Head and Neck Squamous Cell Carcinoma (HNSCC) Protein Profiles Associated with Tumor Response to Treatment

Bissan Ahmed[1], Paul Debbage[2], Sergej Skvortsov[3], Peter Lukas[3] and Ira Skvortsova[3,*]

[1]*Department of Nuclear Medicine, Jules Bordet Institute, Brussels, Belgium;* [2]*Department of Anatomy, Histology and Embryology;* [3]*Department of Therapeutic Radiology and Oncology, Innsbruck Medical University, Innsbruck, Austria*

Abstract: Head and neck squamous cell carcinoma (HNSCC) remains one of the most aggressive malignancies, characterized by limited therapeutic success and poor outcome. Despite continuous development of novel therapeutic strategies, disease-free survival in patients with advanced HNSCC has not been improved during the last 30 years. Evidently, a deeper understanding is needed of the molecular mechanisms underlying both intrinsic and acquired HNSCC cell resistance to currently existing therapeutic approaches. Proteome analysis is a powerful method which can provide deep insight into the molecular basis underlying HNSCC cell survival despite cytotoxic anti-tumor treatment (chemo-, radiotherapy). Evaluation of the protein profiles of cells obtained from locally recurrent or metastatic tumors can allow researchers to identify key protein players which regulate the HNSCC response to therapy. Additionally, subcellular fractionation and isolation of various cell organelles followed by proteomic analysis can provide data about intracellular protein localization, translocation and function following anti-cancer therapy. This review article discusses the protein patterns in HNSCC cells responsible for the radio- and chemo-resistance of these tumors and which result in the carcinoma cell survival and HNSCC recurrence.

Keywords: Head and neck squamous cell carcinoma (HNSCC), Proteomics, Biomarker, Therapeutic target, Treatment resistance.

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide and is still responsible for a quarter of a million deaths annually [1]. Overall, HNSCC accounts for more than 550,000 cases annually worldwide [2]. Males are affected significantly more than females with a ratio ranging from 2:1 to 4:1. The incidence rate in males exceeds 20 per 100,000 in regions of France, Hong Kong, the Indian subcontinent, central and eastern Europe, Spain, Italy, Brazil and among African Americans in the United States. Mouth and tongue cancers are more common in the Indian subcontinent, nasopharyngeal cancer is more common in Hong Kong, and pharyngeal and/or laryngeal cancers are more common in other populations [3, 4]. HNSCC is traditionally associated with older men who smoke and consume alcohol. Alcohol and tobacco use (including smokeless tobacco, sometimes called “chewing tobacco” or “snuff”) are the two most important risk factors for HNSCC, especially for cancers of the oral cavity, oropharynx, hypopharynx, and larynx [5, 6]. At least 75% of HNSCCs are caused by tobacco and alcohol use [7, 8]. Infection with cancer-causing types of human papillomavirus (HPV), especially HPV-16, is also a risk factor for some types of HNSCC, particularly oropharyngeal cancers that involve the tonsils or the base of the tongue [9]. The incidence of oropharyngeal cancers caused by HPV infection is increasing worldwide, while the incidence of oropharyngeal cancers related to other causes is falling [3, 10].

Although methods of HNSCC prevention and diagnosis are continuously being developed, many patients are diagnosed only at advanced stages of tumor development. This markedly reduces the arsenal of anti-tumor treatments available and worsens clinical outcome. Surgery, chemotherapy, and radiation remain the mainstay of patient care [11]. Despite ongoing improvement in traditional treatments, the long-term survival rate, which is 50% at five years after diagnosis, has remained unaltered over the past thirty years [12]. HNSCC tumor resistance to therapy is the major cause of cancer-related deaths, because the disease progresses even after all possible anti-tumor therapeutic options have been tried. It is therefore necessary to elucidate the factors which influence the efficacy of various treatment approaches (radio- and chemotherapy, targeted agents). Definition of the molecular pathways mediating constitutive or acquired cancer aggressiveness and therapy insensitivity would contribute to the development of novel diagnostic, predictive markers and therapeutic targets. HNSCC is a heterogeneous tumor consisting of multiple cancer

*Address correspondence to this author at the Laboratory for Experimental and Translational Research on Radiation Oncology (EXTRO-Lab), Department of Therapeutic Radiology and Oncology, Innsbruck Medical University, Anichstr. 35, A-6020 Innsbruck, Austria; Tel: ++43-512-504-27758;
Fax: ++43-512-504-27756; E-mail: Ira.Skvortsova@i-med.ac.at

E-ISSN: 2410-3802/15

cell subpopulations, so it is necessary to identify the cell subpopulations responsible for HNSCC treatment resistance and recurrence and then to elucidate the molecular properties of these cells. Currently existing proteome-based methods can help to clarify how intracellular and intratumoral signaling pathways are altered in the most resistant HNSCCs.

PROTEOMICS IS A TOOL FOR PROTEIN IDENTIFICATION

Genetic analysis is widely used in cancer research to identify altered genes predicting either a predisposition to cancer development, to disease progression or to the probable tumor response to therapy and thus clinical outcome. However, nucleic acids do not provide full information about the intracellular or intratumoral molecular networks associated with these processes. In contrast, oncoproteomics can determine which molecular pathways are affected and are implicated in tumor development and progression, and thus which molecules could be targeted to improve clinical outcome. Currently, researchers possess a variety of proteome-related approaches that can be applied depending amongst other things on the aim of the investigation, on the type, size or volume of the samples, and on the concentrations of the proteins in the samples.

Proteomic analysis aims to study the structure of proteins (structural proteomics), to determine protein expression in the compared biological samples (differential proteomics) and to elucidate molecular mechanisms underlying disease progression or other intracellular/intratumoral events (functional proteomics) [13]. In the development of cancer-related biomarkers and therapeutic targets, differential and functional proteomics are the most important and actively used approaches. There are two major technical directions in proteomics: the gel-based and the gel-free methods. Although gel-based techniques followed by mass spectrometry are laborious and time-consuming, they identify a wide range of proteins that are specifically or differentially expressed in the samples of interest. Both gel-based approaches, namely one- and two-dimensional gel electrophoresis (1-D and 2-D GE), can identify proteins of different molecular weights, from 10 kDa to more than 300 kDa [14]. In some cases use of 1-D GE can overcome limitations observed during the performance of 2-D-based proteomics. Thus, 1-D SDS PAGE coupled with liquid chromatography-mass spectrometry (LC-MS) helped to identify low abundant proteins with extremely basic or acidic pH, and which were not identified using 2-D gels [14;15]. Despite clear visualization of the differences in expression of proteins and their modifications, 2-D GE has some limitations: proteins are usually poorly detected if they are present in low abundance, or have an extremely basic pH, or a molecular mass less than 10 kDa or greater than 200 kDa. Furthermore, inadequate sample solubilisation adds further difficulties in evaluating protein expression and identifying the spots selected in the 2-D gels. However, standard and differential 2-D GEs followed by mass spectrometry are still widely used by researchers to detect proteins responsible for disease progression or for clinical outcome in cancer patients [16, 17].

Since gel-based methods have a number of limitations, gel-free approaches were developed and mass spectrometry became a core technology of proteomics [16]. Gel-free proteomics does not require protein separation prior to digestion and peptides are fractionated via LC, and it is also possible to use a combination of gel-based separation of proteins and LC-MS-MS. Furthermore, as recently shown, mass spectrometry methods can be combined and the use of MALDI and ESI mass spectrometry substantially improves protein identification in samples [18].

Simultaneous analysis of different proteins and their expression can be evaluated using protein microarrays and MALDI imaging [14, 19]. The protein microarray or protein chip is a proteomic tool which allows fast and effective screening of thousands of different proteins. Forward-phase protein array analysis is often performed using fluorophore-labelled antibodies to capture proteins and to compare them, similarly to the 2-D DIGE technique [14]. In contrast, the reverse-phase protein array immobilizes the protein samples on a surface, followed by application of antibodies to detect specific epitopes. The protein microarray is very useful and attractive for biomarker discovery and for fast screening of proteins which are candidates to become biomarkers.

MALDI imaging is another new and attractive method to compare protein expression in different tissues [19]. Direct application of MALDI-TOF-MS in tissue slices does not require tissue homogenization and the method allows not only protein identification but can also visualize protein distributions both in the tumor and in the adjacent normal tissues. MALDI imaging has other strengths, for example it can be used in principle to evaluate protein patterns at the

subcellular level in organelles [20, 21]. Despite its high capacity to evaluate protein expression and alterations in molecular pathways directly in the tumor tissues, this method does have pronounced limitations. Firstly, the time needed to prepare and process fresh frozen or formalin-fixed paraffin-embedded tumor tissues for MALDI imaging can markedly reduce the efficacy of protein identification. Secondly, the so-called phenomenon of ion (ionization) suppression occurs in tumor tissues analyzed by use of MALDI imaging [19]; it is not specific for MALDI imaging and can accompany any mass spectrometric analysis. It can compromise the results of MALDI imaging analysis because one protein may be more easily ionized than another that is present in the same tumor tissue [19, 22]. Further validation of the proteins identified by MALDI imaging is essential, and can be carried out by a range of different methods, including immunohistochemistry, ELISA, flow cytometry, and immunofluorescence analysis.

SAMPLES FOR PROTEOME ANALYSIS

HNSCC Cell Lines: In Vitro Samples for the Study of HNSCC Treatment Resistance

The selection of samples for proteome-based discovery of predictive biomarkers or therapeutic targets is the most critical point in translational cancer research. In general, HNSCC sampling for proteomic approaches can be conducted in either of two directions: pre-clinical and clinical. Pre-clinical samples include material obtained *in vitro* or *in vivo*.

In vitro cell line models, either primary or immortalized, allow the molecular mechanisms of cancer cell resistance to chemotherapeutic agents and/or ionizing radiation to be investigated comprehensively. Immortalized cell lines are particularly advantageous for the identification of proteins associated with cancer cell treatment resistance because their cellular composition is homogeneous. In contrast to tumor tissues, the cell lines do not contain the several additional cell types that belong to the connective tissues, the blood or the immune system. The researcher can therefore be assured that any proteins identified by use of proteomics-based methods are specific only for the cancer cells and are therefore implicated in intracellular pathways associated with functions occuring in the cancer cells. Next, immortalized carcinoma cell lines are widely used to obtain cells with acquired treatment resistance, which are optimal for studies aimed at elucidating the molecular mechanisms related to the treatment resistance or better response of carcinoma cells. Differences in the protein patterns found in treatment-resistant and treatment-sensitive carcinoma cells are restricted to the mechanisms of therapy resistance. For example, recent reports on HNSCC radiation resistance showed clear differences in the protein profiles of radio resistant HNSCC cells as compared to radiation sensitive cells [23-25]. Both research groups indicated that radiation resistant HNSCC cells were characterized by up-regulation of proteins participating in the DNA damage response, cell cycle regulation, cell adhesion, migration and motility. All these intracellular processes delineate cancer stem cell (CSC)-specific molecular signatures. It has also recently been shown that CSCs are characterized by activation of pathways related to the DNA damage response [26, 27]. Thus, expression of ATM, Chk1 and Chk2 DNA repair and cell cycle-associated proteins are affected in CSCs, resulting in CSC resistance to DNA-damaging agents (*e.g.*, radiotherapy, platinum compounds). Furthermore, proteomic-based studies aiming to elucidate the molecular background for radiation or chemo resistance have shown that therapy resistant carcinoma cells are characterized by up-regulation of scavengers of reactive oxygen species (ROS), which also contribute to the treatment-caused DNA damage [25, 28]. Since key proteins regulating carcinoma cell resistance to therapeutic approaches have been identified, it is possible to develop novel therapeutic agents inhibiting either expression or activity of these proteins. It is believed that concomitant use of conventional therapeutic approaches and compounds targeting activated pro-survival pathways in carcinoma cells can markedly improve therapy outcome in cancer patients.

Since radio- and chemo-resistant cell lines are usually enriched for CSCs, it was logical to suggest that CSCs possess the highest expression of the ROS scavengers. Indeed, recent reports have clearly demonstrated that due to the over expression of ROS scavengers, CSCs reveal very low ROS concentrations compared to the bulk of tumor cells and even to normal non-malignant cells [29]. This fact is a partial explanation for the reduced DNA damage seen in treatment resistant CSCs.

Unfortunately, the exact mechanisms of CSC therapy resistance are still poorly understood. A possible way forward is the recently described idea of isolating CSCs from *in vitro* cultivated cell lines and examining them by proteomic analysis to obtain a list of

the proteins associated with treatment resistance and with enhanced pro-survival activities [30, 31]. Furthermore, subcellular fractionation of cultured CSCs could be used to improve our knowledge of the subcellular localization and translocation of these proteins. Indeed, our research group has recently demonstrated that enhanced Rac1 expression in the nuclei of HNSCC cells can predict a lower sensitivity of HNSCC cells to ionizing radiation and platinum compounds [24]. Similarly, EGFR translocation to the nuclei is associated with an enhancement of DNA repair and with consequent cell resistance to DNA-damaging agents [32;33]. Attenuation of Rac1 expression and inhibition of its activity in chemo-radio resistant HNSCC cells improved cytotoxic effects of ionizing radiation and cisplatin [24].

Since there is no a single opinion as to which membrane proteins can be used as markers for HNSC CSCs, membrane proteomics is under deep investigation. Analysis of the membrane proteins underlying differences in molecular properties between HNSCC spheroid-forming and adherent cells has demonstrated that two types of network are affected in spheroid-forming cells enriched for CSCs, these are: the EGFR - CD44 - HSP70 - HSP90 and the integrin alpha-6 - integrin beta-4 - SLC3A2 pathways [34]. These data correspond to our own data, which show dysregulation of these pathways in radio- and chemo-resistant HNSCC cell lines enriched for CD44-positive CSCs [23, 35]. It is likely that there is no unique or single marker identifying HNSC CSCs; it is more likely that CSCs are characterized by expression of several different markers associated with their functional capacities.

Nowadays exosomes are organelles of interest which can be studied in HNSCC by use of proteomics [36]. Identification of the protein profiles of these extracellular organelles is more useful than analyzing the cellular or tumoralsecretomes. The main advantage is that the cellular secretome contains not only proteins secreted by the carcinoma cells, but also enzymes deriving from cells which are dying or damaged during cultivation under stress conditions. Isolation of the exosomes can help to avoid contamination with non-exosome-specific proteins and makes proteomic-based identification of the secreted proteins more accurate. Exosomal proteins can influence the activity of intratumoral immune T-cells, thus supporting the immune escape of tumor cells [37]. These proteins can also enhance the invasive capacities of carcinoma cells [38], they can change the migration of endothelial cells and they can serve as pro-angio- and vasculogenic agents [35]. One further reason why protein profiling of exosomes should be an issue of interest is that exosomal proteins can serve as a source of biomarkers that can be found in the biological fluids of cancer patients (plasma, serum, saliva).

Experimental In Vivo Samples to Study HNSCC Progression

Despite the fact that almost all studies identifying perturbations in molecular pathways associated with the HNSCC response to chemo- and radiotherapy were performed using carcinoma cell lines, *in vivo* xenograft models can also be utilized. Experimental animal models allow determination of how the response to therapy is regulated within the tumor. In order to know why HNSCC patients respond differently to combinations of radiotherapy and cetuximab, xenograft tumors were investigated using 2D-DIGE followed by MALDI-TOF/TOF [39]. It was found that cetuximab effectively inhibited the growth of tumors having an epithelial phenotype characterized by overexpression of epithelial markers (E-cadherin) and decreased expression of mesenchymal markers (vimentin and N-cadherin), whereas HNSCC with mesenchymal phenotypes were resistant to this EGFR blocker. However, cetuximab failed to enhance the tumor response to ionizing radiation if HNSCC with an epithelial phenotype had initiated the epithelial-to-mesenchymal transition programme, accompanied by up-regulation of the c-myc protein. In contrast, tumors that were initially mesenchymal but had initiated the change toward an epithelial phenotype, with c-myc down-regulation, showed significant improvement of their response to radiation upon treatment with cetuximab [39]. Although proteomic analyses can identify the perturbed intratumoral proteins, most researchers prefer to evaluate protein profiles of carcinoma cells using cell lines first and then proceed to validate the intratumoral roles of these proteins by use of *in vivo* xenograft models.

An especially interesting and important research area is the use of experimental animal models to elucidate the molecular mechanisms underlying the metastatic spread of malignant tumors. There are at least two possible ways to identify proteins associated with tumor metastatic spread: (1) to study protein patterns of the xenograft tumors obtained using primary or immortalized cell lines with known metastatic capacities and (2) to compare the proteomes of tumor cells obtained from both primary and secondary metastatic tumors. Both methods can provide data

about the activated and repressed pathways associated with the high metastatic potential of malignant tumors. Analysis of the literature shows that the first method, using animal models, is widely used by researchers to determine the metastasis-related proteome, whereas the second approach is preferably performed in clinical samples to compare the protein patterns of primary and secondary tumors. It has recently been reported that the metastatic potential of HNSCC depends on the expression of mesenchymal markers and CSC-associated proteins (Oct4, ALDH1) [40]. These results correspond to our own data showing that HNSCC expressing CSC-related proteins (Notch1, CD44+/CD24-/ALDH1+) have enhanced metastatic abilities [35]. Another report by Huang *et al*. demonstrated that the metastatic abilities of nasopharyngeal carcinoma were associated with phosphorylation of lysine-rich CEACAM1 co-isolated protein (LYRIC) [41]. Indeed, LYRIC is described to be a part of the tight junction complex [42]. Since tight junctions create an intercellular barrier and an intramembrane diffusion fence, reduction of these junctions due to LYRIC phosphorylation can initiate metastatic processes [43]. Intratumoral identification of the proteins associated with the enhancement of HNSCC metastatic potential can help clinicians to reschedule therapy towards administration of more aggressive and toxic treatments.

Patients' Material for Evaluation of the HNSCC-Related Proteome

A number of clinically relevant samples can be obtained from patients for proteomic analyses, and used to elucidate the molecular mechanisms underlying HNSCC responses to chemo-radiotherapy (Figure **1**). Evaluation of the protein profiles in such tissue samples is a powerful approach, because by comparing the protein patterns in malignant tissues and healthy mucosa in both primary and secondary tumors, and in tumors with good and poor therapy responses, one can obtain a long list of putative biomarkers to predict treatment outcome in the patients (Table **1**). Unfortunately, use of biopsy materials obtained from cancer patients can sometimes be compromised in the protein profiling by tumor heterogeneity, in particular because the samples compared derive from tumor regions containing different cancer cell subpopulations [44]. It is best to increase the number of tissue samples examined in order to avoid (or at least to reduce) any mistakes in defining protein expression in the tumors with different treatment responses or in primary and metastatic tumor tissues. Since CSC theory is increasing in popularity and can explain tumor heterogeneity, researchers try to obtain CSC subpopulation(s) from tumor tissues of interest. CSCs can be isolated from tumor tissues by a variety of methods, including fluorescence (FACS) and magnetic (MACS) activated cell sorting, laser cell capture microdissection, or three-dimensional spheroid/ organoid cell culture. It remains an important challenge to determine whether the sets of proteins found in CSCs are similar in primary and secondary (metastatic or locally relapsed) tumors. The following questions need to be answered: are CSCs obtained from recurrent or metastatic tissues more aggressive than CSCs in primary tumors? If yes, which molecular pathways are affected in the more aggressive CSCs? Which proteins can indicate CSC aggressiveness? Clinicians aiming to be more successful in the treatment of HNSCC face several very urgent challenges: (1) to identify the key regulator(s) of CSC activities in order to to use it (them) as biomarker(s) and/or therapeutic target(s) to predict and overcome treatment resistance; (2) to find effective ways to image the proteins once they have been found; (3) to use protein imaging to evaluate the efficacy of treatment in eliminating CSCs; (4) to employ protein imaging to determine the regions of the malignant tumors which contain CSCs and therefore require additional treatment by ionizing radiation with higher energy (so called LET-painting irradiation); (5) to develop novel radiolabeled antibodies targeting CSCs. Zhu *et al*. (2014) recently reported the use of proteomic analysis to identify biomarkers and protein networks in hypopharyngeal HNSCC [45]. They found that Sp1, c-myc and p53 crosstalk is implicated in HNSCC carcinogenesis and metastatic spread.

Next, the tumor secretome can provide information equally as important as the cellular secretome, about the proteins secreted by primary or secondary tumors. Proteins identified in the secretome can also be sought in biological fluids such as serum, plasma or saliva. Despite the very limited published information about tumor secretome analysis in HNSCC patients, some putative circulating biomarkers have been identified by proteomic methods. Thus fascin, found amongst 75 other proteins in the HNSCC interstitial fluids, has been mentioned as a factor discriminating between healthy individuals and HNSCC patients [46]. These authors confirmed that fascin concentrations are elevated in serum obtained from HNSCC patients as compared to healthy persons, thus validating fascin's diagnostic value. Additionally, elevated expression of fascin in primary HNSCC indicated a higher risk of HNSCC metastatic spread [47]. Therefore, this protein can also

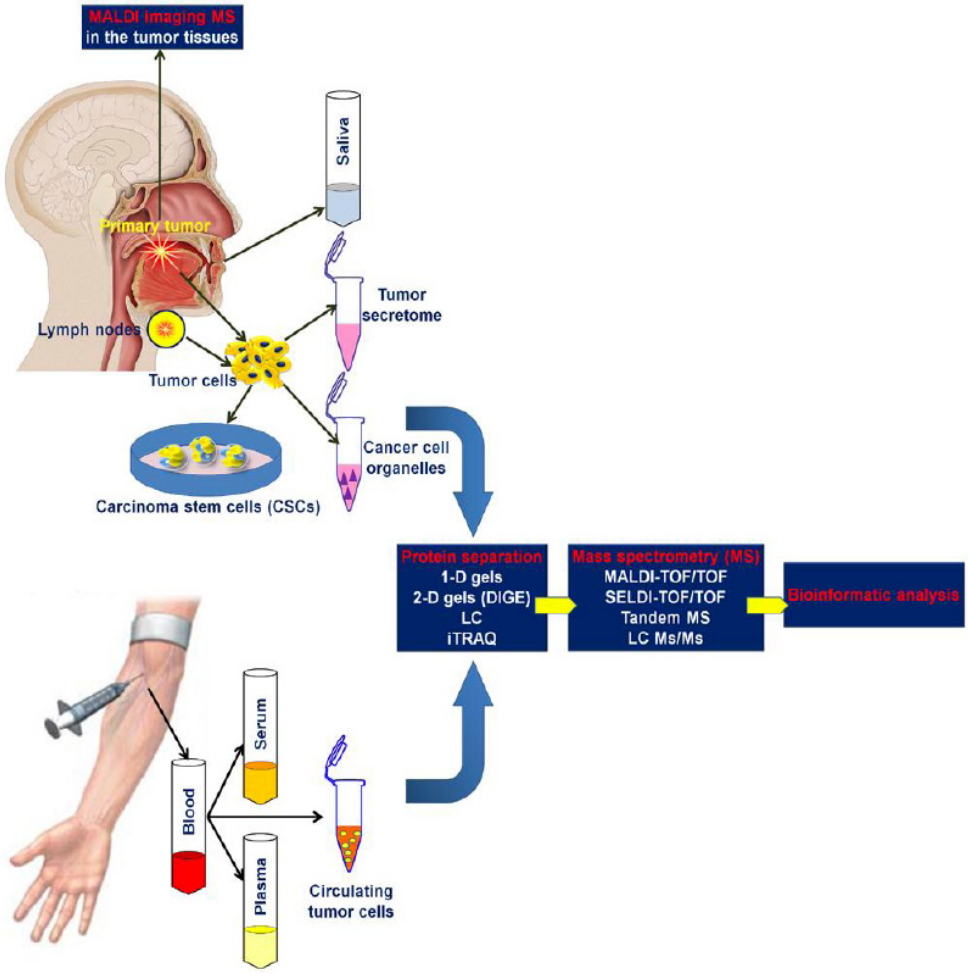

Figure 1: Schematic representation of sample collection for proteomic analysis in HNSCC patients.

Identification of proteins associated with treatment resistance in HNSCC patients can be carried out on samples obtained from the oral cavity (saliva) or from the primary or secondary (metastatic) tumors. Tissue pieces obtained from the tumor can be analysed directly by use of MALDI imaging mass spectrometry or can be further processed in order to obtain populations of the bulk tumor cells and/or of CSCs. All the types of tumor cell subpopulations can be processed to isolate subcellular organelles or to collect secretome. Blood is usually used to obtain plasma and serum for proteomics. However, blood could also be used to harvest circulating cancer cells. All the types of HNSCC samples mentioned are then processed for protein separation followed by mass spectrometry and bioinformatics analysis.

be considered as a stratification factor for HNSCC patients, indicating high and low risks of metastatic spread. Fascin has been shown to be implicated in the PI3K-Akt-related chemoresistance of breast cancer cells [48], therefore it can be assumed that evaluation of the intratumoral fascin expression might help to predict a possibly reduced chemosensitivity in HNSCC patients.

Since HNSCC tumors can excrete proteins and these tumors are located either directly in the oral cavity or very near to it, it would be logical to try to analyse protein expression in saliva. Similarly as for tumor samples, the salivary proteome may be used effectively for early detection and monitoring of malignant tumors [49-51]**.** Salivary proteomics is a very promising approach to identify HNSCC-related biomarkers for the following reasons: sample collection is non-invasive, the volume of samples collected is unrestricted, and saliva contains a high concentration of proteins including those deriving from the tumor and its adjacent tissues. Recently it has been shown that increased levels of soluble CD44 in saliva help to distinguish malignant from benign diseases of the oral cavity [52, 53]. A number of other proteins have also been reported as potential biomarkers for HNSCC. Thus, MRP14, CD59 (protectin), cytokeratin 19 fragment Cyfra 21-1, M2BP, profilin 1, autoantibodies to p53, and cancer antigen 125 were all found in malignant lesions in the oral cavity [53, 54]. Such proteins, once identified, can be proposed for further validation as promising diagnostic tools. Biomarkers discovered using salivary proteomics can usually be applied for cancer detection, but little is known about any relationship between salivary protein profiles and treatment outcome in HNSCC patients. To our

knowledge only one report contains data about a possible link between proteins found in the saliva of HNSCC patients and the response to DNA-damaging agents [55, 56]. Patients with cancer of the oral cavity showed alterations in the levels of reactive nitrogen species and of antioxidants that could cause alterations in the concentrations of salivary free radicals, and thus alter the response to treatment, in particular to treatment employing DNA-damaging agents. Since salivary proteomics is a powerful tool, which provides a framework for discriminating between malignant and benign disease, and opening new perspectives for discovery of biomarkers capable of predicting response to treatment, the development of salivary protein databases would be very useful for both researchers and clinicians. Currently a WEB-site 'Salivary Proteome Knowledge Base' is being developed (http://www.skb.ucla.edu/cgi-bin/hspmscgi-bin/welcome_c.cgi) and promises to be a platform where salivary proteomes can be analysed for protein profiles and protein significance.

Salivary proteomics has a deep relationship with serum proteomics. Salivary and serum proteomes show very similar alterations in protein expression in HNSCC patients [51]. We suggest that serum proteomics can be used not only for cancer detection, but also for prediction of tumor response and evaluation of the tumor damage upon anti-cancer treatment [57, 58]. Thus, acute phase proteins (fibrinogen, haptoglobin, hepcidin, SAA), complement factors (CO3, CO4A), protease inhibitors (AACT, ANT3, CYTC, ITIH4), cytokines (CCL13, CXCL7, OSTP, PLF4, S10AC) and antibacterial defense factors (DEF1, DEF3, DEFB1) have been found in serum obtained from HNSCC patients receiving radiotherapy [59]. It has also been shown that serum protein profiles depend on the dose and volume of irradiation.

Blood samples intended for proteomic analysis can be processed to isolate the circulating tumor cells (CTC). Since CTC serve as a factor possessing prognostic impact for disease-free survival in HNSCC patients, it is logical to assume that evaluation of the CTC protein patterns could help to understand the molecular mechanisms underlying disease progression and tumor response to therapy [60]. Unfortunately, CTC is a rare cell subpopulation and only 1 CTC can be detected in 1 mL blood [61]. It is therefore a big challenge to study protein expression in the small protein samples obtained from CTC populations. However, the current development of proteomic techniques allows analyse of even very small samples having low protein concentrations.

CONCLUSION

The problem of the effective treatment of malignant tumors is very complicated and requires comprehensive treatment approaches to protect patients from local or distant tumor recurrences. Unfortunately, until today a "one-size-fits-all" therapy concept is used to manage HNSCC. Since this non-personalized treatment method cannot effectively prevent recurrence, it is logical to suggest that it should be replaced by personalized medicine. On the one hand, personalized medicine can help to avoid overtreatment of HNSCC patients with a lower risk of tumor relapse and on the other hand it can accelerate therapy schedules in patients having a higher risk of HNSCC recurrence. Biomarkers predicting the risk of HNSCC relapse after radio- and/or chemotherapy should be discovered and introduced into clinical practice for better stratification of HNSCC patients. Evaluation of the molecular features of HNSCC showing different tumor responses to therapy can help to identify candidate proteins to become predictive biomarkers. Furthermore, determining the proteins associated with HNSCC treatment resistance can provide the background for development of potential therapeutic targets. Novel targeting therapeutics might help to repress firstly pathways related to the enhanced survival capacities of the HNSCC cells and also other mechanisms which protect tumors from ionizing radiation and chemotherapeutic compounds. Additionally, identification of the molecules responsible for tumor resistance to radiotherapy would provide the basis to develop imaging methods able to visualize tumor regions containing subpopulations of treatment-resistant cells. Intratumoral imaging can markedly improve radiotherapy results because it enables an increased dose of irradiation to be applied to the tumor regions containing resistant cells, yet concurrently to spare the surrounding tissues from radiation damage.

Proteomics serves as one of the 'omics' approaches that can be used for development of predictive biomarkers and therapeutic targets. Proteomics possesses a number of opportunities to process a variety of HNSCC samples for protein identification and for evaluation of resistance-specific pathways. However, correct selection of the samples that should be examined using proteomics is an important issue. Currently, researchers use both pre-clinical (*in vitro* and *in vivo* HNSCC samples) and clinical samples (blood, serum, plasma, tumor pieces, saliva, primary cell culture, isolated CSCs and circulating tumor cells) to conduct proteomics. We suggest that concurrent

Table 1: Proteins Associated with HNSCC Carcinogenesis, Disease Progression and Response to Treatment. Protein Functions were taken from the Uni Prot (www.uniprot.org) Database and Provided in the Table

Sample	Protein	Protein's Function	Proteome Approach	Ref.
Tissue	Annexin A1	Innate and adaptive immune response; epithelial differentiation and growth regulation; Ca2+-dependent phospholipid-binding proteins; inflammation	DIGE + MS 2DE+MS	[62-64]
	Heat shock protein 27	Intracellular signal transduction, negative regulation of apoptotic process, positive regulation of angiogenesis, immune response	2DE + MALDI ToF MS	[64]
	Lamin A/C	Negative regulation of apoptosis, cellular response to hypoxia, cellular protein metabolic processes, regulation of protein localization to nuclei	2DE + MALDI ToF MS	[64]
	Interleukin 1 receptorantagonist	Immune and inflammatory response	2DE + MALDI ToF MS	[64]
	Serine protease inhibitor clade B5 (serpinB5)	Extracellular matrix organization, morphogenesis of epithelium, negative regulation of endopeptidase activity, regulation of epithelial cell proliferation	2DE + MALDI ToF MS	[64]
	Stathmin 1	Intracellular signal transduction, regulation of cytoskeleton organization	2DE + MALDI ToF MS	[63-66]
	Superoxide dismutase 2	Reactive oxygen species metabolic process, regulation of mitochondrial membrane potential, activation of MAPK activity	2DE + MALDI ToF MS	[64]
	Stratifin (14-3-3 proteinsigma)	Positive regulation of cell growth, positive regulation of epidermal cell differentiation, regulation of cyclin-dependent protein serine/threonine kinase activity, signal transduction, regulation of apoptosis	2DE + MS	[63;66]
	S100 calcium-binding protein A9	Actin cytoskeleton reorganization, autophagy, inflammatory response, innate immune response, positive regulation of cell growth, positive regulation of NF-kappaB transcription factor activity, regulation of integrin biosynthetic process	2DE+MS	[66]
	Actin-related protein 2/3 complex (P21-ARC)	Structural constituent of cytoskeleton, small GTPase mediated signal transduction, ephrin receptor signaling pathway	2DE+MS	[66]
	Enolase	Glycolysis, small molecule metabolic process		[66]
	Hetero-geneous nuclear ribonucleo-protein K(hnRNPK)	Positive regulation of receptor-mediated endocytosis, RNA processing, RNA splicing, signal transduction	LC+tandem MS	[67]
Plasma	Fibrinogen α-chainfragment	Cellular adhesion, proliferation, and migration of protein during carcinogenesis	MALDI ToF/ToF + Mascot identification	[68]
	Immunoglo-bulin gamma-3 chain C region	Innate immune response, complement activation, classical pathway	Nano-LC ESI MS/MS + Mascot identification	[69]
	Complementcompon entC4a	Innate immune response, inflammatory response, complement activation	MALDI ToF/ToF + CID and LIFT acquired MS/MS	[69]
	Immunoglo-bulin kappachain C region	Immune response, complement activation, receptor-mediated endocytosis	Nano-LC ESI MS/MS + Mascot identification	[69]
Serum	Heat shock protein 70	Stress response, membrane organization, cell cycle regulation,	2DE + MALDI ToF MS	[70]
	Soluble form of ICAM-1 (sICAM1)	Regulation of cellular adhesion	2DE + MALDI ToF MS	[70]
	Serum amyloid A protein (SAA)	Activation of MAPK activity, positive regulation of cell adhesion, receptor-mediated endocytosis	2DE + MALDI ToF MS	[70]

proteomics-based evaluation of the different samples could markedly improve identification of the proteins associated with HNSCC resistance to treatment.

FUTURE VIEW

It is generally accepted that HNSCC is a disease with poor prognosis and unfavourable treatment outcome, due to the limited tumor response to radio- and chemotherapy and which results in early or late tumor recurrence. It seems that the currently existing management of HNSCC patients should be markedly re-directed towards the development of personalized medicine. Under the term "personalized medicine" we usually understand that each cancer patient should be diagnosed and treated individually. Clearly, detecting the expression of proteins associated with tumor aggressiveness, and with inclination for tumor relapse owing to higher survival rates of carcinoma cells, can provide oncologists with the information required to administer augmented and more effective therapeutic schedules. In order to know which proteins are related to the tumor's capacity to survive anti-tumor treatments, it is necessary to elucidate the molecular profiles of such tumors. Furthermore, clarification of the key players in HNSCC aggressiveness can help not only to discover novel predictive biomarkers, but also to consider these molecules as potential therapeutic targets. The pharmaceutical industry in collaboration with academic institutions tries to develop novel targeting therapeutics (small molecules, antibodies) blocking the intracellular pathways which help cells to overcome treatment toxicity. However, the majority of these agents eliminate only the bulk tumor cells, whereas the cells with higher pro-survival abilities remain undamaged. It is logical to suggest that the next efforts of researchers should be devoted to elucidating the molecular patterns of the CSCs responsible for disease progression. Furthermore, knowledge about CSC specific protein profiles can be used as a background to develop imaging techniques visualising intratumoral CSC localization. These CSC-based imaging techniques can be used by radiation oncologists to individualise radiation therapy schedules. Thus, it is suggested that in addition to the conventional photon-based therapy usually used in the treatment of HNSCC, boost proton- or carbon ion-based radiation therapy could also be applied to eliminate CSC agglomerates more effectively. It is certain that proteomics can serve as a very powerful method to identify the most promising molecular candidates for use as predictive biomarkers, and as therapeutic and imaging targets.

KEY ISSUES CONSIDERED IN THE REVIEW ARTICLE:

- HNSCC is a highly aggressive malignant type of tumor characterized by an inclination for local recurrence despite the use of a variety of anti-tumor therapeutic approaches, such as surgery, radiotherapy, chemotherapy and targeting agents.
- Despite enormous efforts by researchers and clinical oncologists, therapy outcomes in HNSCC patients have not significantly changed during the last three decades.
- Proteomic analysis can be used to determine the molecular mechanisms underlying HNSCC chemo- and radioresistance with subsequent recurrence after treatment.
- HNSCC can relapse due to the specific protein profiles associated with constitutive or acquired chemo- and/or radioresistance in carcinoma cells.
- The protein patterns of radio- and chemo-resistant carcinoma cells are very similar to the protein signatures in CSCs.
- Correct choice of biological samples for further proteomic analysis, whether tumor tissue, isolated carcinoma cells and their organelles, CSCs, circulating tumor cells, or biological fluids (cancer cell or tumor tissue derived secretomes, plasma and serum, saliva) can provide a framework for biomarker discovery and the development of potential therapeutic targets.
- Prediction of treatment resistance in HNSCC patients can provide the basis for re-directing treatment schedules towards the use of more aggressive treatments.

REFERENCES

[1] Le JM, Squarize CH, Castilho RM. Histone modifications: Targeting head and neck cancer stem cells. World J Stem Cells 2014; 6:511-25. http://dx.doi.org/10.4252/wjsc.v6.i5.511

[2] Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010; 60:277-300. http://dx.doi.org/10.3322/caac.20073

[3] Chaturvedi AK, Anderson WF, Lortet-Tieulent J, Curado MP, Ferlay J, Franceschi S *et al*. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. J ClinOncol 2013; 31:4550-9. http://dx.doi.org/10.1200/JCO.2013.50.3870

[4] Sankaranarayanan R, Masuyer E, Swaminathan R, Ferlay J, Whelan S. Head and neck cancer: a global perspective on epidemiology and prognosis. Anticancer Res 1998; 18:4779-86.

[5] Gandini S, Botteri E, Iodice S, Boniol M, Lowenfels AB, Maisonneuve P *et al*. Tobacco smoking and cancer: a meta-analysis. Int J Cancer 2008; 122:155-64.

http://dx.doi.org/10.1002/ijc.23033

[6] Boffetta P, Hecht S, Gray N, Gupta P, Straif K. Smokeless tobacco and cancer. Lancet Oncol 2008; 9:667-675. http://dx.doi.org/10.1016/S1470-2045(08)70173-6

[7] Blot WJ, McLaughlin JK, Winn DM, Austin DF, Greenberg RS, Preston-Martin S *et al*. Smoking and drinking in relation to oral and pharyngeal cancer. Cancer Res 1988; 48:3282-7.

[8] Tuyns AJ, Esteve J, Raymond L, Berrino F, Benhamou E, Blanchet F *et al*. Cancer of the larynx/hypopharynx, tobacco and alcohol: IARC international case-control study in Turin and Varese (Italy), Zaragoza and Navarra (Spain), Geneva (Switzerland) and Calvados (France). Int J Cancer 1988; 41:483-91. http://dx.doi.org/10.1002/ijc.2910410403

[9] Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E *et al*. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J ClinOncol 2011; 29:4294-301. http://dx.doi.org/10.1200/JCO.2011.36.4596

[10] Hama T, Tokumaru Y, Fujii M, Yane K, Okami K, Kato K *et al*. Prevalence of human papillomavirus in oropharyngeal cancer: a multicenter study in Japan. Oncology 2014; 87:173-82. http://dx.doi.org/10.1159/000360991

[11] Digue L, Pedeboscq S. [Quality assurance in head and neck medical oncology]. Bull Cancer 2014; 101:486-95.

[12] Partridge M, Li SR, Pateromichelakis S, Francis R, Phillips E, Huang XH *et al*. Detection of minimal residual cancer to investigate why oral tumors recur despite seemingly adequate treatment. Clin Cancer Res 2000; 6:2718-25.

[13] Naylor S, Kumar R. Emerging role of mass spectrometry in structural and functional proteomics. Adv Protein Chem 2003; 65:217-248. http://dx.doi.org/10.1016/S0065-3233(03)01021-0

[14] Alvarez-Chaver P, Otero-Estevez O, Paez de la CM, Rodriguez-Berrocal FJ, Martinez-Zorzano VS. Proteomics for discovery of candidate colorectal cancer biomarkers. World J Gastroenterol 2014; 20:3804-24. http://dx.doi.org/10.3748/wjg.v20.i14.3804

[15] Lim SR, Gooi BH, Singh M, Gam LH. Analysis of differentially expressed proteins in colorectal cancer using hydroxyapatite column and SDS-PAGE.ApplBiochemBiotechnol 2011; 165:1211-1224. http://dx.doi.org/10.1007/s12010-011-9339-3

[16] Hudler P, Kocevar N, Komel R. Proteomic approaches in biomarker discovery: new perspectives in cancer diagnostics. ScientificWorldJournal 2014; 2014:260348. http://dx.doi.org/10.1155/2014/260348

[17] Zhang JT, Liu Y. Use of comparative proteomics to identify potential resistance mechanisms in cancer treatment. Cancer Treat Rev 2007; 33:741-56. http://dx.doi.org/10.1016/j.ctrv.2007.07.018

[18] Yang Y, Zhang S, Howe K, Wilson DB, Moser F, Irwin D. A comparison of nLC-ESI-MS/MS and nLC-MALDI-MS/MS for GeLC-based protein identification and iTRAQ-based shotgun quantitative proteomics. J Biomol Tech 2007; 18:226-37.

[19] Kalinowska-Herok M, Pietrowska M, Walaszczyk A, Widak P. MALDI Imaging Mass Spectrometry - A Novel Approach in Biomedical Research of Tissues. Curr Proteomics 2013; 10:76-82. http://dx.doi.org/10.2174/15701646112099990002

[20] Schwamborn K. Imaging mass spectrometry in biomarker discovery and validation. J Proteomics 2012; 75:4990-8. http://dx.doi.org/10.1016/j.jprot.2012.06.015

[21] Schwamborn K, Caprioli RM. MALDI imaging mass spectrometry-painting molecular pictures. MolOncol 2010; 4:529-38. http://dx.doi.org/10.1016/j.molonc.2010.09.002

[22] Tomlinson L, Fuchser J, Futterer A, Baumert M, Hassall DG, West A *et al*. Using a single, high mass resolution mass spectrometry platform to investigate ion suppression effects observed during tissue imaging. Rapid Commun Mass Spectrom 2014; 28:995-1003. http://dx.doi.org/10.1002/rcm.6869

[23] Skvortsov S, Jimenez CR, Knol JC, Eichberger P, Schiestl B, Debbage P *et al*. Radioresistant head and neck squamous cell carcinoma cells: intracellular signaling, putative biomarkers for tumor recurrences and possible therapeutic targets. RadiotherOncol 2011; 101:177-82. http://dx.doi.org/10.1016/j.radonc.2011.05.067

[24] Skvortsov S, Dudas J, Eichberger P, Witsch-Baumgartner M, Loeffler-Ragg J, Pritz C *et al*. Rac1 as a potential therapeutic target for chemo-radioresistant head and neck squamous cell carcinomas (HNSCC). Br J Cancer 2014; 110:2677-87. http://dx.doi.org/10.1038/bjc.2014.221

[25] Bansal N, Mims J, Kuremsky JG, Olex AL, Zhao W, Yin L *et al*. Broad phenotypic changes associated with gain of radiation resistance in head and neck squamous cell cancer. Antioxid Redox Signal 2014; 21:221-36. http://dx.doi.org/10.1089/ars.2013.5690

[26] Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB *et al*. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 2006; 444:756-60. http://dx.doi.org/10.1038/nature05236

[27] Blanpain C, Mohrin M, Sotiropoulou PA, Passegue E. DNA-damage response in tissue-specific and cancer stem cells. Cell Stem Cell 2011; 8:16-29. http://dx.doi.org/10.1016/j.stem.2010.12.012

[28] Skvortsova I, Skvortsov S, Stasyk T, Raju U, Popper BA, Schiestl B *et al*. Intracellular signaling pathways regulating radioresistance of human prostate carcinoma cells. Proteomics 2008; 8:4521-33. http://dx.doi.org/10.1002/pmic.200800113

[29] Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN *et al*. Association of reactive oxygen species levels and radioresistance in cancer stem cells. Nature 2009; 458:780-3. http://dx.doi.org/10.1038/nature07733

[30] Skvortsov S, Debbage P, Skvortsova I. Proteomics of cancer stem cells. Int J RadiatBiol 2014; 90:653-8 http://dx.doi.org/10.3109/09553002.2013.873559

[31] Skvortsov S, Debbage P, Cho WC, Lukas P, Skvortsova I. Putative biomarkers and therapeutic targets associated with radiation resistance. Expert RevProteomics 2014; 11:207-14. http://dx.doi.org/10.1586/14789450.2014.893194

[32] Dittmann K, Mayer C, Fehrenbacher B, Schaller M, Raju U, Milas L *et al*. Radiation-induced epidermal growth factor receptor nuclear import is linked to activation of DNA-dependent protein kinase. J BiolChem 2005; 280:31182-9. http://dx.doi.org/10.1074/jbc.m506591200

[33] Dittmann K, Mayer C, Rodemann HP. Inhibition of radiation-induced EGFR nuclear import by C225 (Cetuximab) suppresses DNA-PK activity. RadiotherOncol 2005; 76:157-61. http://dx.doi.org/10.1016/j.radonc.2005.06.022

[34] Yan M, Yang X, Wang L, Clark D, Zuo H, YeD *et al*. Plasma membrane proteomics of tumor spheres identify CD166 as a novel marker for cancer stem-like cells in head and neck squamous cell carcinoma. Mol Cell Proteomics 2013; 12:3271-84. http://dx.doi.org/10.1074/mcp.M112.025460

[35] Arnold CR, Abdelmoez A, Thurner G, Debbage P, Lukas P, Skvortsov S *et al*. Rac1 as a multifunctional therapeutic target to prevent and combat cancer metastasis. Oncoscience 2014; 1:513-21.

[36] Principe S, Hui AB, Bruce J, Sinha A, Liu FF, Kislinger T.

Tumor-derived exosomes and microvesicles in head and neck cancer: implications for tumor biology and biomarker discovery. Proteomics 2013; 13:1608-23. http://dx.doi.org/10.1002/pmic.201200533

[37] Mrizak D, Martin N, Barjon C, Jimenez-Pailhes AS, Mustapha R, Niki T *et al*. Effect of nasopharyngeal carcinoma-derived exosomes on human regulatory T cells. J Natl Cancer Inst 2015; 107:363. http://dx.doi.org/10.1093/jnci/dju363

[38] Aga M, Bentz GL, Raffa S, Torrisi MR, Kondo S, Wakisaka N *et al*. ExosomalHIF1alpha supports invasive potential of nasopharyngeal carcinoma-associated LMP1-positive exosomes. Oncogene 2014; 33:4613-22. http://dx.doi.org/10.1038/onc.2014.66

[39] Skvortsova I, Skvortsov S, Raju U, Stasyk T, Riesterer O, Schottdorf EM *et al*. Epithelial-to-mesenchymal transition and c-myc expression are the determinants of cetuximab-induced enhancement of squamous cell carcinoma radioresponse. RadiotherOncol 2010; 96:108-15. http://dx.doi.org/10.1016/j.radonc.2010.04.017

[40] Masood R, Hochstim C, Cervenka B, Zu S, Baniwal SK, Patel V *et al*. A novel orthotopic mouse model of head and neck cancer and lymph node metastasis.Oncogenesis 2013; 2:e68. http://dx.doi.org/10.1038/oncsis.2013.33

[41] Huang D, Li Y, Liu N, ZuS, Baniwal SK, Patel V *et al*. Identification of novel signaling components in N,N'-dinitrosopiperazine-mediated metastasis of nasopharyngeal carcinoma by quantitative phosphoproteomics. BMC Cancer 2014; 14:243. http://dx.doi.org/10.1186/1471-2407-14-243

[42] Britt DE, Yang DF, Yang DQ, Flanagan D, Callanan H, Lim YP *et al*. Identification of a novel protein, LYRIC, localized to tight junctions of polarized epithelial cells. Exp Cell Res 2004; 300:134-48. http://dx.doi.org/10.1016/j.yexcr.2004.06.026

[43] Martin TA, Jiang WG. Tight junctions and their role in cancer metastasis.HistolHistopathol 2001; 16:1183-95.

[44] Ieni A, Barresi V, Branca G, Licata L, Caruso RA, Tuccari G. Changes in human epidermal growth factor receptor 2 status between primary breast/gastric carcinomas and synchronous metastatic lymph nodes: how can we explain them?. J Cancer Metastasis Treat . 2015; 1: 21-6.

[45] Zhu G, Cai G, Liu Y, Tan H, Yu C, Huang M *et al*. Quantitative iTRAQ LC-MS/MS Proteomics Reveals Transcription Factor Crosstalk and Regulatory Networks in Hypopharyngeal Squamous Cell Carcinoma. J Cancer 2014; 5:525-36. http://dx.doi.org/10.7150/jca.9207

[46] Lee LY, Chen YJ, Lu YC, Liao CT, Chen IH, Chang JT *et al*. Fascin is a circulating tumor marker for head and neck cancer as determined by a proteomic analysis of interstitial fluid from the tumor microenvironment. ClinChem Lab Med 2015. http://dx.doi.org/10.1515/cclm-2014-1016

[47] Papaspyrou K, Brochhausen C, Schmidtmann I, Fruth K, Gouveris H, Kirckpatrick J *et al*. Fascinupregulation in primary head and neck squamous cell carcinoma is associated with lymphatic metastasis. Oncol Lett 2014; 7:2041-6.

[48] Ghebeh H, Al-Khaldi S, Olabi S, Al-Dhfyan A, Al-Mohanna F, Barnawi R *et al*. Fascin is involved in the chemotherapeutic resistance of breast cancer cells predominantly via the PI3K/Akt pathway. Br J Cancer 2014; 111:1552-61. http://dx.doi.org/10.1038/bjc.2014.453

[49] Jarai T, Maasz G, Burian A, Bona A, Jambor E, Gerlinger I *et al*. Mass spectrometry-based salivary proteomics for the discovery of head and neck squamous cell carcinoma. PatholOncol Res 2012; 18:623-8. http://dx.doi.org/10.1007/s12253-011-9486-4

[50] Jou YJ, Lin CD, Lai CH, Chen CH, Kao JY, Chen SY *et al*. Proteomic identification of salivary transferrin as a biomarker for early detection of oral cancer. Anal ChimActa 2010; 681:41-8. http://dx.doi.org/10.1016/j.aca.2010.09.030

[51] Vidotto A, Henrique T, Raposo LS, Maniglia JV, Tajara EH. Salivary and serum proteomics in head and neck carcinomas: before and after surgery and radiotherapy. Cancer Biomark 2010; 8:95-107.

[52] Franzmann EJ, Reategui EP, Pedroso F, Pernas FG, Karakullukcu BM, Carraway KL *et al*. Soluble CD44 is a potential marker for the early detection of head and neck cancer. Cancer Epidemiol Biomarkers Prev 2007; 16:1348-55. http://dx.doi.org/10.1158/1055-9965.EPI-06-0011

[53] Hu S, Arellano M, Boontheung P, Wang J, Zhou H, Jiang J *et al*. Salivary proteomics for oral cancer biomarker discovery. Clin Cancer Res 2008; 14:6246-52. http://dx.doi.org/10.1158/1078-0432.CCR-07-5037

[54] Tavassoli M, Brunel N, Maher R, Johnson NW, Soussi T. p53 antibodies in the saliva of patients with squamous cell carcinoma of the oral cavity. Int J Cancer 1998; 78:390-1. http://dx.doi.org/10.1002/(SICI)1097-0215(19981029)78:3<390::AID-IJC23>3.0.CO;2-9

[55] Bahar G, Feinmesser R, Shpitzer T, Popovtzer A, Nagler RM. Salivary analysis in oral cancer patients: DNA and protein oxidation, reactive nitrogen species, and antioxidant profile. Cancer 2007; 109:54-9. http://dx.doi.org/10.1002/cncr.22386

[56] Shah FD, Begum R, Vajaria BN, Patel KR, Patel JB, Shukla SN *et al*. A review on salivary genomics and proteomics biomarkers in oral cancer. Indian J ClinBiochem 2011; 26:326-34. http://dx.doi.org/10.1007/s12291-011-0149-8

[57] Chung CH, Seeley EH, Roder H, Grigorieva J, Tsypin M, Roder J *et al*. Detection of tumor epidermal growth factor receptor pathway dependence by serum mass spectrometry in cancer patients. Cancer Epidemiol Biomarkers Prev 2010; 19:358-65. http://dx.doi.org/10.1158/1055-9965.EPI-09-0937

[58] Maher SG, McDowell DT, Collins BC, Muldoon C, Gallagher WM, Reynolds JV. Serum proteomic profiling reveals that pretreatment complement protein levels are predictive of esophageal cancer patient response to neoadjuvantchemoradiation. Ann Surg 2011; 254:809-16. http://dx.doi.org/10.1097/SLA.0b013e31823699f2

[59] Widlak P, Pietrowska M, Polanska J, Rutkowski T, Jelonek K, Kalinowska-Herok M *et al*. Radiotherapy-related changes in serum proteome patterns of head and neck cancer patients; the effect of low and medium doses of radiation delivered to large volumes of normal tissue. J Transl Med 2013; 11:299. http://dx.doi.org/10.1186/1479-5876-11-299

[60] Wikner J, Grobe A, Pantel K, Riethdorf S. Squamous cell carcinoma of the oral cavity and circulating tumour cells. World J ClinOncol 2014; 5:114-24. http://dx.doi.org/10.5306/wjco.v5.i2.114

[61] Chan CM, Au TC, Chan AT, Ma BB, Tsui NB, Ng SS *et al*. Advanced technologies for studying circulating tumor cells at the protein level. Expert Rev Proteomics 2013; 10:579-89. http://dx.doi.org/10.1586/14789450.2013.858021

[62] Zhou G, Li H, DeCamp D, Chen S, Shu H, Gong Y, *et al*.2D differential in-gel electrophoresis for the identification of esophageal scans cell cancer-specific protein markers. Mol Cell Proteomics 2002; 1:117-24. http://dx.doi.org/10.1074/mcp.M100015-MCP200

[63] Cheng AL, Huang WG, Chen ZC, Peng F, Zhang PF, Li MY, *et al*. Identification of novel nasopharyngeal carcinoma biomarkers by laser capture microdissection and proteomic analysis. Clin Cancer Res 2008; 14:435-45. http://dx.doi.org/10.1158/1078-0432.CCR-07-1215

[64] Koike H, Uzawa K, Nakashima D, Shimada K, Kato Y, Higo M, *et al*. Identification of differentially expressed proteins in oral squamous cell carcinoma using a global proteomic approach. Int J Oncol 2005; 27:59-67. http://dx.doi.org/10.3892/ijo.27.1.59

[65] Kouzu Y, Uzawa K, Koike H, Saito K, Nakashima D, Higo M, *et al*. Overexpression of stathmin in oral squamous-cell carcinoma: correlation with tumour progression and poor prognosis. Br J Cancer 2006; 94:717-23. http://dx.doi.org/10.1038/sj.bjc.6602991

[66] Sewell DA, Yuan CX, Robertson E. Proteomic signatures in laryngeal squamous cell carcinoma. ORL J OtorhinolaryngolRelat Spec 2007; 69:77-84. http://dx.doi.org/10.1159/000097406

[67] Ralhan R, Desouza LV, Matta A, Chandra TS, Ghanny S, Dattagupta S, *et al*.iTRAQ-multidimensional liquid chromatography and tandem mass spectrometry-based identification of potential biomarkers of oral epithelial dysplasia and novel networks between inflammation and premalignancy. J Proteome Res 2009; 8:300-9. http://dx.doi.org/10.1021/pr800501j

[68] Cheng AJ, Chen LC, Chien KY, Chen YJ, Chang JT, Wang HM, *et al*.Oral cancer plasma tumor marker identified with bead-based affinity-fractionated proteomic technology. ClinChem 2005; 51:2236-44. http://dx.doi.org/10.1373/clinchem.2005.052324

[69] Gomes CP, Freire MS, Pires BR, Vasconcelos EA, Rocha TL, Grossi-de-Sa MF, *et al*. Comparative proteomical and metalloproteomical analyses of human plasma from patients with laryngeal cancer. Cancer ImmunolImmunother 2010; 59:173-81. http://dx.doi.org/10.1007/s00262-009-0741-4

[70] Liao Q, Zhao L, Chen X, Deng Y, Ding Y. Serum proteome analysis for profiling protein markers associated with carcinogenesis and lymph node metastasis in nasopharyngeal carcinoma.ClinExp Metastasis 2008; 25:465-76. http://dx.doi.org/10.1007/s10585-008-9152-8

Received on 21-05-2015 Accepted on 17-06-2015 Published on 01-07-2015

http://dx.doi.org/10.15379/2410-3802.2015.02.2