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New advances in molecular approaches to head and neck squamous cell carcinoma

Nivedita Sahu, BA and **Jennifer R. Grandis, MD**
Departments of Otolaryngology and Pharmacology & Chemical Biology, University of Pittsburgh School of Medicine and University of Pittsburgh Cancer Institute

Abstract

Head and neck squamous cell cancer (HNSCC) is the sixth most common cancer in the world. Despite advances in combined modality therapy, poor outcomes continue to be observed in the form of locoregional recurrence, metastasis, and development of second primary tumors. Because tumors vary in their molecular and genetic etiology and because often times there is already deregulation at the molecular level in otherwise histopathologically normal tissue, risk stratification using clinical and pathologic criteria alone has proved to be inadequate. In this article, the reader will gain an appreciation for the current advances in biomarker discovery via advanced technology and data interpretation in microarray analysis and proteomics. In addition, other molecular targets, aside from EGFR, are discussed in the context of their promising role in predicting recurrence, response to therapy, survival and overall prognosis.

Keywords

Head and neck squamous cell carcinoma (HNSCC); biomarker; microarray; proteomics; epidermal growth factor receptor (EGFR)

Introduction

Head and neck squamous cell cancer (HNSCC) is the sixth most common cancer in the world with over 560,000 new cases diagnosed annually and over 300,000 deaths per year[1]. Surgery, chemotherapy, and radiation remain the mainstay of patient care. Despite advances in combined modality therapy, poor outcomes continue to be observed in the form of locoregional recurrence, metastasis, and development of second primary tumors, with the 5 year overall survival rate at less than 50%. As the molecular pathways involved in the development and progression of HNSCC become elucidated, it is clear that HNSCC is a heterogeneous group of cancers, stemming from varying molecular and genetic alterations. The diagnosis and prognosis of HNSCC is largely based on clinicohistopathology classification, mainly tumor, node, metastasis (TNM) staging via clinical and radiographic assessment, surgical margin status, and histologic morphology and differentiation. Because tumors vary in their molecular and genetic etiology and because often times there is already deregulation at the molecular level in otherwise histopathologically normal tissue, risk stratification using clinical and pathologic criteria alone has proved to be inadequate. The emerging role of molecular biomarkers in detection, diagnosis, response to treatment and prognosis has the potential for more accurate tumor staging and to facilitate management of HNSCC subtypes.

Correspondence should be addressed to: Jennifer Rubin Grandis, M.D., F.A.C.S., The Eye and Ear Institute, Suite 500, 200 Lothrop Street, Pittsburgh, PA 15213. Phone: 412-647-5280; FAX: 412-647-0108, jgrandis@pitt.edu.

A biomarker is any objectively measured characteristic that may provide an indication of an outcome in question (i.e. response to treatment, metastasis, survival, etc.) [2]. Human papilloma virus (HPV) and epidermal growth factor receptor (EGFR) are currently the two most well studied and frequently used biomarkers in HNSCC. Several other potential targets have also been studied and show promise in predicting disease course. In addition, advanced technology and data interpretation in microarray analysis and proteomics have led to the identification of molecular genetic signatures with biomarker potential in predicting recurrence, metastasis, response to therapy, and survival.

Microarray Analysis in HNSCC Prognosis

Gene expression profiling of primary tumors via microarray technology has played a significant role in enhancing our understanding of HNSCC molecular pathogenesis. Microarray analysis is useful in distinguishing gene expression differences among normal and malignant tissue and many individual genes involved in the oncogenic process have been discovered and studied via this method. Microarrays have been utilized in both the study of known genes and the discovery of new genes. However, what was once a screen for molecular markers of disease by identifying differentially expressed genes between normal and malignant tissue, microarray analysis has more recently evolved into the characterization of gene sets that identify subgroups of HNSCC. These unique molecular signatures are currently being studied as indicators and predictors of disease and disease progression, response to therapy, and survival (Table 1).

Microarray chips permit the study of tens of thousands of genes at once, making this an efficient strategy to characterize HNSCC molecular signatures. Gene based analysis is one method, which can be supervised or unsupervised. Supervised gene-based studies use microarray analysis of predetermined genes of interest to determine differences in expression between two groups. Unsupervised analysis is used to screen for differentially expressed genes between two groups from a large pool of cDNAs. Gene-based analysis has been used to assess differences in expression of individual genes whereas pathway-based analysis, in which functionally related sets of genes are studied, allows examination of differential gene expression in their biological context. Identifying any single common pathway from generated gene lists is challenging since selected genes are usually involved in multiple cellular processes. Because generated gene lists do not explicitly expose any one molecular pathway, Gene Set Enrichment Analysis (GSEA) is used to interrogate expression data to determine the predominant biological processes at work.

One study used microarray analysis to generate a 75-gene list and then applied GSEA to identify enriched gene sets involved in molecular pathways that differed between high-and low-risk groups. High-risk tumors were those that had statistically significant decreased recurrence free survival. The genes sets involved in epithelial to mesenchymal transition (EMT), nuclear factor-kappaB (NF-kB) signaling activation, and cell adhesion were found to be most significant in the high-risk group. EMT and NF-kB gene sets, specifically, were highly predictive of recurrence free survival, suggesting that they could be used as a reliable prognostic biomarker of disease recurrence in HNSCC. Of note, gene sets prominent in the low-risk group were involved in mRNA splicing, cell cycle regulation, and mRNA processing [3].

Another group using a reference dataset in addition to their own dataset of 11 metastasized and 11 non-metastasized HNSCC tumors, validated gene sets that are associated with the propensity for tumors to metastasize. Supervised pathway-based analysis showed differential expression of genes involved in the extracellular matrix remodeling and the hypoxia induced angiogenesis and invasion pathways between the two groups [4]. This

study also highlighted that results from different microarray studies can be more readily compared if a supervised, pathway-based analysis is used. Results from gene expression studies using different microarray platforms can still be compared with this method since it draws on the biological functionality of gene groups, rather than solely individual genes themselves.

More recently, investigators used a novel method in which they applied spectral clustering (SC) and gene ontology (GO) via the Database for Annotation, Visualization and Integrated Discovery (DAVID) to microarray data in order to identify gene sets prognostic of HNSCC survival. SC analysis of a microarray data set generates multiple vectors that embody the different dimensions within a given set and highlights heterogeneous gene expression aspects among samples. Each vector is the result of simultaneous reordering of the gene and sample data matrix in which the most important genes are found at the top and bottom of the vector gene list; and each vector pair (gene + sample) represents different existing elements of variance. SC is an unsupervised analysis of microarray data that avoids bias inherent in supervised pathway based analysis. Further, applying GO to the SC generated vector gene lists identifies enriched gene sets that are representative of major biologic pathways. Thus, the combined approach of applying SC and GO to microarray data allows for an unbiased approach to elucidating important pathways implicated in HNSCC.

Thurlow et al. performed microarray analysis on 14 normal epithelium and 71 HNSCC samples from patients whose outcomes were known. Two important vectors that encompassed the most variance among the dataset were identified via spectral analysis, V2 and V3. V2 separated normal from HNSCC samples, while V3 underscored the heterogenic nature of the HNSCC group. GO applied to SC generated vectors showed that the gene lists were primarily comprised of genes involved in focal adhesion and extracellular matrix remodeling (FA) as well as cytokine-cytokine receptor (CR) pathways. GO applied to V2 and V3 specifically found that V2CR genes were related to innate immune response whereas V3CR genes were involved in the adaptive response. Cox survival analysis used to determine the prognostic significance of these gene sets found the FA and CR gene sets to be significantly prognostic of patient survival in HNSCC. The FA gene set predicted poor outcome, whereas the adaptive immune response CR gene set (V3CR) predicted good outcome, which was not dependent on HPV status. Interestingly, the adaptive immune response gene set was a better prognostic indicator of favorable outcome in the oropharyngeal samples of this cohort compared to HPV markers. Aside from FA and CR gene sets, the relationship between the adaptive and innate immune response in determining outcome seems to be important in that a high ratio of V3CR/V2CR expression was found to be independently prognostic of survival. These findings were also confirmed in an independent data set [5]. Also of interest, the FA and CR prognostic gene sets outperformed current clinical and histopathological parameters in predicting survival outcome [6].

Other investigators used gene sets to predict response to chemoradiation (CRT) and radiation therapy (RT). They identified 167 probe sets that differed in gene expression between complete responders and non-responders to CRT or RT in 14 HNSCC patients undergoing therapy with curative intent. 142 of these probe sets, when further subjected to six different prediction algorithms, showed 93%–100% prediction accuracy of response to therapy. Genes involved in cell adhesion and motility, regulation of transcription, cell proliferation, and apoptosis were found to be differentially expressed in complete and non-responders to therapy. Of note, the investigators found the ankyrin repeat domain 17 gene (ANKRD17), which plays a role in DNA mismatch repair pathways, to be up-regulated in non-responder tumor samples. These results show potential in the use of microarray analysis and genetic expression profiling as markers of prognosis of response to chemoradiation and radiation therapy [7].

Together these studies demonstrate the emerging role of gene expression profiling in the development of unique HNSCC signatures to use as biomarkers in the predictors of response to therapy as well as in the prognostication of disease free survival and overall survival. Genes involved in alterations of cell-cell adhesion, cell motility, extracellular remodeling and epithelial-to-mesenchymal transition (EMT) highlight the importance of microenvironment changes in prognosis as they contribute to tumor recurrence, metastasis, and thus poor response to therapy and overall survival. Pathways involved in EMT, such as loss of cell-cell adhesion and cell motility, are the same as those central to the metastatic process. Interestingly, NF-kB is one of the molecules involved in EMT related cellular occurrences, demonstrating the concerted orchestration in the development of aggressive cancers. In addition, the immune responses as well as the hypoxia-angiogenesis pathways are crucial in their effects on tumor growth, recurrence, and metastasis.

Genetic microarray analysis has the potential to study the expression of tens of thousands of genes at once in a cell or tissue sample. Its use in the clinical setting has already emerged in breast cancer diagnostics and management. The Oncotype DX test analyzes the expression pattern of 21 different genes specific to breast cancer to calculate a Recurrence Score, which characterizes the tumor as low, intermediate, or high risk. This score is used to estimate the risk of recurrence within 10 years from initial diagnosis and to tailor individual treatment accordingly. In HNSCC, validated correlation to known outcomes may allow the identification of definitive genetic profiles, that can be used in a similar manner as the Oncotype DX test, to both accurately diagnose and predict the prognosis and response to therapy of new malignancies.

Microarray analysis evaluates differences in gene expression, mainly mRNA transcription. However, the functional outcome of gene expression, in the form of the post-translationally modified protein, is not distinctly evaluated. The number of mRNA transcripts does not always correlate with protein expression and quantity. In addition microarray analysis must become standardized so that studies can be compared and large datasets can be generated and validated. While this technology remains promising, gene patterns and molecular signatures need to be further studied and validated in larger cohorts in order to be applied in clinical practice.

Serum and Tissue Proteomics

Proteomics accounts for post-transcriptional and translational modifications and may be more accurate in reflecting the functional biological state of tumors, and thus perhaps a better biomarker discovery tool, than genomic analysis alone. A few methods such as(i)two-dimensional differential in gel electrophoresis (2-D DIGE), which is limited by its poor resolution of low molecular weight proteins; (ii) surface enhanced laser desorption/ ionization time-of-flight mass spectrometry (SELDI-TOF-MS), which is based on ionization characteristics and mass-to-charge ratio; and (iii) matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS), which is based on molar quantity, have been used alone or in combination to date. Proteomic analysis by SELDI-TOF-MS has been shown to differentiate, with high sensitivity and specificity, both HNSCC tumors from normal tissue as well as pre-and post-treated HNSCC [8], [9], [10], [11]. Research in the use of proteomics in both detection, for diagnosis and screening purposes, and post-treatment surveillance has recently been of great interest (Table 2).

In one prospective study, serum samples collected from 143 HNSCC patients were analyzed by 2-D DIGE in combination with MALDI-TOF-MS and correlated with disease status. Recurrent HNSCC was significantly associated with underexpression of kininogen and serine protease inhibitors C-1 inhibitor, serine/cysteine proteinase inhibitor clade G member

1, and kininogen and angiotensinogen; and overexpression of thiol-specific antioxidant proteins (TSA), apolipoprotein A1, proapolipoprotein, and epidermal cytokeratin 2. These protein expression differences in protease inhibitors, kininogen, TSA, and apolipoproteins are prospective markers of recurrence that have the potential to identify those tumors that are more aggressive and also have the potential to be used in disease surveillance [12].

In another study, 2D-DIGE analysis was used to examine protein expression patterns in HNSCC and matched normal tissue. Proteomic characterization revealed significant differences in 17 proteins, four of which have not previously been studied. Among the novel proteins, glutathione synthetase was found to be up-regulated whereas cornulin, guanylate binding protein 6, and heat shock 70 kDa protein 5 were down-regulated. Cornulin was the highest ranked differentially expressed protein and loss of expression was validated in an independent cohort. Cornulin, otherwise known as squamous epithelial heat shock protein 53 and member of the calcium binding S-100 family, is involved in the stress response of squamous epithelia. Reduced expression may make squamous epithelia more vulnerable to repeated chemical and infectious offenses that eventually lead to carcinogenesis. Other notable differences in protein expression included the up-regulated keratin-17, which has been associated with metastasis [13]; and proliferating cell nuclear antigen, which has been associated with poor prognosis in other studies [14]. Other markers previously reported and confirmed to be down-regulated in HNSCC compared to normal tissue in this study included cytokeratins 4, 8, and 13, keratin-4, annexin A1, transglutaminase 3, cystatin-B, and leukocyte elastase inhibitor (Serpin B1) [15]. Using 2D-DIGE, the authors were able to identify abnormal under-and overexpression of structural and cell proliferation proteins and those involved in the oxidative stress responses in HNSCC.

Proteomic analysis of normal epithelia and well, moderately, and poorly differentiated HNSCC has revealed significant differences at each stage that may provide insight into the molecular pathology of HNSCC progression. In one study, the relative expression of proteins in expected cellular functions and pathways, such as structural, signal transduction and cell cycle regulation, and extracellular matrix degradation and migration, had a unique pattern with histopathological correlation. Vimentin, which is involved in EMT, was the most unique to HNSCC samples. The well differentiated (WD) tumors differentially expressed cytoskeletal and Notch pathway proteins, such as Delta 4 and Delta 1; while the moderately differentiated (MD) groups expressed placental cadherin and protocadherin γ A6. In contrast, poorly differentiated (PD) tumors expressed AF1q, a potential oncoprotein, as well as other proteins involved in fatty acid metabolism and membrane trafficking. The stem cell protein PIWIL3 was found in only normal and WD tumors; oncoprotein DJ-1 in both WD and MD; and JWA, a protein involved in cell migration, and signal transducer and activator of trancription 3 (STAT3) along with its activator, Janus-activated kinase 2, were common to MD and PD tumors.

Prohibitin, which plays a role in Ras activation of the Raf/MEK/ERK pathway [16], cell adhesion, and cell migration, andEVI-5 were found only in tumor samples. Prohibitin and EVI-5 likely contribute to deregulated cell proliferation in HNSCC and are candidates for further studies as to their use of biomarker potential. Of note, the prominent protease in tumor samples was cathepsin D, which has been studied in breast cancer where its expression levels correlate with metastasis and poor survival [17]. HSP27, HSP70, glutathione S-transferase, and integrin β_4 were other proteins which were overexpressed in malignancies compared to normal controls [18].

Evaluation of histologically normal mucosa in HNSCC may be able to predict the development of second primary tumors and local recurrence. A prospective pilot study used SELDI TOF MS analysis protein profiling of biopsies from five sites between the

oropharynx and esophagous in 24 HNSCC patients. The highest ranking proteins, differentiating normal from HNSCC mucosa included Cystatin A, followed by peaks with m/z10464 and m/z12337 (both unidentified), S100-A8, and by two other unidentified peaks with m/zvalues of 12910 and 3640. Together, these six protein peaks were able to identify those mucosal sites more prone to carcinoma development at the molecular level, even if they appeared to be histologically benign. The authors were able to predict tumor recurrence and progression-free survival using these protein profiles. This study suggests that proteomic analysis of additional biopsies taken from other regional sites at the time of initial tumor workup could allow for individualized HNSCC prognostication in the future [19].

Another study used multidimensional liquid chromatography and tandem mass spectrometry to analyze isobaric mass tag labeled HNSCC and control samples to detect differences in protein expression. Structural proteins, in addition to signaling proteins and transcription factors, were found to be among those consistently differentially expressed in HNSCC. Overexpressed YWHAZ, stratifin, and S100-A7 were the most important in discrimination and when used in combination, had a sensitivity of 92% and a specificity of 91% in distinguishing HNSCC from normal tissue. This finding was validated in an independent cohort where sensitivity = 92% and a specificity = 87%. These three proteins merit further investigation into their usefulness as biomarkers for screening and diagnosis in HNSCC [20].

These cumulative findings suggest that proteins involved in signal transduction and proliferation as well as oxidative stress responses are important in the development of HNSCC. Additionally, deregulation in proteins involved cell structure, adhesion, migration, and extracellular matrix remodeling are associated with increased recurrence, metastasis, and poor survival. Identification of differential protein patterns, similar to genomic signatures, has the potential to stratify patients according to the aggressiveness of their disease.

Molecular Targets: EGFR and Beyond

Epidermal growth factor receptor (EGFR) has been studied extensively as a therapeutic target as well as a prognostic biomarker in HNSCC. EGFR is a trans-membrane tyrosine kinase receptor that is overexpressed, early in HNSCC carcinogenesis [21]. EGFR ligand binding causes homo-or heterodimerization, with other members of the human epidermal growth factor receptor family includingErbB2, resulting in autophosphorylation and activation of multiple downstream signaling pathways; most notably the PI3K/Akt, Ras/Raf/MEK/ERK1/2, and STAT pathways. Aberrations in EGFR and these signaling pathways lead to uncontrolled cell proliferation, contributing to cancer development and progression.

More than 90% of all HNSCC overexpress EGFR where increased levels are associated with poor prognosis in the form of high locoregional failure and decreased survival [22], [23], [24]. In 2002, Ang et al. found that the overall survival and disease free survival were significantly lower and that locoregional recurrence was significantly higher in patients that harbored tumors with high EGFR expression when compared to those with low EGFR expression. Interestingly however, EGFR levels did not correlate with distant metastasis [23]. EGFR gene amplification, as detected via FISH, is one potential mechanism of overexpression where increased EGFR copy numbers has been associated with worse progression free and overall survival [25].

Because of its central role in HNSCC carcinogenesis, several methods of targeted therapy towards EGFR have been developed. Broadly, these therapies include monoclonal antibodies, tyrosine kinase inhibitors, antisense oligonucleotides, and small interfering RNAs that respectively aim at attacking EGFR at both its extracellular and intracellular

domain as well as at the translational stage. At this time, cetuximab, an EGFR monoclonal antibody, is the only approved EGFR targeting drug for use. Cetuximab in conjunction with RT has been shown to be effective in locoregionally advanced HNSCC [26]. Cetuximab also showed a survival benefit in the EXTREME study when used as a first line agent in combination with platinum-based CT in recurrent and metastatic HNSCC [27].

Despite repeated evidence that EGFR overexpression is a poor prognostic factor in HNSCC, neither the levels of EGFR or EGFR gene amplification have been consistently associated with clinical response to EGFR targeting. The only exceptions were a phase III cetuximab plus platinum trial where patients with low EGFR expression had a marginally better response to cetuximab [28]and a phase I EGFR antisense gene therapy study where patients whose tumors expressed higher levels of EGFR were most likely to respond to treatment [29].

More recently, the application of EGFR expression status to predict the occurrence of a second primary lesion has been examined. A study analyzing EGFR expression in primary laryngeal HNSCC, found there to be a significant positive correlation between EGFR expression and the development of subsequent primary tumors [30]. The phosphorylation and mutational status of EGFR in HNSCC prognosis was also recently investigated in a prospective study. EGFR sequencing as well as Western analysis to determine EGFR expression and hyper-phosphorylation was performed on malignant tissue from 82 consecutive patients that had not previously been treated with EGFR targeted therapy. EGFR hyper-phosphorylation was found to be associated with increased number of lymph node metastases and higher nodal stage of disease. Even after accounting for overexpression, tumors with hyper-phosphorylated EGFR relapsed significantly earlier compared to those without [31]. To date, EGFR phosphorylation status has not been well studied as a marker of response to EGFR targeted therapy. While EGFR expression appears to be a consistent marker of poor prognosis, its utility in predicting response to of EGFR targeted therapies remains incompletely understood.

Other Potential Molecular Targets

In addition to EGFR, signal transducers and activators of transcription (STAT) proteins play a major role in the signaling pathways involved in HNSCC, among other carcinomas. STAT3, in particular, is overexpressed or constitutively activated in many HNSCCs [32]. Tyrosine phosphorylation of STAT3 in the cytoplasm results in homo-or heterodimer formation followed by translocation to the nucleus where STAT3 dimers activate anti-apoptotic and pro-proliferative genes. Previous studies have reported that pSTAT3 expression in the primary HNSCC tumor is associated with reduced survival, alone or in the context of expression of coordinate signaling pathways [33], [34]. A recent study showed that nuclear localization of STAT3 in HNSCC tumors was associated with a favorable prognosis. High expression of STAT3 in the nucleus versus the cytoplasm was found to be associated with longer progression free survival and lower risk of death in two independent cohorts [35]. STAT3 has also been studied in the context of molecular targeted therapy. Both *in vitro* and *in vivo* studies demonstrate the anti-tumor effects of STAT3 targeting using oligonucleotide decoys[36], [37], which bind to STAT3 thereby preventing its binding to promoter sequences and activation of pro-proliferative genes, in the treatment of HNSCC. However, the main obstacle in the development of STAT3 decoy remains the limited ability to withstand serum endo-and exonucleases, which currently prevent systemic administration. Nevertheless, STAT3 shows great potential in its use as both a biomarker for disease stratification as well as a molecule for targeted therapy development.

Hypermethylation of E-cadherin may also be an independent marker of improved HNSCC survival. Promoter hypermethylation of cadherin type 1 gene (CDH1), which encodes E-cadherin, inhibits E-cadherin expression. Although loss of E-cadherin has been associated with aggressive HNSCC disease, hypermethylation of CDH1 has significantly better overall survival when compared to tumors without hypermethylation; even after controlling for HPV status, age, sex, and stage [38].

Several other groups have studied the role of a variety of molecules associated with carcinogenesis in predicting recurrence, decreased response to therapy, and poor overall survival and prognosis in HNSCC (Table 3). One relatively large (N = 444) longitudinal, prospective cohort study, which controlled for age, sex, smoking, stage, site of origin, and comorbidities, found increased pretreatment serum interleukin-6 (IL-6) levels to be an independent predictor of both recurrence and poor survival. Thus, pretreatment IL-6 levels could be a biomarker for earlier detection of relapse [39]. Serum cytokine and angiogenic factor (CAF) profiling also seems to be useful in determining recurrence. In a phase II induction therapy trial of carboplatin, paclitaxel, and cetuximab, baseline and post-treatment levels of several tumor hypoxia regulated CAFs were related to patient outcome. Eight CAFs in particular were categorized as “highrisk”. These included VEGF, IL-4, IL-8, osteopontin, growth-related oncogene-α, eotaxin, granulocyte colony stimulating factor, and stromal cell derived factor-1α. An increase in six or more of these high risk CAFs was 73% specific and 100% sensitive for predicting disease progression and strongly associated to shorter time to relapse [40]. In a multivariate analysis examining 140 locally advanced HNSCCs which were surgically resected and radiated postoperatively, Pattje et al. found phosphatase and tensin homologue deleted on chromosome 10 (PTEN) to be an independent predictor of worse locoregional control after accounting for lymph node metastases and extranodal spread. *In vitro* studies by the same group also found that increased PTEN expression was related to radiation resistance [41]. Others compared the expression of HLA class I antigen, transporter associated with antigen processing (TAP), and tapasin in primary and matched metastatic HNSSC lesions. These proteins are involved in antigen presentation and eliciting the cytotoxic T cell immune response. Although down regulation occurred across this panel of proteins in both groups, the decrease was more significant in metastatic tissue. Decreased HLA class I antigen levels in metastatic samples were found to be independent markers of reduced progression free survival and poor prognosis [42]. SERPINB13, which belongs to the serpin family of intracellular serine protease inhibitors, was examined in a cohort of 99 HNSCCs. It was found that SERPINB13 was either partially or completely downregulated in 75% of samples compared to normal epithelial tissue; confirming previous findings of mRNA and protein downregulation of this molecule. When correlated to outcome, the authors found that low protein levels of SERPINB13 were associated with poor tumor differentiation, lymph node metastasis, and decreased disease free and overall survival. It appears that downregulation of SERPINB13 may be yet another biomarker for reduced survival in HNSCC [43]. The role of ANO1, a calcium-activated chloride channel (CaCC) has also been investigated in HNSCC. The investigators found that ANO1 gene amplification and overexpression are involved in cellular processes that increase metastatic potential; making ANO1 a novel target to prevent metastasis and improve patient survival in HNSCC[44].

Taken together, these studies are just a small sampling of the various molecules currently under investigation in their roles as biomarkers. They all share common features that make them attractive candidates of pursuit. Whether the above mentioned molecules are overexpressed, underexpressed, or vary in their localization, it appears that differences exist in these parameters when HNSCC is compared to normal mucosa. Furthermore, these differences have been analyzed and statistically correlated to available clinical outcome data in order to elucidate significant relationships, which suggest a potential for each studied

molecule to be a biomarker of some sort (i.e. recurrence, radiation therapy resistance, survival) in HNSCC. Also, a number of these molecules have been studied in preclinical models (i.e. STAT3) in which targeting is associated with antitumor effects; showing promise in the development of future HNSCC targeted therapies.

Conclusion

HNSCC is a heterogeneous disease despite the presence of common histologic features. Most patients present with advanced disease, thus the disease free survival and overall survival remain suboptimal. The overall 5-year survival rate has not changed significantly in the last twenty years, despite continued advances in the field. It is important to develop reliable methods of elucidating the specific features that are critical to tumor progression for each patient to achieve the promise of personalized medicine. EGFR overexpression is a poor prognostic marker but to date, has not consistently predicted response to EGFR targeted therapy. HPV has emerged as a distinct biological entity that portends favorable prognosis and response to therapy, regardless of the specific therapeutic regimen. Gene discovery efforts including microarray analyses and advances in bioinformatics has allowed for recognition of differential gene expression. Elucidation of the different molecular signatures of HNSCC will help to unravel the distinct underlying mechanisms in this heterogeneous group of cancers as well as provide a source of novel molecules for future research and the development of targeted therapies. In addition to microarrays, newer proteomic approaches are also under investigation. Ideally, the development of clinically useful methods to assess HNSCC tumors can be identified to allow individual HNSCC patients to receive the therapy most likely to be effective in their individual tumor.

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References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005; 55:74–108. [PubMed: 15761078]
2. Atkinson A, Colburn W, DeGruttola V, DeMets D, Downing G, Hoth D, et al. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001; 69:89–95. [PubMed: 11240971]
3. Chung CH, Parker JS, Ely K, Carter J, Yi Y, Murphy BA, et al. Gene expression profiles identify epithelial-to-mesenchymal transition and activation of nuclear factor-kappaB signaling as characteristics of a high-risk head and neck squamous cell carcinoma. Cancer Res. 2006; 66(16): 8210–8. [PubMed: 16912200]
4. Hensen EF, De Herdt MJ, Goeman JJ, Oosting J, Smit VT, Cornelisse CJ, et al. Gene-expression of metastasized versus non-metastasized primary head and neck squamous cell carcinomas: a pathway-based analysis. BMC Cancer. 2008; 8:168. [PubMed: 18544165]
5. Chung CH, Parker JS, Karaca G, Wu J, Funkhouser WK, Moore D, et al. Molecular classification of head and neck squamous cell carcinomas using patterns of gene expression. Cancer Cell. 2004; 5:489–500. [PubMed: 15144956]
6. Thurlow JK, Pena Murillo CL, Hunter KD, Buffa FM, Patiar S, Betts G, et al. Spectral clustering of microarray data elucidates the roles of microenvironment remodeling and immune responses in survival of head and neck squamous cell carcinoma. J Clin Oncol. 2010; 28(17):2881–8. [PubMed: 20458058]
7. Dumur CI, Ladd AC, Wright HV, Penberthy LT, Wilkinson DS, Powers CN, et al. Genes involved in radiation therapy response in head and neck cancers. Laryngoscope. 2009; 119(1):91–101. [PubMed: 19117295]

8. Wadsworth JT, Somers KD, Stack BC Jr, Cazares L, Malik G, Adam BL, et al. Identification of patients with head and neck cancer using serum protein profiles. Arch Otolaryngol Head Neck Surg. 2004; 130:98–104. [PubMed: 14732777]
9. Gourin CG, Xia ZS, Han Y, French AM, O'Rourke AK, Terris DJ, et al. Serum protein profile analysis in patients with head and neck squamous cell carcinoma. Arch Otolaryngol Head Neck Surg. 2006; 132:390–397. [PubMed: 16618908]
10. Gourin CG, Moretz WH III, Weinberger PM, Xia ZS, Liu Z, Terris DJ, et al. Serum protein profile analysis following definitive treatment in patients with head and neck squamous cell carcinoma. Arch Otolaryngol Head Neck Surg. 2007; 133:1125–1130. [PubMed: 18025317]
11. Freed GL, Cazares LH, Fichandler CE, Fuller TW, Sawyer CA, Stack BC Jr, et al. Differential capture of serum proteins for expression profiling and biomarker discovery in pre-and posttreatment head and neck cancer samples. Laryngoscope. 2008; 118:61–68. [PubMed: 18043497]
12. Gourin CG, Zhi W, Adam BL. Proteomic identification of serum biomarkers for head and neck cancer surveillance. Laryngoscope. 2009; 119(7):1291–302. [PubMed: 19444892]
13. Toyoshima T, Vairaktaris E, Nkenke E, Schlegel KA, Friedrich WN, Ries J. Cytokeratin 17 mRNA expression has potential for diagnostic marker of oral squamous cell carcinoma. J Cancer Res Clin Oncol. 2008; 134:515–521. [PubMed: 17786476]
14. Pich A, Chiusa L, Navone R. Prognostic relevance of cell proliferation in head and neck tumors. Ann Oncol. 2004; 15:1319–1329. [PubMed: 15319236]
15. Merkley MA, Weinberger PM, Jackson LL, Podolsky RH, Lee JR, Dynan WS. 2D-DIGE proteomic characterization of head and neck squamous cell carcinoma. Otolaryngol Head Neck Surg. 2009; 141(5):626–32. [PubMed: 19861202]
16. Rajalingam K, Wunder C, Brinkmann V, Churin Y, Hekman M, Sievers C, et al. Prohibitin is required for Ras-induced Raf-MEK-ERK activation and epithelial cell migration. Nat Cell Biol. 2005; 7:837–43. [PubMed: 16041367]
17. Liaudet-Coopman E, Beaujouin M, Derocq D, Garcia M, Glondu-Lassis M, Laurent-Matha V, et al. Cathepsin D: newly discovered functions of a long-standing aspartic protease in cancer and apoptosis. Cancer Lett. 2006; 237:167–79. [PubMed: 16046058]
18. Patel V, Hood BL, Molinolo AA, Lee NH, Conrads TP, Braisted JC, et al. Proteomic analysis of laser-captured paraffin-embedded tissues: a molecular portrait of head and neck cancer progression. Clin Cancer Res. 2008; 14(4):1002–14. [PubMed: 18281532]
19. Roesch-Ely M, Leipold A, Nees M, Holzinger D, Dietz A, Flechtenmacher C, et al. Proteomic analysis of field cancerization in pharynx and oesophagus: a prospective pilot study. J Pathol. 2010; 221(4):462–70. [PubMed: 20593486]
20. Ralhan R, Desouza LV, Matta A, Chandra Tripathi S, Ghanny S, Datta Gupta S, et al. Discovery and verification of head-and-neck cancer biomarkers by differential protein expression analysis using iTRAQ labeling, multidimensional liquid chromatography, and tandem mass spectrometry. Mol Cell Proteomics. 2008; 7(6):1162–73. [PubMed: 18339795]
21. Grandis JR, Tweardy D. Elevated levels of transforming growth factor α and epidermal growth factor receptor messenger RNA are early markers of carcinogenesis in head and neck cancer. Cancer Res. 1993; 53:3579–84. [PubMed: 8339264]
22. Grandis JR, Melhem M, Gooding WE, Day R, Holst VA, Wagener MM, et al. Levels of TGF-α and EGFR protein in head and neck squamous cell carcinoma and patient survival. J Natl Cancer Inst. 1998; 90:824–32. [PubMed: 9625170]
23. Ang KK, Berkey BA, Tu X, Zhang HZ, Katz R, Hammond EH, et al. Impact of epidermal growth factor receptor expression on survival and pattern of relapse in patients with advanced head and neck carcinoma. Cancer Res. 2002; 62:7350–6. [PubMed: 12499279]
24. Psyrri A, Yu Z, Weinberger PM, Sasaki C, Haffty B, Camp R, et al. Quantitative determination of nuclear and cytoplasmic epidermal growth factor receptor expression in oropharyngeal squamous cell cancer by using automated quantitative analysis. Clin CancerRes. 2005; 11:5856–62.
25. Chung CH, Ely K, McGavran L, Varella-Garcia M, Parker J, Parker N, et al. Increased epidermal growth factor receptor gene copy number is associated with poor prognosis in head and neck squamous cell carcinomas. J Clin Oncol. 2006; 24(25):4170–6. [PubMed: 16943533]

26. Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, et al. Radiotherapy plus cetuximab for squamous cell carcinoma of the head and neck. N Engl J Med. 2006; 354:567–78. [PubMed: 16467544]
27. Vermorken JB, Mesia R, Rivera F, Remenar E, Kawecki A, Rottey S, et al. Platinum-based chemotherapy plus cetuximab in head and neck cancer. N Engl J Med. 2008; 359(11):1116–27. [PubMed: 18784101]
28. Burtness B, Goldwasser MA, Flood W, Mattar B, Forastiere AA. Eastern Cooperative Oncology Group. Phase III randomized trial of cisplatin plus placebo compared with cisplatin plus cetuximab in metastatic/recurrent head and neck cancer: an Eastern Cooperative Oncology Group study. J Clin Oncol. 2005; 23(34):8646–54. [PubMed: 16314626]
29. Lai SY, Koppikar P, Thomas SM, Childs EE, Egloff AM, Seethala RR, et al. Intratumoral epidermal growth factor receptor antisense DNA therapy in head and neck cancer: first human application and potential antitumor mechanisms. J Clin Oncol. 2009; 27(8):1235–42. [PubMed: 19204206]
30. Farhadieh RD, Salardini A, Rees CG, Russell PJ, Yang JL, Smee R. Protein expression of epidermal growth factor receptor in laryngeal squamous cell carcinoma index tumors correlates with diagnosis of second primary tumors of the upper aero-digestive tract. Ann Surg Oncol. 2009; 16(10):2888–94. [PubMed: 19669840]
31. Hama T, Yuza Y, Saito Y, O-uchi J, Kondo S, Okabe M, et al. Prognostic significance of epidermal growth factor receptor phosphorylation and mutation in head and neck squamous cell carcinoma. Oncologist. 2009; 14(9):900–8. [PubMed: 19726454]
32. Grandis JR, Drenning SD, Zeng Q, Watkins SC, Melhem MF, Endo S, et al. Constitutive activation of Stat3 signaling abrogates apoptosis in squamous cell carcinogenesis *in vivo*. PNAS. 2000; 97:4227–4232. [PubMed: 10760290]
33. Masuda M, Suzui M, Yasumatu R, Nakashima T, Kuratomi Y, Azuma K, et al. Constitutive activation of signal transducers and activators of transcription 3 correlates with cyclin D1 overexpression and may provide a novel prognostic marker in head and neck squamous cell carcinoma. Cancer Res. 2002; 62(12):3351–5. [PubMed: 12067972]
34. Seethala RR, Gooding WE, Handler PN, Collins B, Zhang Q, Siegfried JM, et al. Immunohistochemical analysis of phosphotyrosine signal transducer and activator of transcription 3 and epidermal growth factor receptor autocrine signaling pathways in head and neck cancers and metastatic lymph nodes. Clin Cancer Res. 2008; 14(5):1303–9. [PubMed: 18316548]
35. Pectasides E, Egloff AM, Sasaki C, Kountourakis P, Burtness B, Fountzilas G, et al. Nuclear localization of signal transducer and activator of transcription 3 in head and neck squamous cell carcinoma is associated with a better prognosis. Clin Cancer Res. 2010; 16(8):2427–34. [PubMed: 20371693]
36. Leong PL, Andrews GA, Johnson DE, Dyer KF, Xi S, Mai JC, et al. Targeted inhibition of Stat3 with a decoy oligonucleotide abrogates head and neck cancer cell growth. PNAS. 2003; 100(7): 4138–43. [PubMed: 12640143]
37. Xi S, Gooding WE, Grandis JR. *In vivo* antitumor efficacy of STAT3 blockade using a transcription factor decoy approach: implications for cancer therapy. Oncogene. 2005; 24:970–979. [PubMed: 15592503]
38. Marsit CJ, Posner MR, McClean MD, Kelsey KT. Hypermethylation of E-cadherin is an independent predictor of improved survival in head and neck squamous cell carcinoma. Cancer. 2008; 113(7):1566–71. [PubMed: 18711702]
39. Duffy SA, Taylor JM, Terrell JE, Islam M, Li Y, Fowler KE, et al. Interleukin-6 predicts recurrence and survival among head and neck cancer patients. Cancer. 2008; 113(4):750–7. [PubMed: 18536030]
40. Byers LA, Holsinger FC, Kies MS, William WN, El-Naggar AK, Lee JJ, et al. Serum signature of hypoxia-regulated factors is associated with progression after induction therapy in head and neck squamous cell cancer. Mol Cancer Ther. 2010; 9(6):1755–63. [PubMed: 20530716]
41. Pattje WJ, Schuuring E, Mastik MF, Slagter-Menkema L, Schrijvers ML, Alessi S, et al. The phosphatase and tensin homologue deleted on chromosome 10 mediates radiosensitivity in head and neck cancer. Br J Cancer. 2010; 102(12):1778–85. [PubMed: 20502457]

42. Bandoh N, Ogino T, Katayama A, Takahara M, Katada A, Hayashi T, et al. HLA class I antigen and transporter associated with antigen processing downregulation in metastatic lesions of head and neck squamous cell carcinoma as a marker of poor prognosis. Oncol Rep. 2010; 23(4):933–9. [PubMed: 20204276]

43. de Koning PJ, Bovenschen N, Leusink FK, Broekhuizen R, Quadir R, van Gemert JT, et al. Downregulation of SERPINB13 expression in head and neck squamous cell carcinomas associates with poor clinical outcome. Int J Cancer. 2009; 125(7):1542–50. [PubMed: 19569240]

44. Ayoub C, Wasylyk C, Li Y, Thomas E, Marisa L, Robe A, et al. ANO1 amplification and expression in HNSCC with a high propensity for future distant metastasis and its functions in HNSCC cell lines. Br J Cancer. 2010; 103(5):715–26. [PubMed: 20664600]

Table 1

MicroarrayStudiesin HNSCC

Pathway	Genes	Prognosis	Reference
ECM remodeling	*FAP, PLAU, COL5A1, COL5A3, LAMB1, MSC, LLGL2*	metastasis	Hensen 2008
Focal adhesion, ECM remodeling	*ITGB4, LAMA3, COL3A1, 4A1, 5A1, 5A2, 11A1, FN1, PDGFC, TNC ITGA3, ITGA4, ITGA6, ITGB6, LAMA3, LAMB3, LAMC2, COL4A6, PGF, VEGFC,* and *MET*	Poor survival	Thurlow 2010
ECM remodeling, MMP regulation	*SHC1, GRB2, SOS1, HRAS, RAF1, HRAS, RAC1, PAK1, PAK2, MAP3K4*	metastasis	Hensen 2008
Cell adhesion, motility	*ADAM9, ARHGAP5, ICAM3, LAMA3, MYH10, MYO5A, TNC, and TSPAN7*	Important in response to therapy	Dumur 2009
Cell adhesion	*IL8, JUP, TGFBI, ATP2A2, LOXL2*	High-risk, decreased recurrence free survival	Chung 2006
Epithelial to mesenchymal transition	*INHBA, HIF1A, PROCR, MMP2, PDGFRA*	High-risk, decreased recurrence free survival	Chung 2006
NF-kB signaling activation	*IL8, SERPINE1, KRT14, BCL2A1, MMP1*	High-risk, decreased recurrence free survival	Chung 2006
Hypoxia induced invasion	*HIF1α, MMP2, MMP13, PLAUR, P4HA1*	metastasis	Hensen 2008
Hypoxia induced angiogenesis	*HGF, FGF2, PDGFβ, IL8, PGF*	metastasis	Hensen 2008
Cell proliferation	*TNC, PTHLH, CDC37L1, PCTK2, and SEPT11*	Increased in non-responders to CRT/RT	Dumur 2009
Apoptosis	*TOSO, BAG5, FOSL2, SULF1, and LTB*	Anti-apoptotic pattern increased in non-responders to CRT/RT	Dumur 2009
Integrin Signaling	*TSPAN7*	Increased in non-responders to CRT/RT	Dumur 2009
DNA mismatch repair pathway	*ANKRD17*	Increased in non-responders to CRT/RT	Dumur 2009
V2CR – innate immune response	*CCL3, CSF2, CXCL6, IL6, IL8, PDGFC, TGFB2, VEGF*	Poor survival	Thurlow 2010
V3CR – adaptive immune response	*BLR1, CCL19, CCL21, CCL5, CCR2, CCR5, CCR6, CCR7, CXCR4, IL7, LTB, TNFSF4*	Good survival	Thurlow 2010

Table 2

Proteomic Studies in HNSCC

Protein profile	Significance	Reference
Underexpression: kininogen and serine protease inhibitors C-1 inhibitor, kininogen, angiotensinogen, serine/cysteine proteinase inhibitor clade G member 1	recurrence	Gourin 2009
Overexpression: thiol-specific antioxidant proteins (TSA), apolipoprotein A1 and proapolipoprotein, epidermal cytokeratin 2	recurrence	Gourin 2009
Upregulated: keratin 17, Mn superoxide dismutase, proliferating cell nuclear antigen, glutathione synthetase, ferritin light chain, keratin 5	Increased in HNSCC vs matched normal	Merkley 2009
Downregulated: cornulin, keratin 4, cystatin-B, cytokeratin 13, leukocyte elastase inhibitor (Serpin B1), Annexin A1, cytokeratin 8, guanylate binding protein 6	Decreased in HNSCC vs matched normal	Merkley 2009
Overexpression: stratifin (14-3-3sigma), YWHAZ (14-3-3zeta), S100-A7	3 molecule biomarker to identify HNSCC	Ralhan 2008
Cystatin A, S100A8, and 4 un-identified proteins	Marker to predict recurrence/ progression free survival	Roesch-Ely 2010
Vimentin, prohibitin, EVI-5, cathepsin D	expressed in HNSCC, potential biomarkers	Patel 2008

Table 3

Molecular Targets in HNSCC

Marker	Prognosis	Reference
EGFR amplification and overexpression	Poor survival	Ang 2002
Nuclear STAT3	Increased progression free survival, lower risk of death	Pectasides 2010
Hypermethylation of E-cadherin gene	Improved overall survival	Marsit 2008
Increased IL-6	Recurrence and poor survival	Duffy 2008
Cytokine and angiogenic factors: VEGF, IL-4, IL-8, osteopontin, growth-related oncogene- α, eotaxin, granulocyte colony stimulating factor, and stromal cell derived factor-1α	Recurrence and increased progression	Byers 2010
Increased PTEN	Radioresistance, and worse locoregional control	Pattje 2010
Decreased HLA class I antigen	Decreased disease free survival	Bandoh 2010
Downregulation of SERPINB13	Poor grade, lymph node metastasis, reduced survival	de Koning 2009
ANO1 amplification and overexpression	Increased metastatic potential *(in vitro)*	Ayoub 2010