	21	20	13	14	17
Down Regulated	14	14	15	9	5	5	11	4	8	9
Grand Total	33	25	17	16	23	26	31	17	22	26

Table 2. The table demonstrates the unique, common and total protein counts among the varying carcinomas and their stages

ovarian, endometrial and head and neck cancer subjects. The values exhibited in table 1 and table 2 represent the ratio of disease state to healthy state. Simply stated the ratio for each protein is a result of the log sum integrated area under each peak formed when plotting the intensity versus mass-to-charge ratios for each peptide (Boehm et al., 2007). The value is divided by the corresponding value yielded for that particular protein in the healthy cohort. Significance between ratios is determined by t-test analysis. Those with values greater than 1.000 were considered up-regulated proteins while those with values less than 1.000 were considered down-regulated proteins.

In total, 166 proteins were identified at a confidence level at >95. Of these there were 76 proteins that were determined to be expressed significantly different ($p<0.05$) in the saliva from cancer subjects as compared to the healthy individuals. Table 1 lists the 25 proteins that are unique to each type of carcinoma with their corresponding ratios. Unique being defined as not associated with the other types of carcinoma. Eleven of the unique proteins were up-regulated and fourteen were down-regulated. There were no unique proteins associated with endometrial carcinoma or severe dysplasia of the cervix. Carcinoma of the breast and its associated stages accounted for nearly 58% of the total unique proteins.

Table 3 indicates the function of the associated proteins. Generally, the proteins appear to be diverse with the exception of the cytoskeletal associated proteins which comprised 20% of the protein panel. Additionally, over 50% of the proteins in table 3 are referenced in the literature for the presence of carcinoma in blood and cell supernatants from cancer cell lines (Polanski & Anderson, 2006).

The following table represents the up and down-regulated proteins that over-lapped the various types of carcinomas. Numerous proteins, 21 percent, were common to both squamous cell carcinoma and the adenocarcinoma cancer types. This was particularly noticed among the S100 proteins. There were 13 proteins that were exclusive to the gynecological carcinomas despite the fact that some were squamous cell carcinoma or adenocarcinoma cancer types. These proteins comprised 25% of the protein panel. Table 5 illustrates the function of the proteins listed in table 4.

Gene ID	Accession	Protein Name	Reported Function
A1AT	P01009	Alpha 1 protease inhibitor	Protease inhibitor
ACBP	P07108	AcylCoA binding protein	Transport protein
ANXA3	P12429	Annexin A3	Inhibits phospholipase activity
CO3	P01024	Complement 3 precursor	Activates complement system
COBA1	P12107	Collagen alpha-1 chain	Fibrillogenesis
CRISP3	P54108	Cysteine rich secretory protein	Immune response
CYTC	P01034	Cystatin C	Inhibitor of cysteine proteases
CYTD	P28325	Cystatin D precursor	Protein degradation & inhibitor
HEMO	P02790	Hemopexin precursor	Heme transporter protein
HPTR	P00739	Haptoglobin – 1 related protein	proteolysis
K1C16	P08779	Cytoskeleton 16	Cytoskeleton associated
K1CJ	P13645	Cytoskeleton 10	Cytoskeleton associated
K2C6C	P48666	Cytoskeleton 6C	Cytoskeleton associated
KAC	P01834	Immunoglobulin kappa chain C region	Immune response
KLK	P06870	Kallikrein-1	Cleaves kininogen
LUZP1	Q86V48	Leucine zipper protein-1	Nucleus functioning protein
NGAL	P80188	Neutrophil Gelatinase associated lipocalin	Iron trafficking protein
NUCB2	P80303	Nucleobindin-2 precursor	Calcium binding protein
PERL	P22079	Lactoperoxidase	Transport, antimicrobial
PIP	P12273	Prolactin inducible protein precursor	Secretory actin binding protein
PPIB	P23284	Peptidyl-prolyl cis trans isomerase B	Accelerates protein folding
PROF1	P07737	Profilin-1	Cytoskeleton associated
SCOT2	Q9BYC2	Succinyl CoA	Ketone body catabolism
VEGP	P31025	Lipocalin - 1	Ligand binding protein
ZN248	Q8NDW4	Zinc finger protein 248	Transcriptional regulation

Table 3. The table reveals the protein functions associated with the varying carcinomas and their stages

Proteins		Breast				Ovarian	Endo.	Cervical		H & N	
		Staging									
Gene ID	Accession	Stage 0	Stage I	Stage IIa	Stage IIb	Variable	Variable	Mod.	Severe	Stage 0	Variable
1433S	O70456					1.50	2.14	2.09	2.12		
ADA32	Q8TC27					0.52	0.69				
ALBU	P02768	0.86		0.91	1.46	1.15	1.23			1.94	2.50
ALK1	P03973					2.94		1.33			
AMYS	P04745		0.84	1.11	0.75	0.84	0.47	0.58	0.55	0.78	0.65
ANXA1	P04083	1.54	1.43				3.79	2.04	2.06		0.63
BPIL1	Q8N4F0	1.15				1.94	0.86				1.78
CAH6	P23280	1.46	0.85				1.57				
CYTA	P01040	1.63						1.16			
CYTN	P01037	0.77						0.53	1.16	0.67	0.73
CYTS	P01036	0.80			0.64						0.59
CYTT	P09228		1.14		0.59			0.49		0.62	0.50
DEF3	P59666					3.86	2.61	1.17			

Table 4. Represents the up and down-regulated proteins that over-lapped the various types of carcinomas.

Proteins		Breast				Ovarian	Endo.	Cervical		H & N	
		Staging									
Gene ID	Accession	Stage 0	Stage I	Stage IIa	Stage IIb	Variable	Variable	Mod.	Severe	Stage 0	Variable
DMBT1	Q9UGM3					1.83	1.40	1.22			
ENOA	P06733		0.74	0.81			1.71			1.43	1.55
FABPE	Q01469	1.56							0.55		
FGRL1	Q8N441					0.21	0.44	0.64			
HPT	P00738	0.65			2.22	1.94				2.64	
IGHA1	P01876					1.23	1.55	1.55	1.23		1.34
IGHA2	P01877						1.81	1.65			2.18
IGHG1	P01857	1.26	0.83					1.41		1.48	1.50
IGHG2	P01859		0.82			0.80				1.58	1.89
IGJ	P01591		1.23				1.41			0.77	1.30
K1C13	P13646						3.50	6.21	5.91		0.73
K1C14	P02533					5.34	6.55				
K1CM	P13646	3.92				2.95					
K1CP	P08779			0.13	0.13	2.61					
K22O	Q01546					7.47	5.40				
K2C1	P04264	0.46	0.63	0.33	0.32			2.07	2.22		
K2C4	P19013	4.42				4.47	4.17	5.46	5.91	1.11	0.54
K2C5	P13647					3.08	5.39	2.65	3.49	0.75	
K2C6A	P02538					4.34	5.78				
K2C6E	P48668			0.10	0.09						
LAC	P01842			0.84			1.43	1.51			1.62
LCN1	P31025						0.72	1.33			
LV3B	P80748					1.33		1.26			
MUC	P01871	1.22	0.72								1.45
MUC5B	Q9HC84	1.25	0.71	0.54	1.26	1.39		2.04	1.75		2.15
PERM	P05164		0.72				1.88				
PIGR	P01833	0.82								0.88	1.29
PRDX1	Q06830						1.67	1.22			
S10A7	P31151	2.05				0.67	1.26		0.74	0.76	
S10A8	P05109	1.46	1.36	0.76	1.33		1.49	3.46	3.35	1.42	1.73
S10A9	P06702		1.53	0.67	1.15			3.86	3.10	1.38	1.73
SPLC2	Q96DR5	1.22	1.18	0.87	0.68			0.74	1.22	2.11	
SPRR3	Q9UBC9	1.10	0.87		1.42					1.92	
THIO	P10599	1.51								1.43	
TRFE	P02787	0.80	0.80	0.83						2.00	2.36
TRFL	P02788	1.36	1.19							0.64	
TRY	P07477			1.46	1.66			0.84	0.88		
ZA2G	P25311	0.90						0.91	1.13	1.29	

Table 4. Represents the up and down-regulated proteins that over-lapped the various types of carcinomas.(continuation)

Gene ID	Accession	Protein Name	Reported Function
1433S	O70456	1433 sigma	Signaling
ADA32	Q8TC27	Disintegrin	Role in sperm development
ALBU	P02768	Albumin	Protein transport
ALK1	P03973	Antileukoproteinase	Acid-stable proteinase inhibitor
AMYS	P04745	Amylase	Enzyme
ANXA1	P04083	Annexin A1	Involved in exocytosis
BPIL1	Q8N4F0	Bactericidal/permeability-increasing protein-like 1	Lipid binding
CAH6	P23280	Carbonic anhydrase 6	Hydration of carbon dioxide.
CYTA	P01040	Cystatin-A	Intracellular thiol proteinase inhibitor.
CYTN	P01037	Cystatin-SN	Cysteine proteinase inhibitors
CYTS	P01036	Cystatin-S	Inhibits papain and ficin
CYTT	P09228	Cystatin-SA	Thiol protease inhibitor
DEF3	P59666	Neutrophil defensin 3	Antimicrobial activity
DMBT1	Q9UGM3	Deleted in malignant brain tumors 1	Candidate tumor suppressor gene
ENOA	P06733	Alpha-enolase	Multifunctional enzyme
FABPE	Q01469	Fatty acid-binding protein, epidermal	Keratinocyte differentiation
FGRL1	Q8N441	Fibroblast growth factor receptor 1	Negative effect on cell proliferation
HPT	P00738	Haptoglobin	Combines with free hemoglobin
IGHA1	P01876	Ig alpha-1 chain C region	Immune Response
IGHA2	P01877	Ig alpha-2 chain C region	Immune Response
IGHG1	P01857	Ig gamma-1 chain C region	Immune Response
IGHG2	P01859	Ig gamma-2 chain C region	Immune Response
IGJ	P01591	Immunoglobulin J chain	Immune Response
K1C13	P13646	Cytokeratin 13	Cytoskeleton
K1C14	P02533	Cytokeratin 14	Cytoskeleton
K1CM	P13646	Keratin, type I cytoskeletal 13	Cytoskeleton
K1CP	P08779	Keratin, type I cytoskeletal 16	Cytoskeleton
K22O	Q01546	Keratin, type II cytoskeletal 2 oral	Contributes to terminal cornification
K2C1	P04264	Keratin, type II cytoskeletal 1	Regulate the activity of kinases
K2C4	P19013	Keratin, type II cytoskeletal 4	Cytoskeleton
K2C5	P13647	Keratin, type II cytoskeletal 5	Protein binding
K2C6A	P02538	Keratin, type II cytoskeletal 6A	Protein binding
K2C6C	P48666	Keratin, type II cytoskeletal 6C	Structural molecule activity
K2C6E	P48668	Keratin, type II cytoskeletal 6C	Cytoskeleton organization
LAC	P01842	Lactoperoxidase	Antimicrobial
LCN1	P31025	Lipocalin-1 precursor	Plays a role in taste reception.
LV3B	P80748	Ig lambda chain V-III region LOI	Activates complement pathway
MUC	P01871	Ig Mu Chain	Immune Response
MUC5B	Q9HC84	Mucin-5B	Contribute to the lubricating
PERM	P05164	Myeloperoxidase	Microbiocidal activity
PIGR	P01833	Polymeric immunoglobulin receptor	Binds IgA and IgM at cell surface
PRDX1	Q06830	Peroxiredoxin-1	Involved in redox regulation

Table 5. The table represents the function of the common proteins associated with the various types of carcinomas.

Gene ID	Accession	Protein Name	Reported Function
S10A7	P31151	S100 A7	Interacts with RANBP9
S10A8	P05109	S100 A8	Calcium-binding protein
S10A9	P06702	S100 A9	Calcium-binding protein
SPLC2	Q96DR5	Epithelial carcinoma associated protein-2	Lipid binding protein
SPRR3	Q9UBC9	Small proline-rich protein 3	Protein of keratinocytes.
THIO	P10599	Thioredoxin	Redox activity
TRFE	P02787	Serotransferrin	Iron binding transport proteins
TRFL	P02788	Lactotransferrin precursor	Iron binding transport proteins
TRY	P07477	Trypsin-1	Activity against synthetic substrates
ZA2G	P25311	Zinc alpha-2 glycoprotein	Signaling

Table 5. The table represents the function of the common proteins associated with the various types of carcinomas.(continuation)

3.2 Western blot analyses

The results of the western blot suggest the presence of profilin-1 in saliva. Figure 3 illustrates the presence of profilin-1 protein in both the human submandibular gland cell lysates and in whole saliva.

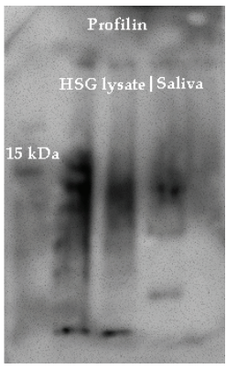


Fig. 3. The western blot indicates the presence of the 15 kDa profilin-1 protein in human submandibular gland cell lysates and stimulated whole saliva

The western blot analyses also revealed the presence of profilin-1 In SKBR3 cell lysates.

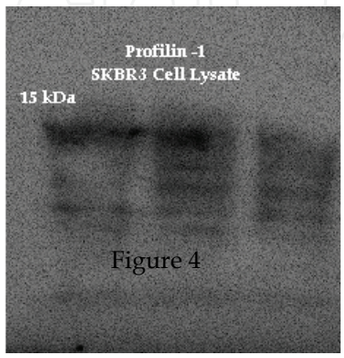


Fig. 4. Also revealed the presence of profilin-1 In SKBR3 HER2/neu receptor positive breast cancer cell lysates.

Figure 5 illustrates the presence of profilin-1 in the saliva sampled from healthy, benign and malignant tumor patients. Profilin is a down-regulated protein in the presence of malignancy and it is visualized by the lighter bands associated with malignancy. It is also worth noting that the Her2/neu receptor negative band is darker than the Her2/neu receptor positive counterpart suggesting further down-regulation of the profilin-1 protein.

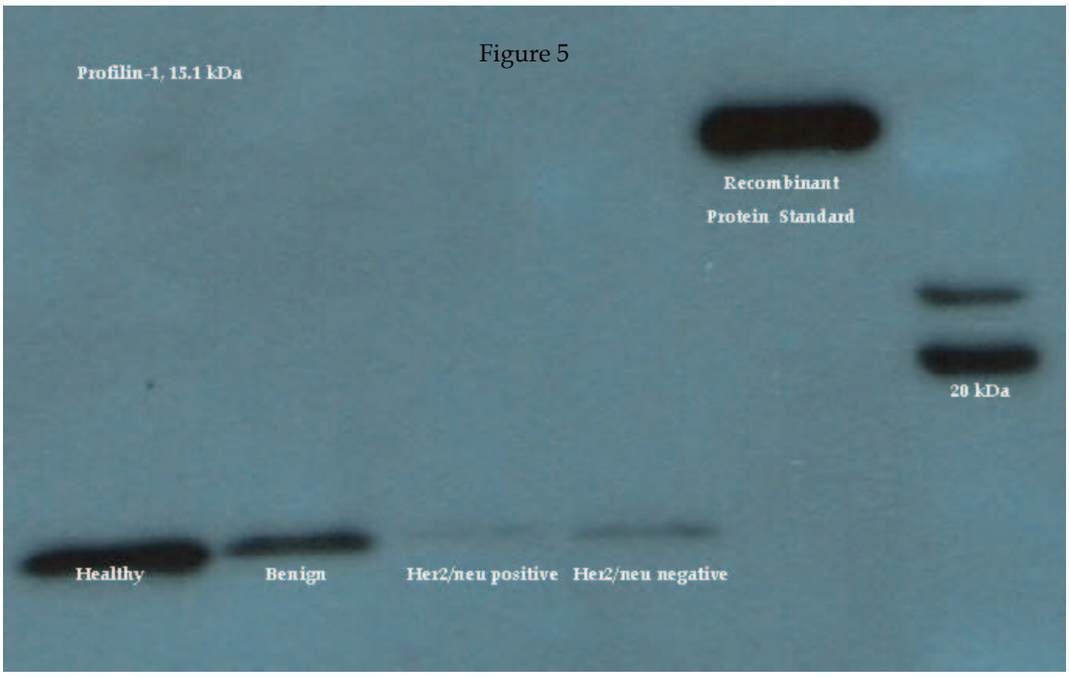


Fig. 5. This figure illustrates the presence and modulation of salivary profilin-1 protein secondary to HER2/neu receptor status.

3.3 Conclusions

It is interesting to note that the salivary protein profile mimics the findings of protein alterations secondary to cancer in serum, tissues and cell lysates that are numerously cited in the literature (Polanski et al, 2006). For example, there are salivary protein alterations in the cytoskeleton phenotype, which are strongly implicated in tumor growth and cancer metastases. Additionally, there are alterations in the metabolic, growth and signaling pathways all of which provide support for the concept that salivary protein profiles are altered secondary to the presence of cancer.

The investigator has spent 15 years investigating the phenomena and postulates, that in the presence of disease, *i.d.*, carcinoma, that there is an over abundance of protein resulting from the rapid growth of the malignancy which in turn, produces a humoral response in the salivary glands. This response results in altered salivary protein concentrations. Another possible explanation is active transport of the proteins of interest. It is plausible that these proteins are secreted into saliva as consequence of localized regulatory function in the oral cavity via signal transduction similar to the proposed explanation of HER-2/*neu* protein in nipple aspirates. These "loop" mechanisms, in health, appear to be in equilibrium both intercellularly and extracellularly with each pathway fulfilling the resultant phenotypic process of growth, proliferation, and differentiation.

The results of this preliminary study suggest that there are two panels of biomarkers that need to be assessed. There are those biomarkers that are unique to each tumor site *i.e.*, breast, cervix, etc. and those that are common to various types of malignancies. The finding may prove to be useful. It may be possible to develop a "global" profile for the overall detection of cancer while using the unique protein identifiers to determine the site of the tumor. Theoretically, by using the biomarkers S100A7, S100A8 and S100A9 you may predict the presence of a malignancy and by using the biomarkers listed in table 1 you, potentially could determine the site of the tumor. This is merely speculation at this time; however, advances in mass spectrometry and microarray technology may make the concept cost effective and logistically feasible.

These results are very preliminary and investigated only the abundant proteins found in saliva. Further research is required to determine the low abundant protein profiles associated with the various carcinomas. Additionally, the peptidome and the fragmetome need to be evaluated to further enhance the profile. Other carcinomas such as lung, colon, pancreas, bladder, skin, brain and kidney at varying stages of development also need to be studied and their resultant profiles added to the catalogue of cancer associated protein profiles. One major question that is essential to answer is, "Are we identifying the primary site of the tumor's origin?" This information is essential for rendering "tailored" treatment regimens.

The results of this study suggest that it is feasible to employ biometric principles for cancer detection. It is obvious from the resultant data that cancer is a very complex disease process involving numerous gene alterations in varying numbers of molecular pathways. This in turn renders the concept of the single biomarker as passé with respect to cancer detection. The multi-marker approach is evident as it avoids the pitfalls of a single marker concept as demonstrated by the current shortcomings of the prostate specific antigen marker for prostate cancer. Due to the overwhelming complexity of the malady high-throughput protein detection coupled with protein modeling in health and disease may yield the tools necessary for life saving early cancer detection.

4. Acknowledgement

The research presented in this chapter was supported by the Avon Breast Cancer Foundation (#07-2007-071), Komen Foundation (KG080928), Gillson-Longenbaugh Foundation and the Texas Ignition Fund. The authors would also like to thank Dr. William Dubinsky for the LC-MS/MS salivary mass spectrometry analyses and Dr. Karen Storthz for her technical expertise concerning the western blot analyses.

5. References

Birkhed D & Heintze U (1989). Salivary secretion rate, buffer capacity, and pH. In: *Human Saliva: Clinical Chemistry and Microbiology* - Volume 1. Tenovuo JO, editor, pp. (25-30), CRC press, Boca Raton, ISBN 0-8493-6391-8.

Costantino JP, Gail MH, Pee D, Anderson S, Redmond CK, Benichou J & Wieand HS (1999): Validation studies for models projecting the risk of invasive and total breast cancer incidence. *J Natl Cancer Inst* , 91. 18, (Sept, 1999), pp. (1541-1548), ISSN 0027-8874.

Delaleu N, Immervoll H, Cornelius J & Jonsson R. Biomarker profiles in serum and saliva of experimental Sjögren's syndrome: associations with specific autoimmune

manifestations. *Arthritis Research & Therapy* 10. 1,(Feb 2008), (R22-R36), ISSN (electronic) 1478-6362.

Ernst DJ & Balance LO (2006). Quality collection: the phlebotomist's role in pre-analytical errors. *Med Lab Obs*, 83. 9, (Sept 2006), pp. (30-38), ISSN 0580-7247.

Gu S, Liu Z, Pan S, Jiang Z, Lu H, Amit O, Bradbury E.M, Hu CA & Chen X (2004). Global investigation of p53-induced apoptosis through quantitative proteomic profiling using comparative amino acid-coded tagging. *Mol Cell Proteomics*, 3.10, (Oct 2004), pp. (998-1008), ISSN 1535-9476.

Hirtz C, Chevalier F, Sommerer N, Raingeard I, Bringer J, Rossignol M & and Deville de Périere D (2006). Salivary protein profiling in type I diabetes using two-dimensional electrophoresis and mass spectrometry. *Clinical Proteomics 2. 2,* (Sept 2006), pp. (117-127), ISSN 1542-6416.

Kallenberg CG, Vissink A, Kroese FG, Abdulahad WH, Bootsma H. What have we learned from clinical trials in primary Sjögren's syndrome about pathogenesis? Arthritis Res Ther. 2011 Feb 28;13(1):205.

Koomen JM, Li D, Xiao L, Liu TC, Coombes KR, Abbruzzese J & Kobayashi R (2005). Direct tandem mass spectrometry reveals limitations in protein profiling experiments for plasma biomarker recovery. *J Prot Res, 4. 3, (*2005), pp. (972-981), ISSN 1535-3893.

Koomen JM, Zhao H, Li D, Abbruzzese J, Baggerly K & Kobayashi R (2004). Diagnostic protein discovery using proteolytic peptide targeting and identification. *Rapid Com Mass Spec,* 18. 21, (Nov 2004), pp. (2537-2548) ISSN 1097-0231.

Moura S, Sousa J, Lima D, Negreiros A, Silva F & Costa L. (2007). Burning mouth syndrome (BMS): sialometric and sialochemical analysis and salivary protein profile. *Gerodontology*, 24, (Sept., 2007), pp. (173–176), ISSN 0734-0664.

Navazesh M, Christensen C (1982). A comparison of whole mouth resting and stimulated salivary measurements. *J Dent Res,* 61. 10, (Oct 1982), pp. 1158-1162, ISSN 0022-0345.

Polanski M & Anderson NL. A list of candidate cancer biomarkers for targeted proteomics (2006*). Biomarker Insights,* 7. 1, (Feb 2006), pp. (1-48), ISSN 1177-2719.

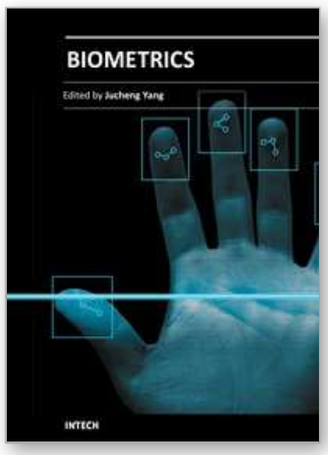
Shevchenko AV, Chernushevic I, Shevchenko A, Wilm M & Mann M (2002). "De novo" sequencing of peptides recovered from in-gel digested proteins by nanoelectrospray tandem mass spectrometry. *Mol Biotech,* 20. 1, (Jan 2002), pp. (107-118) ISSN 1073-6083.

Teisner B, Davey MW & Grudzinskas JG (1983). Interaction between heparin and plasma proteins analyzed by crossed immunoelectrophoresis and affinity chromatography. *Clin Chem Acta*, 127. 3, (Feb 1983) pp. 413-417, ISSN 0009-8981.

Ward LD, Reid, GE, Moritz RL & Simpson RJ (1990). Strategies for internal amino acid sequence analysis of proteins separated by polyacrylamide gel electrophoresis. *J Chroma*, 519. 1, (Oct 1990), pp. (199-216), ISSN 0021-9673.

Wilmarth PA, Riviere MA, Rustvold DL, Lauten JD, Madden TE & David LL (2004). Two dimensional liquid chromatography study of the human whole saliva proteome. *J Prot Res*, 3. 5, (Sept-Oct, 2004), pp. (1017-1023), ISSN 1535-3893.

Wu AJ, Atkinson JC, Fox PC, Baum BJ & Ship JA (1993). Cross-sectional and longitudinal analyses of stimulated parotid salivary constituents in healthy, different-aged subjects. *J Geron*, 41. 5, (Sept,1993); pp. (M219-224), ISSN 1079-5006.



Biometrics

Edited by Dr. Jucheng Yang

ISBN 978-953-307-618-8
Hard cover, 266 pages
Publisher InTech
Published online 20, June, 2011
Published in print edition June, 2011

Biometrics uses methods for unique recognition of humans based upon one or more intrinsic physical or behavioral traits. In computer science, particularly, biometrics is used as a form of identity access management and access control. It is also used to identify individuals in groups that are under surveillance. The book consists of 13 chapters, each focusing on a certain aspect of the problem. The book chapters are divided into three sections: physical biometrics, behavioral biometrics and medical biometrics. The key objective of the book is to provide comprehensive reference and text on human authentication and people identity verification from both physiological, behavioural and other points of view. It aims to publish new insights into current innovations in computer systems and technology for biometrics development and its applications. The book was reviewed by the editor Dr. Jucheng Yang, and many of the guest editors, such as Dr. Girija Chetty, Dr. Norman Poh, Dr. Loris Nanni, Dr. Jianjiang Feng, Dr. Dongsun Park, Dr. Sook Yoon and so on, who also made a significant contribution to the book.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Charles F. Streckfus and Cynthia Guajardo-Edwards (2011). The Use of Salivary as a Biometric Tool to Determine the Presence of Carcinoma of the Breast Among Women, Biometrics, Dr. Jucheng Yang (Ed.), ISBN: 978-953-307-618-8, InTech, Available from: http://www.intechopen.com/books/biometrics/the-use-of-salivary-as-a-biometric-tool-to-determine-the-presence-of-carcinoma-of-the-breast-among-w

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen