Chapter

Salivary Biomarkers to Assess Breast Cancer Diagnosis and Progression: Are We There Yet?

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Abstract

Technological developments are propelling medical diagnostics forward at unprecedented rates. Advancements in genomic, proteomics and cellomics are leading the way for diagnostic tests that will be capable of rapid multi-analyte detection in both laboratory and non-laboratory settings. Currently, proof-of-principle has been demonstrated for salivary biomarkers, whose up and down regulation has been shown to correlate strongly with breast cancer among women. Consequently, the rationale for this chapter is to review the medical literature and present the current research focused on the use of saliva as a diagnostic medium for the study of early breast cancer progression.

Keywords: breast cancer progression, saliva, cancer proteomics, breast cancer, omics, cancer detection, Her2/neu, mass spectrometry, cancer molecular pathways

1. Introduction

Reducing the overall morbidity and mortality rates for carcinoma of the breast is the overall goal of the Department of Health and Human Services [1]. As stated in *Healthy People 2020*, breast cancer is the second leading cause of death in women in the United States, with over 45,000 women dying from the disease each year. Additionally, in the United States, in 2019, it is estimated that 268,600 women and 2670 men will be diagnosed with invasive breast cancer. Additionally, another 62,930 women will be diagnosed with in situ breast cancer [2–7].

Taken together, the chances of a woman being diagnosed with breast cancer during her lifetime have increased. In 1975, the rate was 1 in 11; whereupon today the rate is roughly 1 in 8 [2]. Overall, the number of women being diagnosed continues to increase. This is due in part to the increase in the number of women in age groups at risk of breast cancer. Currently, the median age at which breast cancer is diagnosed is 62 [4–7].

There is also an increase in breast cancer screening which has resulted in a dramatic increase in the incidence of ductal carcinoma in situ (DCIS). DCIS is an early stage, pre-invasive form of breast cancer with a cause-specific survival rate of approximately 100%. The current problem, however, is that it is not possible to distinguish DCIS that will develop into invasive cancer. Consequently, the over-diagnosis and overtreatment of DCIS remains a persistent problem [8, 9].

While breast cancer screening with mammography has been shown in randomized controlled trials to reduce breast cancer-specific mortality, there remains ongoing controversy regarding the value of mammography and its utilization.

Additionally, studies using mathematical modeling suggest that more frequent mammographic screening would further increase patient survival. Analysis of these data suggests a 96% survival rate if patients could receive a mammogram every 3 months. However, due to the costs coupled with the invasiveness of the procedure, this increase in the frequency of mammography is currently not feasible [10].

While advances in imaging have increased breast cancer detectability there are still overarching challenges that need to be addressed. The FY19 BCRP funding announcement succinctly outlines these challenges [11]: (1) prevent breast cancer, (2) identify determinants of breast cancer initiation, risk, or susceptibility, (3) distinguish deadly from non-deadly breast cancers, (4) address the problems of over-diagnosis and overtreatment, (5) identify what drives breast cancer growth; and determine how to stop it, (6) identify why some breast cancers become metastatic, (7) determine why/how breast cancer cells lie dormant for years and then re-emerge, (8) determine how to prevent lethal recurrence, (9) revolutionize treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival, and (10) eliminate the mortality associated with metastatic cancer [11, 12].

The author would like to add two more items: (1) ascertain methods to determine the efficacy of treatment and (2) ascertain methods to determine that the treated patient is not only in *clinical remission*, but in *molecular remission* as well [13, 14]. The latter action items would assure successful treatment.

Taken together, there remains a necessity to identify novel approaches that improve breast cancer screening and early detection. This, in turn, reduces the problems of over-diagnosis and over-treatment and can detect cancers at a point where interventions maybe more effective [1–14].

2. Significant single analyte salivary biomarker studies

There is a paucity of studies in the medical literature concerning the use of saliva for the detection of breast cancer. These reports deal primarily with the identification and quantification of cancer-related proteins, in saliva that were previously discovered to be present in serum or cancer tissue supernatants of diagnosed cancer patients. The importance of these studies establishes the basic groundwork and feasibility of salivary cancer diagnostics and ascertains the basis for continued biomarker research [15].

2.1 Kallikrein

A study that serves to establish the basis for salivary cancer biomarker research methodology comes from a report of kallikrein being used as a diagnostic marker. The investigation reports the use of saliva to detect variations in the concentrations of kallikrein, a regulatory protease, among healthy individuals and patients with malignant breast and gastro-intestinal tumors. The outcome of their investigation revealed elevated concentrations of salivary kallikrein among individuals diagnosed with malignant tumors in comparison with those individuals diagnosed with benign tumors and those from a healthy cohort. Kallikrein was measured by chromogenic tripeptide assay [16].

2.2 Epidermal growth factor

Epidermal growth factor (EGF) is a regulatory growth factor protein responsible for tissue growth and repair [17]. Since EGF overexpression is implicated in tumorigenesis, it may therefore be useful as a tumor marker. Based on this thinking, an additional study [17] demonstrated that EGF concentrations were higher in the saliva of women with primary or recurrent breast cancer in comparison with women lacking a malignancy [17]. The highest concentrations of EGF were found in the local recurrence subgroup, suggesting a potential use for this marker in the post-operative follow-up of diagnosed cancer patients [18].

2.3 The Her2/neu studies

Salivary Her2/neu (e-*erb*B-2) is probably the most thoroughly investigated biomarker [19–21] that is still under current investigation [22–24]. The original investigators [19–21] selected the salivary protein as the biomarker of choice, because this protein was previously shown to be immunohistologically present on the membrane of the ductal epithelium of salivary gland tissues [25, 26]. Additionally, Her2/neu was found in serum [27] and, therefore, provided the investigators with a basis for analytical humoral comparison [28]. Collectively, these points provided the basis for the selection of Her2/neu as a salivary biomarker. CA 15-3 was the gold standard for comparison in both the saliva and serum specimens [19–21].

The results of these studies demonstrated elevated concentrations of the Her2/neu and CA 15-3 proteins in the saliva and serum of all three groups of women. The salivary and serological levels of Her2/neu among the cancer patients were significantly higher ($p < 0.001$) than the salivary and serum levels of healthy controls and benign tumor patients. Additionally, the Her2/neu protein was found to be equal to or to surpass the ability of CA 15-3 to detect cancer patients. The sensitivity and specificity were 87 and 65%, respectively, with a cut-point of 100 Units/ml. These results were comparable with the serum levels of Her2/neu [19–21]. Tumor staging and receptor status were also assessed. Her2/neu increased with staging, but not with Her2/neu status. The finding was later supported by other researchers [23, 29].

Concurrent with the aforementioned investigation, studies to determine the relationship between Her2/neu protein concentrations and various demographic and clinical variables we conducted. These variables potentially could confound the results of any salivary biomarker research. Consequently, the effects of race, age, weight, BMI, tobacco usage, alcohol consumption systemic illnesses (e.g., diabetes, hypertension, etc.), the use of prescription medications, hormonal status and estrogen usage [30], salivary flow rates, marker contributions from epithelia present in saliva, and the presence of periodontal disease on salivary Her2/neu concentrations were assessed. Edentulous patients were also assessed to control for the effects of periodontal disease. These studies show that these potentially confounding variables exact no effect on salivary Her2/neu concentrations [19–21].

Two other questions that needed to be answered are as follows: could the pungent chemotherapeutics used in cancer treatment adversely affect Her2/neu salivary concentrations and secondly could tumor removal and post-chemotherapy events modulate, in this case lower, salivary Her2/neu concentrations. In response, a study was conducted to answer the aforementioned questions and to establish the utility of the salivary Her2/neu in monitoring patients diagnosed with carcinoma of the breast before and after treatment.

Twenty-five patients with various stages of carcinoma of the breast were followed through the course of treatment. ELISA assays for Her2/neu and CA 15-3 were performed on their serum and stimulated whole-saliva specimens. These baseline samples

were collected from all patients prior to the administration of any adjunct therapy or surgery and were sequentially sampled during therapy and after their treatment. As noted in **Figure 1**, the outcome of the study demonstrated the modulation of Her2/neu concentrations in response to chemotherapeutic treatment. Additionally, it may be useful to detect post-treatment recurrence. Indeed, salivary Her2/neu may have the potential to track treatment success and guide therapy. The salivary Her2/neu determinations also mirrored those for serum Her2/neu and CA 15-3 [31].

Finally, a clinical experiment was conducted to ascertain the reliability and repeatability of detecting Her2/neu in both 9 healthy men and 10 healthy women over a 5-day time-period [32]. The individuals were saliva sampled at 9 AM., 4 PM. and 9 PM. over the 5-day period. The samples were stored at −80°C and later assayed by ELISA for the Her2/neu protein. The results exhibited no demonstrable salivary Her2/neu differences regarding gender, day or time of collection. Inter and intra correlation coefficients did decrease in the 4 PM. and 9 PM. collections suggesting that morning to afternoon collections may be optimal for collecting salivary Her2/neu specimens [32].

2.3.1 Other supporting experiments

Other technologies were employed to support the aforementioned clinical findings [32]. Using Her2/neu antibodies, the authors executed several Western blot experiments. The first of these experiments compared saliva and serum concentrations of Her2/neu in specimens from among seven healthy subjects. The blot demonstrated protein presence at a range of 170–185 kDa, with denser bands being revealed in the salivary samples. A second Western blot to ascertain which salivary gland(s) were producing the salivary Her2/neu. Saliva collected from the parotid and submandibular glands, and total whole saliva, were sampled from two healthy individuals. The results

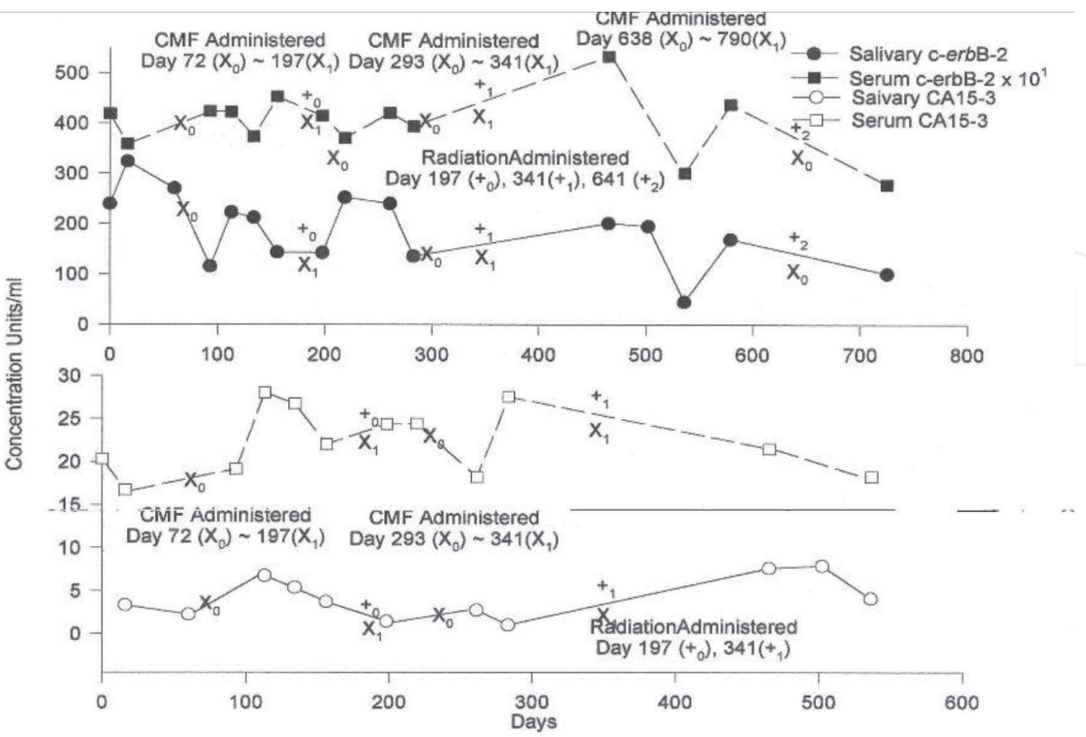

Figure 1.
An example of post-operative monitoring. The upper graph represents salivary and serum c-erbB-2 concentrations across time of treatment. The lower graph represents salivary and serum CA 15-3 concentrations across time of treatment. As illustrated, the c-erbB-2 concentrations are in concordance with the commonly used CA 15-3 cancer biomarker [31].

suggest that all the major salivary glands were contributors of Her2/neu salivary secretions regardless if the glands were serous (parotid) or mixed (submandibular) saliva producers. A third experiment compared Her2/neu salivary secretions between three individuals diagnosed with carcinoma of the breast and two healthy controls. The Western blot demonstrated denser 170–185-kDa bands among the cancer subjects as compared with healthy controls. The conclusions derived from these experiments suggest that Her2/neu is increased in saliva secondary to carcinoma of the breast [33].

An animal study was also conducted determine if Her2/neu can be detected from a remote areas of the body [34]. Male rat saliva does not contain salivary Her2/neu. Consequently, two male Sprague-Dawley rats, each weighing between 300 and 500 g, were used. One experimental animal received 200 μl, and the other, 500 μl, of encapsulated Her2/neu protein. The capsulated Her2/neu proteins were placed in the peritoneum of each rat. Prior to capsule placement, baseline serum and saliva samples were taken. Samples were also taken 20, 68, 140, 188, 308 and 356 hours post-placement. Saliva flow was induced by administration of ophthalmic pilocarpine prior to sampling. All samples were kept at −20°C. Antibody detection was performed using a modified double capture ELISA system. The animal receiving the higher antibody concentration showed a markedly greater salivary level of the antibody than the other (peak 24.158 vs. 18.313 HNU/ml at 308 and 188 hours post-implantation, respectively). Baseline values were below detection for both animals. These results appear to indicate that Her2/neu may be detected from remote areas of the body [34].

A different type of platform, surface-enhanced laser desorption/ionization mass spectrometry (SELDI), was employed to confirm the presence and alteration of Her2/neu in saliva [35]. The results of the preliminary SELDI assays were encouraging. The weak cation exchange (WCX @ pH 3.5) biochip demonstrated the most potential for profiling salivary proteins. Interestingly, a protein cluster in the range of 170 kDa (**Figure 2**) was more prevalent in cancer patient saliva samples than in

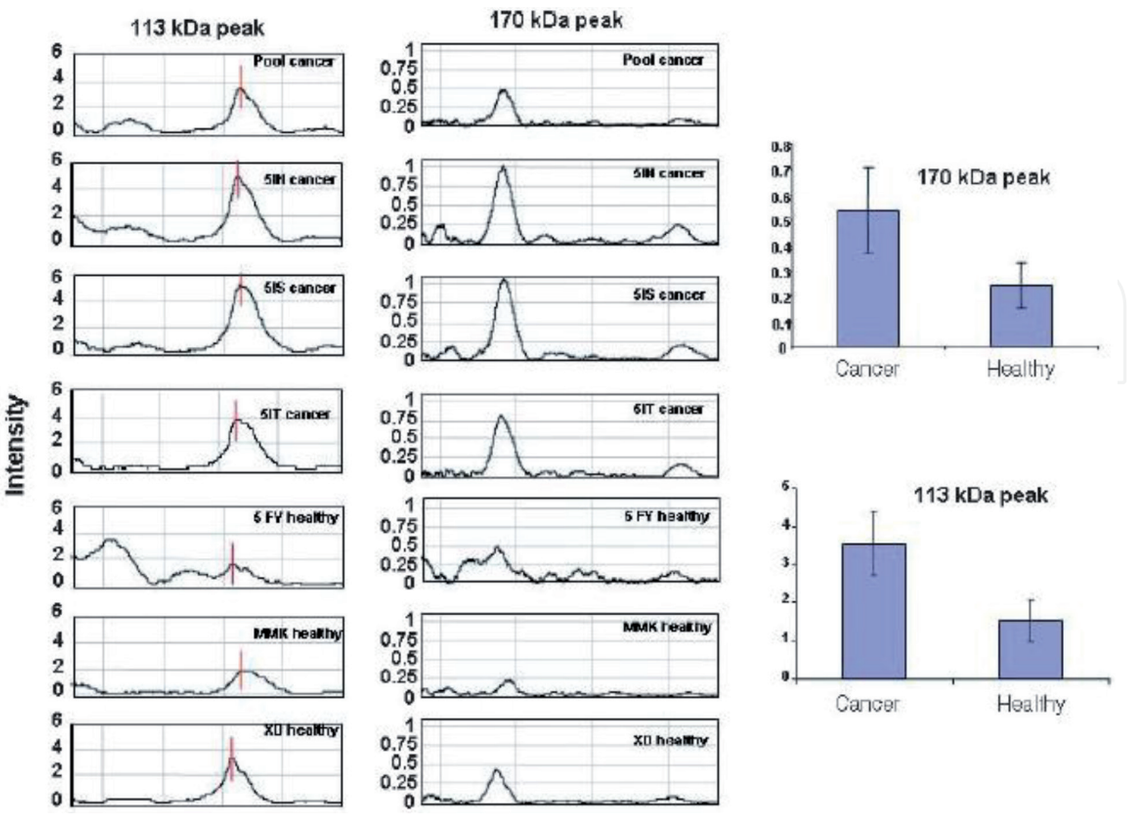

Figure 2.
The 113 and 117 kDa spectral peaks in saliva. Additionally, bar graphs to the right show mean values for the 113 and 117 kDa spectral peaks [35].

samples from normal subjects [36]. Also of note, one of the donors in the normal group (XD) is known by ELISA to have quite high Her2/neu levels and stands out from the other two normal donors in this analysis. These similar results were also demonstrated by serum and the extract from the SKBR-3 breast cancer cell lines with SELDI analyses.

In addition, an increase of the proteins in this range from cancer patient saliva, especially in the range of approximately 113 kDa, can be seen in **Figure 2**. One possible cancer-related biomarker is the extracellular domain (ECD) of Her2/neu receptor, which is approximately 110 kDa (deglycosylated), based on two-dimensional and Western blot gel analyses.

3. Proteomics studies for salivary biomarkers and their findings

Mass spectrometry and liquid chromatography have enabled the researchers to analyze complex peptide mixtures with the ability to detect proteins differing in abundance by over eight orders of magnitude. Isotopic labeling coupled with liquid chromatography tandem mass spectrometry (IL-LC-MS/MS) is often used to characterize the salivary proteome. 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.

A PubMed.gov database search yielded a paucity of manuscripts (15) using LC/MS/MS mass spectrometry. The majority of the manuscripts were composed by the author of this chapter [37–40]. In these manuscripts, the researchers used an Applied Biosystems QStar® XL LC/MS/MS mass spectrometer equipped with an LC Packings high performance liquid chromatography (HPLC) for capillary chromatography. The HPLC was coupled to the mass spectrometer by a nanospray ESI head for maximal sensitivity. 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 either by peptide similarity or sequence analysis with a high degree of confidence [41–44].

The researchers employed iTRAQ reagents [37–39] that are amino reactive compounds. The real advantage of using iTRAQ is that the tags remain intact through TOF MS analysis. This in turn enables the tags to be revealed during collision-induced dissociation by MS/MS analysis. Thus in the MS/MS spectrum for each peptide, there is a pattern indicating the quantity of that peptide from each of the different protein pools. Essentially all of the peptides in a mixture are labeled by the reaction; numerous proteins 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 because of the large number of peptides that can be used for protein identification.

Pooled samples were used in these studies with full knowledge of the advantages and shortcomings for the technique [45]. Considering that, the exploratory findings would have to be validated using other laboratory methodologies, the researchers decided to use the pooled sample technique. Sample size for the pooled specimens was also calculated [45]. The sample size for each pool was 10 subjects. Additionally, the authors used a *positive control* (benign tumors), which is not found in many of the breast cancer biomarker experiments using saliva as the diagnostic media. To enhance the study, the same samples were assayed by a *blinded outside laboratory* [37].

Using the LC-MS/MS platform, the researchers used a three-prong approach to obtain salivary protein profiles of cancer patients. The first was to determine if the

technology could detect the smallest ductal carcinoma lesions (Stage 0, Stage I) and compare their profiles to those of a positive control (fibroadenomas) and healthy individuals [37]. The second parameter was to determine protein profile differences between lymph node positive and lymph node negative cancer patients [38]. Third experiment was to ascertain differences between Her2/neu receptor positive and Her2/neu receptor negative individuals [39]. Taken together, these three major parameters influence tumor progression.

The initial study compared the protein profiles of four cohorts: healthy, benign tumor, Stage 0 and Stage I [37]. The PathwayStudio® software, a bioinformatics software package was used to determine the fold increase/decrease and p-values. p-Values equal to or less than 0.001 were used to select the best biomarker candidates. The number of proteins for each group is shown in **Table 1**.

The panel (**Table 1**) has putative markers that are both up and/or down regulated and have varying cellular functions. These proteins are validated in cell studies and found altered in the presence of carcinoma of the breast.

The aim of the second study was to compare the salivary protein profiles of pooled saliva specimens from individuals diagnosed with ductal carcinoma of the breast

Comparison	Upregulated	Downregulated	Total markers
Healthy vs. benign	19	10	29
Healthy vs. Stage 0	15	15	30
Healthy vs. Stage I	9	17	26
Benign vs. cancers	9	6	15

Table 1.
The up- and downregulated proteins for each cohort.

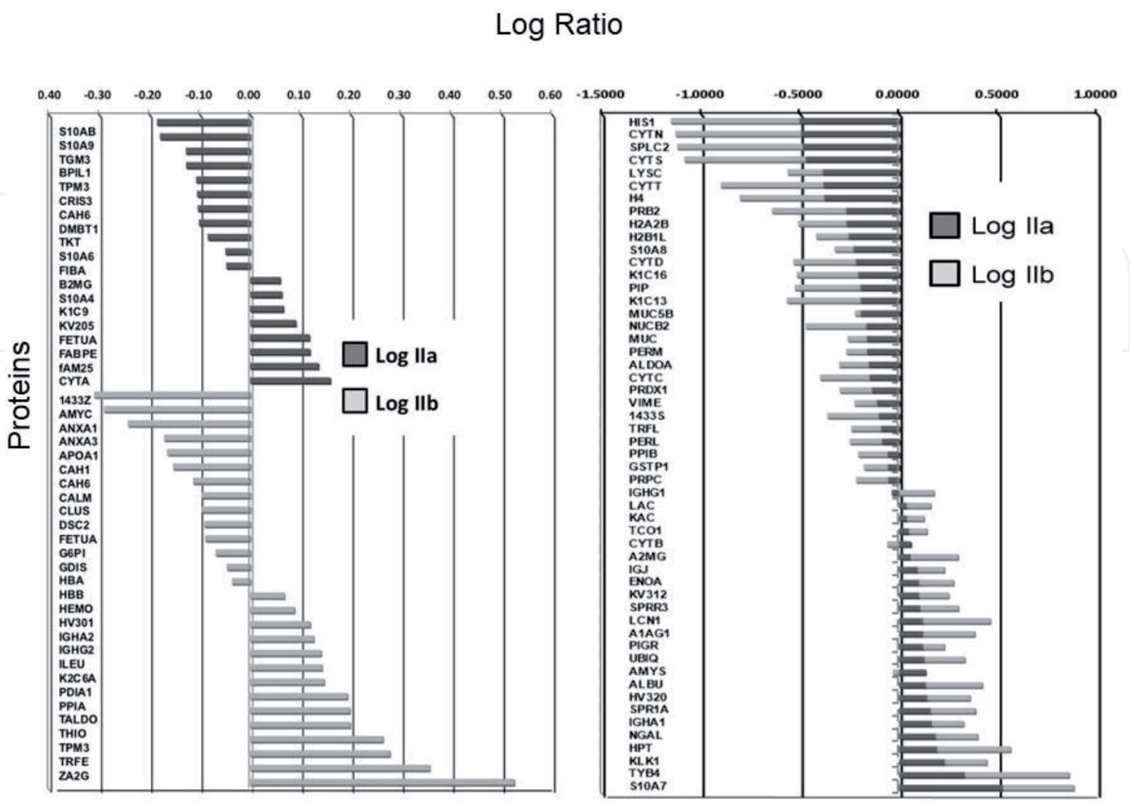

Figure 3.
The differential expression of salivary proteins common and different to both Stage IIa and Stage IIb [38].

with and without lymph node involvement [38]. Three pooled (n = 10 subjects/pooled specimen) stimulated whole saliva specimens from women were analyzed. One pooled specimen was from healthy women, another pooled specimen was from women diagnosed with Stage IIa ($T_2N_0M_0$) invasive ductal carcinoma (IDC) without lymph node involvement and one pooled specimen was from women diagnosed with Stage IIb ($T_2N_1M_0$) with lymph node involvement. Experimentally, saliva from each of the pooled samples was trypsinized and the peptide digests labeled with the appropriate iTRAQ reagent. The results are shown in **Figure 3**. The analyses yielded approximately 174 differentially expressed proteins in the saliva specimens. Fifty-five proteins were common to both cancer stages while there were there were 20 proteins unique to Stage IIa and 28 proteins that were unique to Stage IIb. The proteins are listed in **Figure 3**. The study suggests that salivary proteomic profiles may be useful in determining lymph node involvement among cancer patients [38].

The third aspect was to compare the salivary profiles from subjects diagnosed with breast cancer that were either Her2/neu receptor positive or negative. As previously mentioned, two pooled saliva specimens underwent proteomic analysis. One pooled specimen was from women diagnosed with Stage IIa, Her2/neu receptor positive breast cancer patients (n = 10) and the other was from women diagnosed with Stage IIb, Her2/neu receptor negative (n = 10). The pooled samples were trypsinized and the peptides labeled with iTRAQ reagent. Specimens were analyzed using a LC-MS/MS mass spectrometer. The results yielded approximately 71 differentially expressed proteins in the saliva specimens. There were 34 up-regulated proteins and 37 down regulated proteins. **Figure 4** provides a visualization of the saliva protein differences between positive and negative Her2/neu receptor status [39].

Validation of the makers resulting from these studies was performed in a number of studies [46–49]. The results of all these studies plus numerous others were finally comprised into a manuscript, which presents a *catalogue of salivary proteins* that have been altered in the presence of ductal carcinoma of the breast. These findings are supported by other proteomic analysis of breast cancer cell lines, breast cancer tissues, tissue microenvironment and serum [40]. Additionally, nearly 29% of the panel of proteins has been technically validated by either Western blot or by ELISA. A breakdown of these proteins has also been analyzed according staging and Her2/neu receptor status. All of the proteins were sorted according to their *function*. A *pathway analysis* was also employed. The investigators have also found that the protein concentrations can be modulated while undergoing cancer treatment and respond differently according pathological cell type [40].

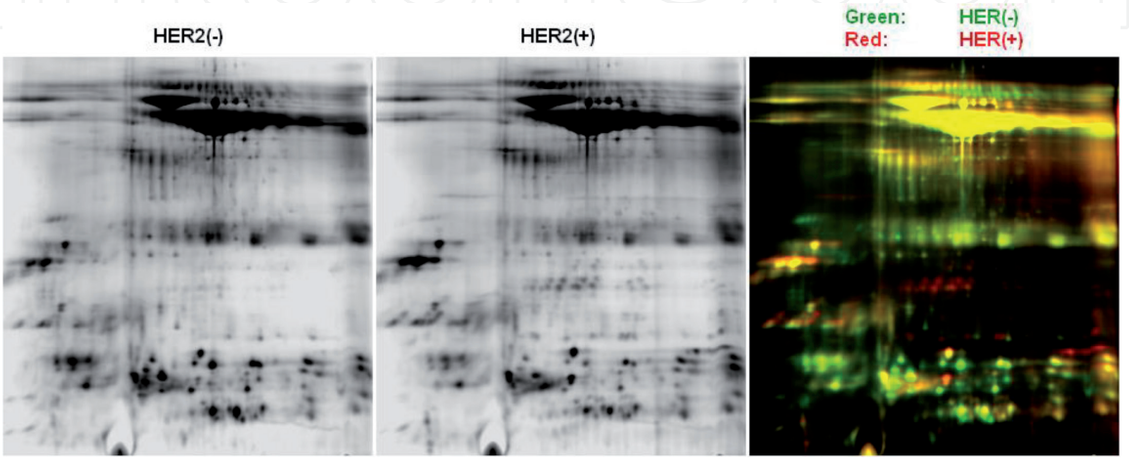

Figure 4.
The protein profiles for HER2/neu receptor positive and HER2/neu receptor negative samples. As shown in the far right red and green dyed gel comparisons, there are numerous differences between the two profiles [39].

One major result of these and other studies is the standardization of saliva collection and preparation for storage [50]. Additionally, standards for proteomic analysis have been established [51].

4. Salivary transcriptome: mRNA and miRNA

In a number of studies conducted by Wong et al., they discovered the presence and utility of mRNA in saliva for the detection of breast cancer [52–55]. These findings not only provided researchers with a new source of biomarkers, but also a new method for cross-validating proteomic findings [55]. Taken together, these studies helped explain preexisting studies and further served to illustrate the complexity of salivary composition [52–55].

Concurrent with these studies, were investigations assessing salivary miRNA as potential biomarkers for breast cancer detection [56, 57]. These studies also demonstrated how gene microarray and quantitative real-time PCR technology, could detect micro-RNA candidates [56, 57]. It has been demonstrated that, secondary to breast cancer, salivary micro-RNA profiles are altered [56, 57].

5. Salivary exosomes and microvesicles

One of the major questions regarding salivary biomarker research is how large proteins, mRNA and miRNA enter the saliva proper. Many proteins are too large to pass through the intercellular spaces of the acinar cells. So how do they enter whole saliva? Additionally, RNAs are intracellular proteins making the problem even more complex.

In 2012, Lau and Wong demonstrated how these particulates might enter the saliva via *exosome—like microvesicles* [58]. The investigators employed an *in vitro* breast cancer model that demonstrated that breast cancer derived exosome—like

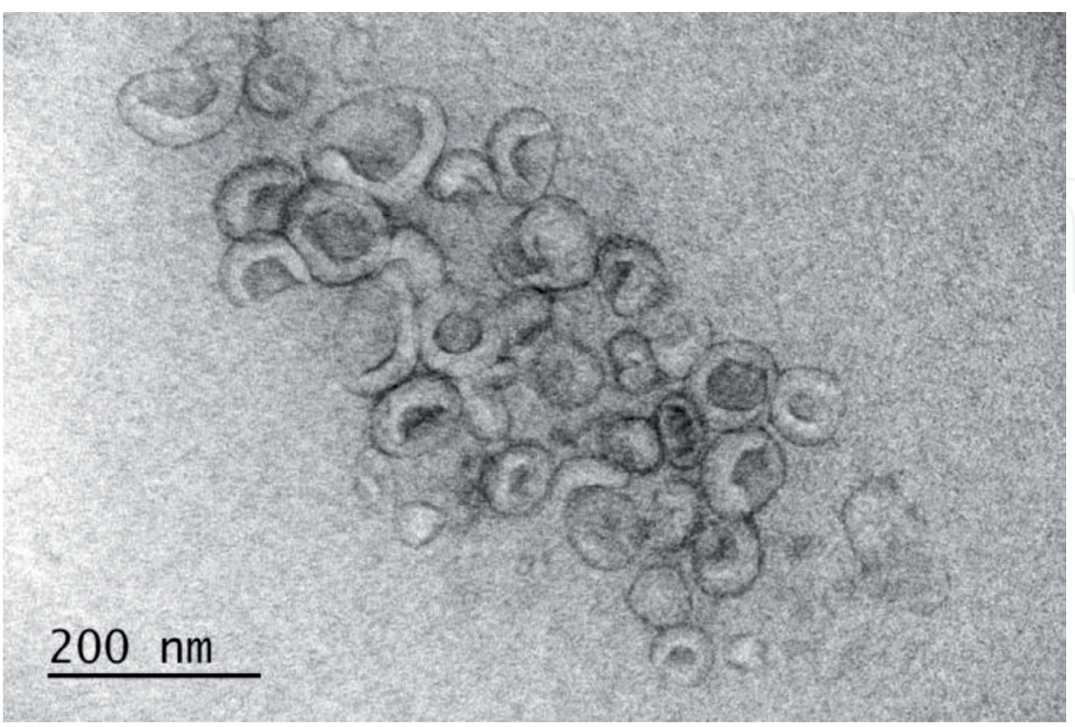

Figure 5.
An electron micrograph of extracellular vesicles (courtesy of Maija Puhka, Ph.D. of the EV CORE and Institute for Molecular Medicine Finland FIMM, University of Helsinki, Finland).

microvesicles interact with salivary gland cells and produce an altered salivary profile. Within these exosome—like microvesicles they found proteins and mRNA. They also found that the exosome—like microvesicles communicated and activated the transcriptional activity of the salivary glands. The result of this activity altered the composition of salivary gland exosome—like microvesicles and the ensuing salivary secretion [58].

Subsequent research has yielded numerous vesicular structures, which can bind to a suitable receptor on a target cell [59–61]. These bodies include exosomes, ectosomes, apoptotic bodies and extracellular vesicles (**Figure 5**). Once thought to be repositories for cellular waste, these vesicular bodies provide intricate functions in intercellular communication and compound exchange. Current research is demonstrating that these vesicular bodies mediate disease progression. Subsequent research has shown this finding to be of great utility in under-standing altered salivary composition secondary to various disease states [59–61].

It is also worth noting that extracellular vesicles may also explain the findings reported in Section 2.2 of this chapter as Andre et al. extracted Her2/neu from exosomal pellets [62]. This may be the underlying mechanism by which biomarker proteins enter saliva.

6. Metabolites

In a study conducted by Sugimoto et al., the investigators assessed the diagnostic utility *metabolites* in saliva [63]. They conducted a comprehensive metabolite analysis of saliva samples obtained from 215 individuals. Sixty-nine subjects were diagnosed with oral cancer, 18 with pancreatic cancer and 30 with carcinoma of the breast. These cohorts were compared to 11 periodontal disease patients and 87 healthy controls. They used capillary electrophoresis time-of-flight mass spectrometry (CE-TOF-MS). Fifty-seven principal metabolites were identified that accurately predicted the probability of being affected by each individual disease. Additionally they were found that between the known patient characteristics and the quantified metabolites, the profiles manifested relatively higher concentrations in most of the metabolites in all three cancers as compared to those in people with periodontal disease and control subjects. Several metabolites in breast cancer patients yielded a statistically significant difference between breast cancer and healthy controls, including taurine and lysine. However, there were no differences in metabolites between breast cancer and other two cancers. These metabolites are promising biomarkers for cancer screening [63, 64].

7. Conclusions

Based on the volume of evidence provided in this review, the author believes that that saliva may have diagnostic potential and the potential to be used to study breast cancer progression. As described throughout this text, different analytes using various biochemical platforms have all indicated that the constituents in saliva are altered secondary to breast cancer. The logistic utility of this media has been described in numerous manuscripts; however, to date there are no FDA approved salivary diagnostic devices for the detection of breast on the commercial market despite the fact that many of these findings have been patented.

Considering the logistical advantages of salivary diagnostic testing and the deadliness of the disease we are trying to detect, it would be extremely useful to continue to explore the possibility of using saliva as a medium for treatment

efficacy and tumor recurrence. Post-treatment monitoring for breast cancer recurrence is extremely important. The patient may be in *clinical remission* but not necessarily in *molecular remission* [40, 65]. This could be accomplished using a microarray containing salivary marker of the varying cancer pathways associated with carcinoma of the breast [40]. Post-treatment serial sampling would indicate the efficacy and indicate protein changes, which could lead to tumor recurrence.

Besides commercialization, Karachi et al. (1985) presented a lingering problem to the salivary research community [65]. While at N.I.H., he conducted an experiment whereby he removed the submandibular gland of virgin mice 14–22 weeks old. This resulted in a reduced the tumor incidence to 12.8% (n = 39) at the age of 52 weeks and also increased the latency period of mammary tumor development as much as 14 weeks when compared to that of normal mice. Long-term treatment of sialoadenectomized virgin mice with EGF increased the tumor incidence to 33.3%. Moreover, sialoadenectomy of mammary tumor-bearing animals caused a rapid and sustained cessation of tumor growth, but EGF administration quickly restored the rate of tumor growth to a normal level. These results indicate that submandibular gland EGF plays a crucial role in mouse mammary tumorigenesis [65].

The aforementioned study suggests an influence of salivary EGF on tumorigenesis. Additionally, it is known from other salivary studies that EGF is elevated in human saliva secondary to breast cancer [17, 18]. Could the same effect mentioned by Karachi be taking place in humans? [65].

In addition, another study reported an increase in salivary protein rich peptides levels among breast cancer patients [40, 48]. Using a proline rich peptide segment (p1978) from the parent SMR3B protein peptide in saliva, the researchers found that this peptide inhibited the growth of HCC38 triple-negative cancer cells [48]. Perhaps the elevation of in of this peptide is in response to tumor proliferation. It is known that proline-rich-peptides have a high affinity to the Grb2/SH3 domain, which in turn inhibits Ras activation by blocking Sos binding to Grb2 receptor. Taken together both studies suggest feedback systems as illustrated in **Figure 6**.

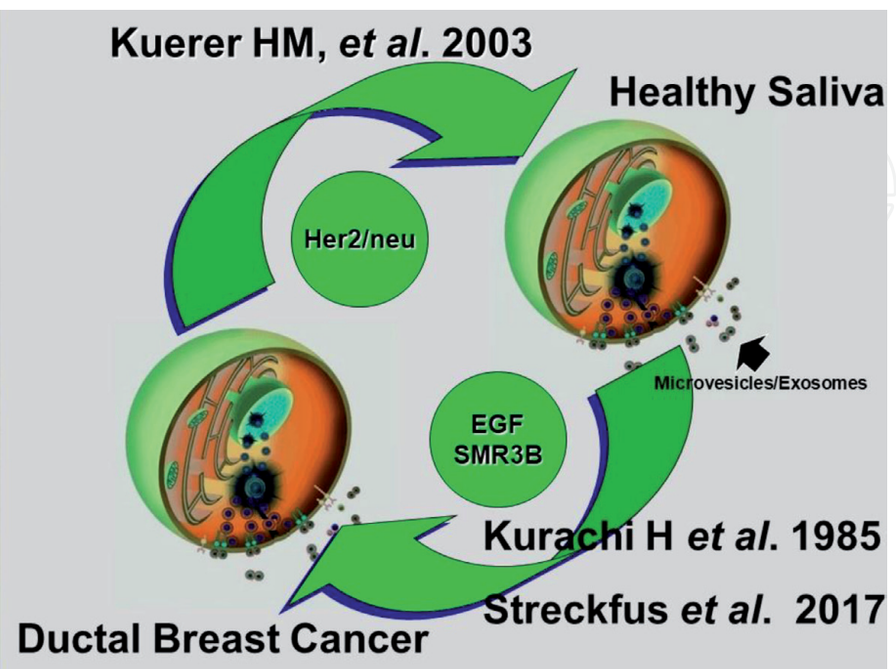

Figure 6.
Feedback systems going from saliva to the tumor [65], from saliva in response to the tumor [48] and from the tumor to healthy breast tissues [66] and saliva [20, 21].

In addition, Kuerer [28] found that healthy contralateral breast aspirates exhibited elevated Her2/neu concentrations secondary to carcinoma in the opposite breast [66]. This supported the findings of Streckfus, where salivary Her2/neu concentrations secondary to carcinoma of the breast [21]. These findings suggest *inter-exocrine gland communication* perhaps by microvesicles and/or exosomes.

Taken together, the author presents sufficient evidence of the interrelationship of exocrine tissues and the possibility of using saliva not only as a diagnostic media, also to study or assess breast cancer progression.

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Conflict of interest

The author has no conflict of interest or declarations to report.

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