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Implications of salivary proteomics in drug discovery and development: a focus on cancer drug discovery

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Human saliva proteomics has proven to be a novel approach in the search for protein biomarkers for non-invasive detection of human cancers. This approach may also have implications within the process of anti-cancer drug discovery. Information from saliva proteomic measurements may contribute to the target discovery and validation, assessment of efficacy and toxicity of candidate drugs, identification of disease subgroups, and prediction of responses of individual patients. In this article, we aim to give a brief overview on human saliva proteome analysis, as well as its applications to cancer biomarker discovery. Potential applications of saliva proteomics in anticancer drug discovery and development will also be discussed.

Human saliva is a mixture of secretions from multiple salivary glands, including the parotid, submandibular, sublingual, and other minor glands lying beneath the oral mucosa. This complex body fluid aids in the execution of multiple physiological functions, such as oral digestion, food swallowing and tasting, tissue lubrication, maintenance of tooth integrity, as well as antibacterial and antiviral protection [1]. In addition to the important role of maintaining homeostasis of the oral cavity system, saliva is also very attractive as a diagnostic fluid, because saliva testing is noninvasive, simple, safe, and cost-effective [2,3]. There has been increasing interest in studying the human saliva proteome and exploring the use of saliva protein biomarkers for the detection of human diseases, such as cancers and autoimmune diseases [4–6]. In this article, we will give a brief overview on human saliva proteomics and its application to the discovery of protein biomarkers for cancer detection. The implications of saliva proteomics in anticancer drug discovery and development will also be discussed. The potential utility of salivary proteome analysis is summarized in Box 1.

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Saliva proteome analysis

Human saliva harbors a wide spectrum of peptides and proteins that constitutes the human salivary proteome. These polypeptides not only play important roles in maintaining oral and general health but may also serve as biological markers to survey normal health and disease status. Therefore, analysis and cataloguing of the human salivary proteome will certainly be of great interest to researchers within the fields of oral biology and saliva-based diagnostics. Mass spectrometry (MS)-based proteomics has been successfully applied to the identification of proteins and their post-translational modifications in human whole and ductal saliva [7–12]. Many of these studies were performed using shotgun proteomics, which is based on multi-dimensional separation, tandem MS (MS/MS), and database searching algorithms. Shotgun proteome analysis is very efficient in cataloguing and profiling of proteins, whereas 2D gel electrophoresis coupled with MS (2-DE/MS) allows mapping of the proteome at the protein level and visualization of protein modifications and isoforms [13,14]. Profiling of salivary glycoproteins and proteins in distinct families has been recently demonstrated. The selective enrichment of glycoproteins, followed by liquid chromatography–tandem MS (LC–MS/MS) profiling, may appear to be a promising approach for finding biomarker and therapeutic targets in

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BOX 1

Potential utility of salivary proteomics at various stages of drug discovery process

Candidate selection and validation
In certain disease models, salivary proteomics can be used to establish diagnostic and prognostic capability of the proteins and indicate particularly useful molecules to monitor the disease.

Clinical trials
For certain diseases, especially local diseases, saliva proteomics can be run in parallel with established techniques to evaluate the efficacy and safety of the approach in patients.

Post-launch personalized medicine
Salivary proteomics may help to establish the most useful patient subgroup for treatment and allows simplified long-term follow-ups.

Clinical diagnosis and prognosis
If successfully validated, salivary protein markers may allow early diagnosis and improve likelihood of successful treatment. Simple detection devices need to be developed for the clinical use of those markers.

cancers [15]. Analysis and characterization of cystatins, histatins, proline-rich proteins, and their fragments in saliva provides further insight in the assessment of their functions in the oral cavity [16–20]. In addition, salivary proteome databases (http://www.hspp.ucla.edu, http://fields.scripps.edu/public/project/saliva/) have been established to centralize the acquired proteomic data and annotate the identified saliva proteins. These databases are fully accessible to the public for the query of the identified proteins, which are linked to public protein databases. With the data deposited and centralized, we can start to integrate large-scale datasets from a variety of laboratories and also conduct comparative analysis of saliva proteome to other body fluid proteomes. Once we have a general catalogue of the human saliva proteome, saliva proteomics can therefore, in principle, provide a molecular profiling approach towards a deeper understanding of oral biology and related disease pathogenesis. Because of its ready accessibility, saliva is also an attractive medium for noninvasive diagnosis and/or prognosis of human diseases. Proteomic analysis of saliva over the course of disease progression could reveal valuable biomarkers for early detection and monitoring of disease status. Similarly, profiling of saliva proteins before and after pharmacological treatments may provide clues regarding drug efficacy and toxicity.

Salivary protein biomarkers for human cancer detection

The term, biomarker, refers to measurable and quantifiable biological parameters that can serve as indicators for health and physiology-related assessments, such as pathogenic processes, environmental exposure, disease diagnosis and prognosis, or pharmacologic responses to a therapeutic intervention. Analyzing the proteomic content of tissues or fluids over the course of disease progression could reveal 'protein signatures' indicative of specific disease status. Such 'signatures' may be used extensively in future medical diagnostics. Saliva diagnostics are very attractive because of noninvasive sample collection and simple sample processing. For patients, the noninvasive collection procedure for saliva dramatically reduces anxiety and discomfort and simplifies procurement of repeated samples for monitoring over time [21]. Compared to tissue biopsies, saliva is an easily accessible fluid and, therefore, a large number of saliva samples can be enrolled for clinical proteomic studies. This allows enough statistical power for a robust study design, and true signatures can be unveiled for disease detection.

Some preliminary studies have suggested that salivary proteins can serve as biomarkers for human cancer detection. The soluble fragments of human epidermal growth factor receptor-2 (HER2) and cancer antigen CA 15-3 were found to be significantly up-regulated in the saliva samples of breast cancer patients compared with those of healthy controls and patients with benign tumors [22,23]. Testing of HER2 in saliva may be promising for monitoring progression and recurrence of breast cancer [24]. Saliva from patients with oral squamous cell carcinoma (OSCC) or head and neck squamous cell carcinoma (HNSCC) contains signature proteins, such as TNF-alpha, interleukin (IL)-1, IL-6, IL-8, CD44, fibronectin, defensin-1, hyaluronidase, telomerase, cytokeratin 19 fragment (CYFRA 21-1), tissue polypeptide antigen, and cancer antigen CA125, which are potentially useful as diagnostics, if successfully validated [25–33]. Using subtractive proteomics and immunoassays, we have recently identified and validated five protein biomarkers for OSCC detection, including Mac-2 binding protein, catalase, calgranulin B, Profilin, and CD59 [34].

Early detection is a key question that needs to be addressed in almost all types of cancers. In OSCC, if the cancer is detected at T1 stage, the five-year survival rate is over 80%, compared to 20–40% if the cancer is diagnosed at later stages (T3 and T4). Several preliminary studies have shown the potential of salivary proteins for early detection of OSCC. The levels of certain proangiogenic, proinflammatory cytokines, such as TNF-alpha, IL-1, IL-6, and IL-8, were found to be significantly increased in the saliva of patients with oral premalignant lesions (OPMLs) compared to controls, and also significantly elevated in the saliva from patients with OSSCs compared to those with OPMLs [30,35]. The levels of the cytokines, TNF-alpha, IL-1-alpha, IL-6, IL-8, and vascular endothelial growth factor (VEGF) were also found at elevated levels in saliva from patients with oral lichen planus compared to normal controls. These studies suggest that NF-kappaB-dependent inflammatory cytokines may have diagnostic potential for monitoring disease activity in patients with premalignant lesions [36–38].

The clinical utility of these putative biomarkers has been limited, so far, because of questions with respect to their overall accuracy. Further retrospective validation of these candidates needs to be conducted, preferably, using a different technology platform. There is also concern about using inflammatory proteins (e.g. cytokines) as disease biomarkers. Perhaps a rational approach, when designing a biomarker study, would be to include a second inflammatory disease group, in addition to a healthy control group, to reveal the truly cancer-associated alterations. For instance, patients with periodontal disease can be enrolled as a second control group, in order to test the diagnostic value of those inflammatory proteins in OSCC. Tumor-specific autoantibodies could be present in human saliva for cancer detection. For instance, the p53 autoantibody level in saliva was found to corre-

late with the serum levels in OSCC; monitoring of the salivary levels of p53 antibody may offer a specific method for detection of a subset of OSCC with p53 aberrations [39]. Proteomics tools, especially protein arrays, may be used to discover specific antibodies as potential biomarkers for OSCC/HNSCC or, possibly, other cancers. In addition, many discovered biomarkers or therapeutic targets in human cancers are glycoproteins. Targeting the glycoproteins in saliva may prove to be a promising approach to cancer biomarker discovery. Lastly, the development of quantitative proteomics tools, especially isotope labeling/tandem MS and protein arrays [40,41], represents significant advances in proteomics. These quantitative profiling technologies only requires small amount of clinical samples for analysis. With these novel tools, we can obtain the much-needed quantitative information on saliva proteins associated with disease pathogenesis.

Pharmacoproteomics

Pharmacoproteomics refers to applications of proteomics used in the drug industry, including target identification and validation, discovery of efficacy and toxicity biomarkers, and investigations into mechanisms of drug action or chemo-resistance. Although a few new anticancer drugs have reached the market, more than 80% of drugs for all indications entering clinical development do not get marketing approval, with many failing late in development often in Phase III trials, because of unexpected safety issues or difficulty in determining efficacy. These factors contribute to the high costs of cancer drug development and clearly show the need for faster, more cost-effective strategies for evaluating drug effectiveness and safety and defining patient subgroups that can benefit from these treatments [42]. Proteomics may have a potential role in these applications, because proteins are the primary effectors of drug action and proteomic analysis represents a global approach to monitoring protein alterations in response to drug administration.

Human cancer is a very heterogeneous disease and multiple cellular and etiological pathways are involved in the process of oncogenesis. This implies that multiple protein molecules should be simultaneously targeted as an effective strategy to counter the disease. Apart from helping us to understand the molecular pathogenesis, the cancer-associated proteins identified by proteomics may serve as potential therapeutic targets or may be used to classify patients for clinical trials. Proteome analysis may also offer a strategy to validate the targets. For instance, investigation of cellular proteomes perturbed with matrix metalloproteinase (MMP) inhibitor drugs suggests that, in addition to identification of new MMP substrates, quantitative proteomics can provide valuable information for target validation and drug efficacy before commitment to clinical trials [43].

Those proteins whose levels are modified in response to drug administration could provide vital clues with respect to drug effectiveness and toxicity. These proteins, if constantly validatable, may serve as efficacy or toxicity biomarkers to guide clinical trial studies. Similarly, analyses of protein profiles before and after pharmacological treatments could also confirm the mechanism of drug action and provide insight for new drug discovery [44]. In addition, particular biomarkers (e.g. HER2) may be used to classify patient subgroups and therefore customize therapeutic strategies for specific patients, which could eventually lead to personalized therapy for individual cancer patients. The positive outcome of these pharmacoproteomics applications may potentially reduce the time and cost of clinical research while concomitantly increasing patient safety and reducing the risk associated with the development of new therapies.

Efficacy biomarkers

In oncology, a special class of extensively evaluated biomarkers of efficacy (surrogate endpoints) that generally correlate with desired clinical outcomes, can be used as a basis for corporate decisions, as well as for gaining accelerated provisional regulatory approval of a drug [45]. In general, these efficacy biomarkers must be mechanistically linked to the disease process and significantly capture the treatment effect of a drug. The pretreatment variability of an efficacy biomarker must be small compared to the changes in the biomarker concentration or activity produced by therapy. The effect of drugs on a proposed efficacy biomarker must be sustained, to allow determination of efficacy [46–48]. These biomarkers can impact drug development strategies, thereby aiding corporate decision-making about advancing compounds and improving the productivity of the overall development process. Saliva testing is a noninvasive procedure that can be repeated on the same patients without causing significant distress. This makes it especially well suited for time-course experiments. Identification of protein expression changes through salivary proteomics, particularly secreted proteins, may allow the development of simple procedures to assay drug efficacy in preclinical and clinical phases. Alternatively, a proven drug efficacy biomarker, previously discovered and validated in tissue biopsies, may be further tested in saliva samples from the patients with the same type of cancer. If successfully evaluated and validated, a simple saliva assay of the drug efficacy biomarker can then be established for the entire drug development process.

Classification of cancer patients and prediction of drug response

The development and administration of molecular-targeted therapeutics requires diagnostic tools to monitor and predict individual response to therapy, in order to maximize drug efficacy and minimize the risk or severity of adverse events. Accurate prediction of an individual patient's drug response is an important prerequisite of personalized medicine. Once a compound is in clinical trials, data on molecular profiles associated with response to therapy would be a tremendous resource. This information base could be used to determine why a drug fails in a particular patient, to formulate guidelines for patient stratification in subsequent trials to improve the likelihood of establishing safety and efficacy, and to develop diagnostics that could be marketed with a drug that is specific to a particular patient subgroup [49]. Saliva proteome analysis during preclinical or exploratory clinical development may allow the discovery of candidate markers for the prediction of drug response. A proof-of-principle study has been demonstrated to measure proteinase activity in saliva and proteinase inhibition after systemic treatment with different proteinase inhibitors. After treatment, drug concentration could be determined in saliva and concomitant decreases in salivary proteinase activity were observed, demonstrating that saliva testing could be used to assess the *in vivo* modulation of their targets by these inhibitor drugs [50].

In a recent study, the levels of TNF-alpha, IL-1-alpha, IL-6, and IL-8 were found to be significantly decreased following dexamethasone treatment of those patients with OLP; IL-1-alpha and IL-8 were detected at levels that were not significantly significant from controls [51]. These preliminary investigations indicated that salivary testing of proteins might be applicable for monitoring the therapeutic response of patients, though one should exercise a degree of caution when using inflammatory proteins as biomarkers for therapeutic response.

Cancer patients can also be classified, based on altered protein expression profiles, and subsequently, statistical methodologies can be used to develop predictors for patient subgroups. These predictors can help define what patients may benefit from the targeted therapy [48]. A good example would be trastuzumab, a humanized monoclonal antibody targeted against HER2 [52]. This oncoprotein is over-expressed in 25–30% of patients with breast cancer and is associated with a more aggressive clinical course and shortened survival in these patients. In clinical practice, ~20% of breast cancer patients identified by this biomarker are chosen for the trastuzumab treatment. Even though FDA-approved immunohistochemical and flurorescence *in situ* hybridization (FISH) methods are now available for assessing HER2 over-expression, these methods are only semi-quantitative and interpretation could be operator-dependent [53]. Since HER2 is present in human saliva [22,23], incorporation of this marker in both clinical trials and practice might assist in the classification of breast cancer patients. The established assays for salivary proteins, such as HER2, can help determine which patient subgroups are most likely to benefit from such a molecular-targeted therapy.

Toxicity biomarker

Toxicoproteomics is the use of global protein expression technologies to detect up-regulation and down-regulation of genes associated with drug toxicity risk; it allows for better understanding of the effects of environmental and genetic factors, both in episodes of acute exposure to toxicants and in the long-term development of disease [54]. The rising costs of drug development and high failure rate at later stages of development are putting pressure on pharmaceutical companies to reduce clinical attrition and maximize decision-making at the preclinical stage. One of the keys to reducing the cost and time of drug development is early and accurate safety evaluation of candidate drugs. Therefore, toxicity (safety) biomarkers are clearly required for screening compounds in preclinical studies and clinical trials for determining target organ toxicities. Serum proteomic patterns produced by MS have been linked to the major organ toxicities of candidate drugs [54]. Similarly, saliva proteomic patterns or biomarkers may be revealed for *in vivo* toxicity (safety) assessment when molecular-targeted chemotherapy for OSCC/HNSCC reaches the clinic. Incorporation of high-throughput screening of these toxicity biomarkers in saliva from patients with OSCC/HNSCC or other cancers into preclinical studies such as hit-to-lead screening, lead screening, and preclinical validation might be possible.

Summary and perspective

Current efforts in human saliva proteomics are mainly devoted to the identification and cataloguing of proteins in human whole and glandular saliva based on shotgun proteomics and 2-DE/MS approaches. With a comprehensive list of saliva proteins at hand, a next step would be to identify significant changes in saliva protein composition associated with disease processes. These alterations may represent potential diagnostic and/or prognostic markers that could be used in a clinical context for noninvasive detection and monitoring of human diseases. The application of the salivary proteomics may be explored for early detection of human cancers, predicting aggressiveness and prognosis, and surveillance for cancer recurrence, which may eventually lead to simple clinical tools for early detection and monitoring of cancers such as OSCC/HNSCC.

Saliva proteomics is undoubtedly a promising approach for the identification of protein markers for certain human diseases. However, there are several issues that could complicate the development of biomarkers into useful diagnostic and prognostic agents. First, whole saliva, in contrast to serum, is not constant in its composition because of changes in salivary flow and a differential contribution from the different salivary glands, or other sources, within the oral cavity [55]. Physiological variations in the salivary proteome between individuals or even within one individual exist [56,14], and, therefore, determining minor changes in certain disease-relevant markers remains a major challenge. This suggests the need for a systematic study of the variables affecting the production of protein content in human saliva and means of standardization of sample collection and handling procedures for saliva biomarker studies. Secondly, human saliva contains a large number of proteins differing by the extraordinary dynamic range. How to deplete the highly abundant proteins such as amylase and proline-rich proteins needs to be addressed. Thirdly, considering multifactorial etiology and heterogeneity of oncogenic pathways complicate building biomarker prediction models, robust study design and statistical strategies should be strictly followed in clinical proteomic studies in order to reveal valuable prediction models forecasting malignant potential [57–59]. Most tumors result from an inter-dependent series of genetic alterations, rather than a single decisive event. Thus, a prediction model often needs to seek a set of potential predictors, which collectively improve the prediction power. Finally, it is also challenging to translate markers from proteomic investigations into real-world diagnostic or prognostic applications. Approval of use of a marker or set of markers for a given clinical decision relies on the results of a large-scale multicentric clinical trial (e.g. TAILORx and MINDACT trials) [60,61], and approval of the use of the detection technology for that purpose. Microfluidics-based platforms are currently being developed under a National Institute of Dental and Craniofacial Research (NIDCR) initiative, which may lead to simple detection devices for the clinical use of salivary protein markers [21].

There is an enormous need to develop more effective and less toxic therapeutic approaches to treat cancer. The opportunities for pharmacoproteomics to impact the study of differential gene expression applied to drug discovery and optimization can be remarkable. These advances will likely include the discovery of new drug targets, the confirmation of expected action(s) of mechanism of a drug, the assessment of drug efficacy and toxicity, and the identification of disease subgroups and prediction of treatment responses of individual patients. These applications may benefit the entire drug discovery and development process

by reducing failure rates at all stages of the development pipeline, fastening the transition from discovery to clinical trials, and facilitating more successful therapies for patient subgroups.

Within the next few years, affordable platforms for quantitative and high-throughput proteomics will be developed. These proteomics tools will be fully investigated on saliva protein biomarker discovery for cancer detection and treatment. Saliva represents a readily accessible body fluid that may be repeatedly sampled for *in vivo* assessment of efficacy and toxicity of anticancer drugs. We are enthusiastic that saliva protein markers will be developed and integrated into clinical trials and practice of molecular-targeted chemotherapies, especially those for OSCC/HNSCC.

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References

1 Mandel, I.D. (1987) The functions of saliva. *J. Dent. Res.* 66, 623–627
2 Tabak, L.A. (2001) A revolution in biomedical assessment: the development of salivary diagnostics. *J. Dent. Educ.* 65, 1335–1339
3 Mandel, I.D. (1990) The diagnostic uses of saliva. *J. Oral Pathol. Med.* 19, 119–125
4 Hu, S. *et al.* (2006) Human body fluid proteome analysis. *Proteomics* 6, 6326–6353
5 Ryu, O.H. *et al.* (2006) Identification of parotid salivary biomarkers in Sjogren's syndrome by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry and two-dimensional difference gel electrophoresis. *Rheumatology (Oxford)* 45, 1077–1086
6 Hu, S. *et al.* Salivary proteomic and genomic biomarkers for primary Sjögren's syndrome. *Arthritis Rheum.* (in press)
7 Wilmarth, P.A. *et al.* (2004) Two-dimensional liquid chromatography study of the human whole saliva proteome. *J. Proteome Res.* 3, 1017–1023
8 Hu, S. *et al.* (2005) Large-scale identification of proteins in human salivary proteome by liquid chromatography/mass spectrometry and two-dimensional gel electrophoresis-mass spectrometry. *Proteomics* 5, 1714–1728
9 Xie, H. *et al.* (2005) A catalogue of human saliva proteins identified by free flow electrophoresis-based peptide separation and tandem mass spectrometry. *Mol. Cell. Proteomics* 4, 1826–1830
10 Yates, J.R. *et al.* (2006) Performance of a linear ion trap-Orbitrap hybrid for peptide analysis. *Anal. Chem.* 78, 493–500
11 Guo, T. *et al.* (2006) Characterization of the human salivary proteome by capillary isoelectric focusing/nanoreversed-phase liquid chromatography coupled with ESI-tandem MS. *J. Proteome Res.* 5, 1469–1478
12 Hu, S. *et al.* (2007) Human saliva proteome analysis. *Ann. N.Y. Acad. Sci.* 1098, 323–329
13 Hirtz, C. *et al.* (2005) MS characterization of multiple forms of alpha-amylase in human saliva. *Proteomics* 5, 4597–4607
14 Walz, A. *et al.* (2006) Proteome analysis of glandular parotid and submandibular-sublingual saliva in comparison to whole human saliva by two-dimensional gel electrophoresis. *Proteomics* 6, 1631–1639
15 Ramachandran, P. *et al.* (2006) Identification of N-linked glycoproteins in human saliva by glycoprotein capture and mass spectrometry. *J. Proteome Res.* 5, 1493–1503
16 Inzitari, R. *et al.* (2006) Detection in human saliva of different statherin and P-B fragments and derivatives. *Proteomics* 6, 6370–6379
17 Inzitari, R. *et al.* (2005) Different isoforms and post-translational modifications of human salivary acidic proline-rich proteins. *Proteomics* 5, 805–815
18 Messana, I. *et al.* (2004) Characterization of the human salivary basic proline-rich protein complex by a proteomic approach. *J. Proteome Res.* 3, 792–800
19 Castagnola, M. *et al.* (2004) A cascade of 24 histatins (histatin 3 fragments) in human saliva. Suggestions for a pre-secretory sequential cleavage pathway. *J. Biol. Chem.* 279, 41436–41443
20 Lupi, A. *et al.* (2003) Identification of the human salivary cystatin complex by the coupling of high-performance liquid chromatography and ion-trap mass spectrometry. *Proteomics* 3, 461–467
21 Wong, D.T. (2006) Salivary diagnostics powered by nanotechnologies, proteomics and genomics. *J. Am. Dent. Assoc.* 137, 313–321
22 Streckfus, C. *et al.* (2000) The presence of soluble c-erbB-2 in saliva and serum among women with breast carcinoma: a preliminary study. *Clin. Cancer Res.* 6, 2363–2370
23 Streckfus, C. *et al.* (2000) A preliminary study of CA15-3, c-erbB-2, epidermal growth factor receptor, cathepsin-D, and p53 in saliva among women with breast carcinoma. *Cancer Invest.* 18, 101–109
24 Bigler, L.R. *et al.* (2002) The potential use of saliva to detect recurrence of disease in women with breast carcinoma. *J. Oral Pathol. Med.* 31, 421–431
25 Mizukawa, N. *et al.* (1999) Defensin-1, an antimicrobial peptide present in the saliva of patients with oral diseases. *Oral Dis.* 5, 139–142
26 Lyons, A.J. *et al.* (2000) Salivary oncofoetal fibronectin and oral squamous cell carcinoma. *J. Oral Pathol. Med.* 29, 267–270
27 Franzmann, E.J. *et al.* (2003) Expression of tumor markers hyaluronic acid and hyaluronidase (HYAL1) in head and neck tumors. *Int. J. Cancer* 106, 438–445
28 St. John, M. *et al.* (2004) Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. *Arch. Otolaryngol. Head Neck Surg.* 130, 929–935
29 Franzmann, E.J. *et al.* (2005) Salivary soluble CD44: a potential molecular marker for head and neck cancer. *Cancer Epidemiol. Biomarkers Prev.* 14, 735–739
30 Rhodus, N.L. *et al.* (2005) NF-kappaB dependent cytokine levels in saliva of patients with oral preneoplastic lesions and oral squamous cell carcinoma. *Cancer Detect. Prev.* 29, 42–45
31 Zhong, L.P. *et al.* (2005) Detection of telomerase activity in saliva from oral squamous cell carcinoma patients. *Int. J. Oral Maxillofac. Surg.* 34, 566–570
32 Nagler, R. *et al.* (2006) Concomitant analysis of salivary tumor markers - a new diagnostic tool for oral cancer. *Clin. Cancer Res.* 12, 3979–3984
33 Drake, R.R. *et al.* (2005) Serum, salivary and tissue proteomics for discovery of biomarkers for head and neck cancers. *Expert Rev. Mol. Diagn.* 5, 93–100
34 Hu, S. *et al.* (2007) Oral cancer proteomics for the biomarker discovery. *American Association for Cancer Research Annual Meeting*, Los Angeles, USA
35 Brailo, V. *et al.* (2006) The significance of salivary interleukin 6 and tumor necrosis factor alpha in patients with oral leukoplakia. *Oral Oncol.* 42, 370–373
36 Rhodus, N.L. *et al.* (2005) The feasibility of monitoring NF-kappaB associated cytokines. TNF-alpha, IL-1alpha, IL-6, and IL-8 in whole saliva for the malignant transformation of oral lichen planus. *Mol. Carcinog.* 44, 77–82
37 Rhodus, N.L. *et al.* (2005) A comparison of the pro-inflammatory, NF-kappaB-dependent cytokines: TNF-alpha, IL-1-alpha, IL-6, and IL-8 in different oral fluids from oral lichen planus patients. *Clin. Immunol.* 114, 278–283
38 Tao, X. *et al.* (2007) Assessment of local angiogenesis and vascular endothelial growth factor in the patients with atrophic-erosive and reticular oral lichen planus. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 103, 661–669
39 Warnakulasuriya, S. *et al.* (2000) Expression of p53 in oral squamous cell carcinoma is associated with the presence of IgG and IgA p53 autoantibodies in sera and saliva of the patients. *J. Pathol.* 192, 52–57
40 Aebersold, R. and Mann, M. (2003) Mass spectrometry-based proteomics. *Nature* 422, 198–207
41 Wulfkuhle, J.D. *et al.* (2006) Technology insight: pharmacoproteomics for cancer – promises of patient-tailored medicine using protein microarrays. *Nat. Clin. Pract. Oncol.* 3, 256–268
42 Kelloff, G.J. and Sigman, C.C. (2005) New science-based endpoints to accelerate oncology drug development. *Eur. J. Cancer* 41, 491–501
43 Butler, G.S. and Overall, C.M. (2007) Proteomic validation of protease drug targets: pharmacoproteomics of matrix metalloproteinase inhibitor drugs using isotope-coded affinity tag labeling and tandem mass spectrometry. *Curr. Pharm. Design* 13, 263–270
44 Sung, F.L. *et al.* (2006) Pharmacoproteomics study of cetuximab in nasopharyngeal carcinoma. *J. Proteome Res.* 5, 3260–3267
45 Floyd, E. and McShane, T.M. (2004) Development and use of biomarkers in oncology drug development. *Toxicol. Pathol.* 32 (Suppl. 1), 106–115
46 Colburn, W.A. (2003) Biomarkers in drug discovery and development: from target identification through drug marketing. *J. Clin. Pharmacol.* 43, 329–341
47 McClelland, C.M. and Gullick, W.J. (2003) Identification of surrogate markers for determining drug activity using proteomics. *Biochem. Soc. Trans.* 31 (Pt 6), 1488–1490
48 Ma, Y. *et al.* (2006) Predicting cancer drug response by proteomic profiling. *Clin. Cancer Res.* 12, 4583–4589

49 Stoughton, R.B. and Friend, S.H. (2005) How molecular profiling could revolutionize drug discovery. *Nat. Rev. Drug Discov.* 4, 345–350

50 Fingleton, B. *et al.* (2004) Proteinase activity in human and murine saliva as a biomarker for proteinase inhibitor efficacy. *Clin. Cancer Res.* 10, 7865–7874

51 Rhodus, N.L. *et al.* (2006) Proinflammatory cytokine levels in saliva before and after treatment of (erosive) oral lichen planus with dexamethasone. *Oral Dis.* 12, 112–116

52 Shak, S. (1999) Overview of the trastuzumab (Herceptin) anti-HER2 monoclonal antibody clinical program in HER2-overexpressing metastatic breast cancer. Herceptin Multinational Investigator Study Group. *Semin. Oncol.* 26 (4 Suppl 12), 71–77

53 Lesko, L.J. and Atkinson, A.J., Jr (2001) Use of biomarkers and surrogate endpoints in drug development and regulatory decision making: criteria, validation, strategies. *Annu. Rev. Pharmacol. Toxicol.* 41, 347–366

54 Petricoin, E.F. *et al.* (2004) Toxicoproteomics: serum proteomic pattern diagnostics for early detection of drug induced cardiac toxicities and cardioprotection. *Toxicol. Pathol.* 32 (Suppl 1), 122–130

55 Dawes, C. (1974) The effects of flow rate and duration of stimulation on the concentrations of protein and the main electrolytes in human submandibular saliva. *Arch. Oral Biol.* 19, 887–895

56 Hardt, M. *et al.* (2005) Assessing the effects of diurnal variation on the composition of human parotid saliva: quantitative analysis of native peptides using iTRAQ reagents. *Anal. Chem.* 77, 4947–4954

57 Ransohoff, D.F. (2002) Bias as a threat to the validity of cancer molecular-marker research. *Nat. Rev. Cancer* 5, 142–149

58 Pepe, M.S. *et al.* (2001) Phases of biomarker development for early detection of cancer. *J. Natl. Cancer Inst.* 93, 1054–1061

59 Hu, J. *et al.* (2005) The importance of experimental design in proteomic mass spectrometry experiments: some cautionary tales. *Brief Funct. Genomics Proteomics* 3, 322–331

60 Sparano, J.A. (2006) TAILORx: trial assigning individualized options for treatment (Rx). *Clin. Breast Cancer.* 7, 347–350

61 Bogaerts, J. *et al.* (2006) Gene signature evaluation as a prognostic tool: challenges in the design of the MINDACT trial. *Nat. Clin. Pract. Oncol.* 3, 540–551