The Prognostic Function of Biomarkers in Head and Neck Squamous Cell Carcinomas (HNSCC)

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Abstract: Objective: HNSCC is one of frequently cancers worldwide. Biomarkers would fulfill all three categories: diagnostic, prognostic and therapeutic which are different from other departed biomarkers. These markers could serve as targets for new therapies, which would probably eventually change the outcome of HNSCC. The objective in this review was to highlight recent research about biomarkers that shows prognostic function for HNSCC. **Methods:** The data used in this review were obtained mainly from the studies reported in PubMed using the key terms"HNSCC","biomarker"and "prognostic". Original articles and critical reviews selected were relevant to tumor pathogenetic and tumorigenic molecular mechanism. **Results:** There are many biomarkers referring to proto-oncogene and tumor suppressor gene, cell cycle regulation, tumor metastasis and immunological markers of virus oncogene. Among them drugs targeting HPV and EGFR have been used for clinic. **Conclusion:** Individual inherited diversity is the basis of tumor markers. Because of complicated signal network and reciprocal cross in diverse pathways in most tumors, various kinds of biomarkers should be specifically combined in time of judging tumor prognosis.

Key words: Head and neck cancer squamous cell carcinomas, Biomarker, Prognosis, Evaluation

Introduction

Head and neck cancer is the fifth common cancer worldwide with approximately 650,000 new cases reported annually(1). There are estimated 35,720 new cases of oral, pharynx cancer and 7,600 expected deaths in USA in 2009. More than 90% head and neck cancer are head and neck cancer squamous cell carcinomas, arising from the epithelium lining the sinonasal tract, oral cavity, pharynx, and larynx.

Cigarette smoking, pipes, smokeless tobacco, and the use of alcohol are the most important risk factors. Their interactions may be synergistic in the development of HNSCC. Although significant therapeutic gains have been demonstrated in a number of randomized controlled trials, therapeutic outcome is far from satisfactory, with relatively high loco-regional failure rates of around 50% after 3 years and an overall 5-year survival rate of 50%. The prognosis of HNSCC is influenced by multitude factors about host and tumor.

Improvements in overall survival in patients with HNSCC rest on early identification of pre-malignant lesions and intervention in patients with different methods at risk prior to development of advanced stage disease. Since epithelial carcinogenesis is a multistep process directed by complex molecular events that involve specific genetic defects in proto-oncogene and tumor suppressor gene, early identification of genetic alteration that may represent early transition into malignant phenotype may be possible through various measures recently mentioned.

For the past few years, many researches have been carried out about tumor pathogenetic molecular mechanism and abnormal molecular signal chain. A large number of tumor biomarkers emerged after the invention of monoclonal antibody technique. Tumor biomarkers are an indicator of normal biological processes, pathological processes or pharmacologic responding to a therapeutic intervention which can be detected and evaluated objectively(2). A biomarker for HNSCC can be derived from several sources but falls into three general sources: primary tissue, surgical margin tissue, and bodily fluid markers. Three basic types of molecules can be extracted from these sources: DNA, RNA, and protein. Tumor biomarkers are synthesized, secreted by tumors or delivered by host responding to tumors. Biomarkers would fulfill all three categories: diagnostic, prognostic, and therapeutic, which are different from other departed biomarkers such as CEA, AFP and PSA in sensitivity and specificity. These markers could serve as targets for new therapies, which would eventually change the outcome of HNSCC (Table 1). This article will review the biomarkers' prognostic functions.

Table 1 Potential prognostic markers in HNSCC

Member		Major classes	Common mutation or gene expression	Correlation with
Other growth factors and HNSCC related gene	Cyclin D1	Cell-cycle regulation	Over expression	Worse prognosis
	EGFR	Signal transduction	Over expression	Poor prognosis
	VEGF	Signal transduction	Over expression	Shorter progression-free survival and overall survival and higher recurrence rate
	MMP	Extracellular matrix degradation	Over expression	Lymph node metastasis and poor overall survival
Oncogen	EBV DNA	duplicating of EBV genome lead to malignant transformation of host cell	Elevated loading	Predicting for metastasis
	E6,E7		HPV positive	Better prognosis
Tumour suppressor gene	P16	Cell-cycle regulation	Loss of expression	Decreased survival
	Rb	Cell-cycle regulation	Deletion, methylations, inactivation	Tumor developing
	P53	Cell-cycle regulation	Missense mutation, nonsense mutation, generearrangement, allelic loss and germinal mutation	Poor prognosis
	PTEN	Cell-cycle regulation	Deletion and methylation	shorter overall survival and event-free survival

Proto-oncogene and Tumor Suppressor Gene

Cyclin D1

Cyclin D1 is a proto-oncogene located on chromosome 11q13 that modulates a critical step in cell-cycle control progression. In the advanced time of G1, it is combined with many kinds of cyclin-dependent kinase (CDK) which can make cell enter the stage of S faster, and eventually lead to cell multiplication. The over-expression of cyclin D1 has been demonstrated in 17–79% of tumour specimens from patients with HNSCC which has been shown to correlate significantly with tumour cell differentiation, style of growth extension and metastasis. Patients with cyclin D1-positive tumors had a worse prognosis compared with those with cyclin D1-negative tumors[3]. However, some researchers found no significant associations between levels of cyclin D1 and survival of patients with HNSCC[4].

p16

As a tumour suppressor gene p16 is located on chromosome 9p21. P16 was important CDK inactivating protein in the cell cycle regulation having multiple suppressing cancer functions. Some genetic alterations such as loss of p16 expression, mostly homozygous deletions and methylations have been considered to associate with decreased survival[5]. Patients with p16 positive oropharyngeal squamous cell carcinoma (OPSCC) exhibited a significantly better overall survival than those with p16 negative tumors. The improved prognosis of p16 positive OPSCC was found after radiotherapy and surgery[6]. However, downregulation of p16 found in 48% of tumours of HNSCC was associated with a more locally advanced tumour and had no prognostic significance for nodal metastasis and survival[7].

Significant correlation with poor clinical outcome measures of recurrence, metastasis and survival was seen when cyclin` D1 amplification and loss of expression of p16 gene occured together than

either alone[8].

Retinoblastoma (Rb)

Rb is one of the important tumour suppressor genes located on chromosome 13p14.2. Rb can regulate cell-cycle progression and apoptosis. Rb, i.e. retinoblastoma gene, is a predisposing gene of retinoblastoma. After making analysis of genetics foundation of puerile retinoblastomas, Knudsone verified that Rb gene mutation is related to the genesis of retinoblastomas[9]. Rb gene is the tumour suppressor gene first cloned and completely sequenced. When tumor is developing, the main mutation styles include deletion, mutation, methylations, loss of expression and functional inactivation owing to the combination of virus and oncoprotein. Deletion of Rb is premise of cell multiplication. Nineteen biomarkers were evaluated for protein expression in HNSCC after concurrent cisplatin-based chemoradiation. The result demonstrated that Rb remained significant independent predictive markers for local control. Patients treated with concurrent chemoradiation with high Rb expression had an increased probability of having a recurrence[10].

Cell Cycle Regulation

P53

P53 is located on chromosome 17p13. The protein of p53 is TP53. P53 is a tumor suppressor gene found first which has high associatively with tumor. In 1979 p53 was separated by Lane and Crawford[11]. Wild p53 is a negative growth factor in cell growth cycle which has important biological function in the procedures of cell cycle regulation, DNA damage reparation, cell differentiation, apoptosis and aging. So it is famed with "cell soldier". The ways of p53 gene mutation include missense mutation, nonsense mutation, generearrangement, allelic loss and germinal mutation. The ways of TP53 functional inactivation have different types. For example, TP53 can be degradated when it binds to E6 of HPV, which is related to HPV dependable tumor such as uterine cervix cancer and oropharyngeal cancer. Malignant transformation of B lymphocyte can take place when TP53 binds to EBNA-5 and BZLE1, which is related to EBV dependable nasopharyngeal carcinoma and lymphoma.

The incidence rate of p53 mutation is 50%-69% in HNSCC. p53 mutation may directly influence DNA binding or interfere with formation of TP53 which easily results in tumor recurrence and poor prognosis[12]. Over-expression of mutated p53 protein is associated with tumorous recurrence, poor disease-free survival rate and decreased disease-free survival in HNSCC[13]. A strong correlation was observed between p53 expression in the primary tumor and in the matched lymphnode metastases. The role of p53 in the lymph node metastases was an independent predictor of regional failure and a poor prognosis in patients with HNSCC[14]. Other studies have shown that TP53 mutation or over-expression does not independently predict clinical outcome in patients with HNSCC[15].

For the past few years, recombinant p53 adenovirus (Ad-p53) in combination with chemoradiotherapy has shown very effective in head and neck tumors. In China, Ad-p53 gene therapy has been classified to tumor clinical therapeutic drugs[16]. MiR-34[17] and miR-29[18] are indispensable important molecules in p53 gene regulation network which may play a tumor suppressor gene or oncogene role in tumorigenesis. Artificial constructing pre-microRNA has become hot spot of gene regulation research.

PTEN

PTEN is located on 10q23.3. PTEN (MMAC1) was the first tumor suppressor gene found and named by Steck in 1997 which having phosphatase activity. The functions of PTEN mainly include regulating cell cycle, inducing tumor apoptosis and inhibiting tumor cell growth, invasion and metastasis. The ways `of PTEN inactivation includes mutation, deletion and methylation, among which the main way is mutation. Somatic mutation or deletion of PTEN has been reported in a variety of tumor types connecting to tumorigenesis. Genetic analysis of PTEN in HNSCC has demonstrated alterations in PTEN, suggesting that PTEN may play a role in HNSCC tumorigenesis. Patients with tongue cancer lacked PTEN expression had a significantly shorter overall survival time and event-free survival time. Lack of PTEN expression may be an independent prognostic indicator for clinical outcome in tongue cancer[19]. PTEN expression in OSCC was related to malignancy grade. Aggressive tumors with a high score of malignancy did not express PTEN, and the PTEN expression was present in the epithelium adjacent to the tumor[20].

EGFR

The EGFR family includes EGFR (c-erbB1 or Her1), c-erbB2 (Her2-neu), c-erbB3 (Her3) and c-erbB4(Her4). They are all transmembrane protein which not only has receptor's function but also directly transforms extracellular signal to intra-cellular effect. So EGFR family is an important regulatory factor of cell growth, differentiation and survival. The abnormality of signal transduction

mediated by EGFR family has significant relation with tumorigenesis and tumor development.

The state of EGFR has been associated with prognosis of HNSCC. Mutations of EGFR are often located on tyrosine kinase. Patients with increased EGFR copy number had a worse median time to progression (TTP) and time to death[21]. Patients without EGFR mutations whose tumor samples showed phosphorylated EGFR had poor prognosis in HNSCC who had not been treated with EGFR molecular targeting therapy[22]. In HNSCC 80%~100% of tumors have increased EGFR protein level. EGFR over-expression correlates with a poor prognosis and decreased overall survival[23]. However, several studies have found no association between EGFR and clinical stage, recurrence or survival[24].

The application of target drug began with Dr. Stanley Cohen's stating about EGF in 1960. Tyrosine kinase inhibitor (TKI) (such as Gefitinib and Erlotinib) and EGFR antibody Cetuximab have been widely used in advanced non-small cell lung cancer, colon carcinoma, and head and neck cancer clinically.

Tumor Metastasis

VEGF

VEGF is an important factor in angiogenesis. VEGF induces proliferation, migration and survival of endothelial cells during tumorous growth by binding to specific tyrosine kinase receptor. VEGF has important function in tumor growth and metastasis. There are six members of the VEGF family including VEGF-A~VEGF-E and placenta growth factor. The receptors of VEGF contain VEGFR-1, VEGFR-2 and VEGFR-3.These receptors are all transmembrane protein which over-expression can result in pathological angiogenesis or lymph vessel production. VEGF-A high expression in HNSCC was correlated to shorter progression-free survival and overall survival and higher recurrence rate[25].Over-expression of VEGF-A and VEGF-C have been correlated to poor overall survival in patients with advanced stage HNSCC[26]. However some studies showed no correlation between VEGF levels and overall survival in native tumor tissue. The results might be attributed to the tumor stroma containing additional VEGF-producing normal cells[27].

The anti–VEGF receptor antibody, bevacizumab, have been approved by the US Food and Drug Administration for first and second line treatment in metastatic colorectal cancer, first-line treatment in advanced non small cell lung cancer, advanced or metastatic renal cell carcinoma, and Her-2-positive advanced breast cancer. But further clinical trial should be done to verify bevacizumab's function in HNSCC.

MMP

MMP family plays an important role in tumor invasion and metastasis. MMP is a family of zinc-dependent proteolytic enzymes that degrade most components of the extracellular matrix including collagen, elastin, fibronectin and gelatin which are very important in tumorous invasion and metastasis in HNSCC. The over-expression MMP members in HNSCC contain MMP-2, MMP-7, MMP-8, MMP-9, and MMP-13. Among them, MMP-2 and MMP-9 are believed to play major roles. The high expression of MMP-2 in HNSCC was related to advanced tumor stage, lymph node metastasis, and bad grade[28]. MMP-9 was over-expressed in 60%-92% of HNSCC which was correlated with poor overall survival[29]. However, there are also some contrary results[30]. Over-expressed MMP-7[31] and MMP-13[32] were related to poor survival.

Sometimes the results of researches about biomarkers are inconsistent. The discordance between these researches may be due to the difference in the standard of detection method, cases selection and the variation of the tissue handling and analysis technique. The reliability of biomarkers must be validated by random clinical test in the last.

Immunological Markers of Virus Oncogene

Epstein Barr Virus (EBV)

EBV is etiological factor of most Nasopharyngeal carcinoma (NPC). In respect of epidemiology, treatment, and potential biomarkers, NPC has distinct entity compared to other HNSCCs. The role of EBV in the pathogenesis of this disease, particularly in endemic populations, makes this virus an attractive candidate as a clinically useful biomarker. Not only the EBV genome but also many kinds of EBV specific antigens are present in the NPC cells. Quantitative analyses of anti-EBV serology and EBV DNA have become means in early detection, di3ease surveillance and evaluating prognosis. EBV DNA can be detected in 95% of NPC. Plasma EBV DNA load is an independent prognostic factor, especially in predicting for metastasis. Plasma EBV DNA showed more superior effects in prognosis compared to other clinical parameters in NPC patients receiving concurrent chemoradiotherapy[33]. The Epstein-Barr Nuclear Antigen gene-1 (EBNA1) can be detected before tumorigenesis which is one of early antigens appearing in patient after EBV infection. It is indispensable to latent infection of EBV, keeping and duplicating of EBV genome. Now it is widely used

clinically to detect EBNA1 antigen-antibody which is looked as serological index of NPC[34].

The Human Papilloma Virus (HPV)

HPV has been accepted as the causative agent in human cervical cancer. There are over 110 different genotypes of HPVs among which genotypes 16 and 18 are considered as "high risk" HPVs. Two main viral oncogene products, E6 and E7, is related to cell transformation and oncogenicity. After E6 and E7 bind to and inactivate the tumor suppressor gene p53 and pRb, respectively, they lead to malignant transformation of host cell.

HPV is also a causative agent in oropharyngeal cell carcinomas. Approximately 20% of HNSCC are HPV positive and about half of the oropharyngeal cell carcinomas are positive for HPV detection. HPV positive tumors demonstrate the loss of Rb and cyclin D1 expression, over-expression of p16, and rarely have p53 mutation. On the other hand, HPV negative tumors have the contradictory characteristic[35]. HPV positive HNSCC population appears to be biologically and clinically different from HPV negative patients. HPV positive patients are seldom likely to be a smoker or addicted to drinking. HPV positive tonsillar tumors have a better prognosis, including a 60%~80% risk reduction of death, than their HPV negative counterparts. This may in part be due to the fact that HPV positive tonsillar tumors tend to occur in non-smokers and non-drinkers, where p53 mutations are uncommon[36]. HPV-positive oropharyngeal cancers also show good outcome[37]. The presence of HPV confers a survival advantage among HNSCC patients, particularly when p53 is wild type[38]. HPV can be detected from not only tumor biopsy but also salivary rinses. Patients with presence of HPV-16 DNA in surveillance salivary rinses are at significant risk for recurrence. Quantitative measurement of salivary HPV-16 DNA has promise for surveillance and early detection of recurrence[39]. Currently, clinical trials for HPV positive patients by lowering treatment intensity are being carried out in order to minimizing unnecessary toxicities.

Now two types of HPV vaccines, Cervarix and Gardasil, have been used worldwide to prevent cervical cancer. These two vaccines have no therapeutic effect and are not appropriate for patients who have suffered from cervical cancer. Clinical trials will be done to validate these two vaccines' therapeutic effect in other HPV-dependent tumor such as partial HNSCCs.

Conclusions

So some tumor biomarkers can be taken as tools to measure tumorous progress because of their distinct characteristic. Many kinds of molecular passageway relating to tumorigenesis and tumor development have been discovered with the advancement of oncobiology and tumor genetics. Besides frequently used biomarkers reviewed above the function of telomerase cannot be ignored of. Up to now telomerase is the most broad-spectrum tumor biomarkers. Telomerase's activity has been reported to be up-regulated in HNSCC. It can be looked as an independent prognostic factor for survival in HNSCC[40]. There are different phenotypic biomarkers in different tumor types. Individual inherited diversity is the basis of tumor markers. In view of thinking highly of the transformation of translational medicine, there will be more biomarkers to be used for tumorous screening, diagnosis, therapy and prognosis. Target of targeted therapy is essentially tumor biomarkers. For the past few years drugs targeting HPV and EGFR have been used for clinic. However the effectiveness of targeted therapy is always between 20% and 50% maybe because there are complicated signal network and reciprocal cross in diverse pathways in most tumors. So developing multiple targets therapeutic alliance seems very important. In addition, biomarkers are different in tumorigenic various stages. For example, prognostic value of colon carcinoma's biomarker has stage specificity. Tumorigenic multiple factor and individual variation decide that various kinds of biomarkers should be specifically combined in time of judging tumor prognosis and predicting curative effect. An assessment scoring system of integrated evaluation should be established to undertake individualized treatment of tumor more objectively.

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