Review Article

Role of tumor markers in oral squamous cell carcinoma: Review of literature and future consideration

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ABSTRACT

Sensitive and reliable early diagnostic markers for oral squamous cell carcinoma (OSCC) remain unavailable. Early identification of recurrence for OSCC is also a challenge. This article reviews the recently identified biomarkers for OSCC such as cytokeratins, p53, tumor necrosis factor alpha (TNF-α), etc., which are of great utility in early diagnosis. In addition, the biomarkers that have been correlated with OSCC tumor malignancy by molecular pathology analysis are also described. This article speaks about selected reaction monitoring (SRM) which might even be applied to monitor differential expression of tumor proteins in blood, saliva, or fresh frozen tissue materials. SRM technique may complement or possibly replace western blotting for biomarker verification and for selection of potential biomarker candidates. This article may help to identify the potential biomarkers for screening and the molecular pathology analysis for high-risk patients of OSCC. Effective screening to identify high-risk patients will allow clinicians to provide an early and appropriate treatment to patients without delay and also reduce the risk of recurrence of OSCC.

Key words: Keratin, oral squamous cell carcinoma, tumor marker

INTRODUCTION

Squamous cell carcinoma (SCC) of the head and neck is the sixth most common cancer worldwide. Despite improvements in the diagnosis and treatment, the 5-year survival rate of advanced head and neck squamous cell carcinoma (HNSCC) has only moderately increased, which is largely due to the high proportion of patients who present with advanced disease stage and the frequent development of relapse and second primary tumors.

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The diagnosis of cancer is based on the analysis of tissue and cytology specimens obtained through several procedures. When a cell becomes cancerous, new antigens unfamiliar to the immune system appear on the cell's surface. The immune system identifies these new antigens, called tumor antigens, as foreign and may be able to contain or destroy the cancerous cells.

A tumor marker can be defined as a molecule that indicates the likely presence of cancer or can also be defined as one that provides information about the likely future behavior of an existing cancer (e.g. ability to metastasize or to respond to therapy).[1] The majority of existing tumor markers are mostly useful in making a clinical decision after initial suspicion of cancer or its behavior which has been already raised by more conventional means.[2]

An ideal tumor marker theoretically should have the following criteria:[3]

1. It should have high sensitivity and specificity.
2. It should have high positive and negative predictive value.
3. It should be able to differentiate between neoplastic and

non-neoplastic disease and show positive correlation with tumor volume and extent
4. It should predict early recurrence and have prognostic value
5. It should be clinically sensitive, i.e. detectable at early stage of tumor
6. Its levels should be preceding the neoplastic process, so can be useful for screening
7. It should be easily assayable

Unfortunately, none of the tumor markers reported to date have the above ideal characteristics.

Tumor markers are potentially useful in the following:
1. Screening for early malignancy
2. Acting as a diagnostic aid for malignancy
3. Determining prognosis in malignancy
4. Predicting therapeutic efficacy
5. Maintaining surveillance following surgical of the primary tumor
6. Removal and monitoring therapy in advanced malignancy.[4]

Limitations of tumor markers

- False elevation may occur in non-neoplastic conditions as many tumor markers are proteins, over-expressed not only by cancer cells but also by normal tissues.
- Many tumor markers are not specific to a particular type of cancer.
- Tumor marker levels are not elevated in every person.
- No simple tests are yet available with sufficient sensitivity and specificity to detect the presence of a cancer.

Tumor markers can be broadly classified based on the type of tissue as follows:[5]

(a) Epithelial markers
- Cell surface markers – Histocompatibility
- Intracellular markers – Cytokeratins
- Basement membrane markers – Type 4 collagen
- Matrix markers – Tenascin
- Membrane antigen – Blood group antigens.

(b) Connective tissue markers
- Intermediate filament proteins – Desmin
- Other filament proteins – Laminin
- Cellular enzymes – Amylase, lysozyme
- Cytoplasmic non-filamentous non-enzymatic proteins – Myoglobin, S100 protein
- Membrane antigen – Leukocyte specific antigen.

(c) Salivary gland markers
- Epithelial markers – Cytokeratins
- Myoepithelial cell markers – Actin, myosin
- Serum acinar cell markers – Salivary amylase
- Myoepithelial cells + acinar cells – S100 protein.

TUMOR MARKERS IN RELATION TO SCC

Albumin

Ecological and observational studies suggest that low serum albumin is associated with higher mortality from cancer. Research conducted over the last decade or so has demonstrated that serum albumin levels (either considered alone or in combination with other parameters) can provide useful prognostic information in a variety of cancers. Pre-treatment serum albumin levels provide useful prognostic significance in cancer.[6]

Autoantibodies as tumor biomarkers

Tumor proteins may induce the formation of autoantibodies which can be detected in patient serum. The approach for these studies is to separate protein lysates from human SCC cell lines, followed by Western Blotting using patient sera. Antigens are then identified using mass spectrometry [e.g. heat shock protein 70 as an early marker for SCC of the esophagus and sideroflexin 3 for oral squamous cell carcinoma (OSCC)]. It has been suggested that these autoantibodies might be used to establish effective new immune therapies, besides using them for early diagnosis of these tumors.

Catalase

Antioxidant enzymes such as superoxide dismutase and catalase can directly counterbalance the oxidant attack and may protect cells against DNA damage. Superoxide dismutase inhibits hydroxyl radicals (OH^-) production; therefore, it acts as an inhibitor at the initiation and promotion stages during carcinogenesis. Studies have shown that erythrocyte superoxide dismutase activity was decreased in oral cancer patients than in healthy individuals and patients with oral lichen planus (OLP). The low activity of erythrocyte superoxide dismutase can be due to the depletion of antioxidant defense system, occurring as a consequence of overwhelming free radicals.[7]

CD44

The CD44 family of receptors includes multiple variant isoforms, several of which have been linked to malignant properties including migration, invasion, and metastasis. Analysis of the expression of standard CD44s and the CD44 variant isoforms v3, v6, and v10 was carried out in the HNSCC cell line, HSC-3. The role of CD44 isoforms in migration, proliferation, and cisplatin resistance was determined.

HSC-3 cells express at least four CD44 isoforms, and these CD44 isoforms mediate migration, proliferation, and cisplatin sensitivity. Compared with primary tumors, a greater proportion of metastatic lymph nodes demonstrated strong expression of CD44 v3, CD44 v6, and CD44 v10, while

expression of standard CD44 was not significantly different in metastatic lymph nodes and primary tumors. Expression of CD44 variant isoforms was associated with advanced T stage (v3 and v6), regional (v3) and distant (v10) metastasis, perineural invasion (v6), and radiation failure (v10). CD44 v6 and CD44 v10 were also significantly associated with shorter disease-free survival.[8]

CD59

CD59 inhibits the complement membrane attack complex by binding C5b678 and preventing C9 from binding and polymerizing. It is present on "self" cells to prevent complement from damaging them. Tumor cells can escape complement-dependent cytotoxicity (CDC) by expressing complement restriction factors (CRFs), CD46, CD55, and CD59. CD46, CD55, and CD59 were highly expressed in HNSCC cells including T1/T2N0M0 stages. The CRF expression was much lower or absent in non-neoplastic squamous epithelia or in the submucosa of both normal and tumor tissues.[9]

Cofilin 1 (CFL1) and neural Wiskott–Aldrich syndrome protein (N-WASP)

The expression patterns of CFL1 and N-WASP in normal esophageal mucosa and esophageal squamous cell carcinoma (ESCC), and their correlation with clinical characteristics have been studied. CFL1 is correlated with the clinicopathological factors in ESCC, such as infiltration depth, lymph node metastasis, and pathological staging. N-WASP is related to lymph node metastasis and pathological staging in ESCC. It has been shown that CFL1 mRNA level was over-expressed in ESCC tissue, while N-WASP mRNA expression level was not different between cancerous tissues and adjacent normal esophageal mucosa. Also, CFL1 mRNA expression was significantly associated with regional lymph node metastasis and pathological staging. There was no correlation between CFL1 and N-WASP mRNA expression and survival.[10]

Cancer antigen 125 (CA 125)

CA 125, a tumor associated glycoprotein of more than 200 kD, was detected by using murine monoclonal antibody OC 125 generated by immunization against histologically well-defined ovarian adenocarcinoma cell line. Elevated CA 125 levels were observed with varying degree of sensitivity in carcinoma of the neck of the womb, cervical carcinoma, and gastrointestinal carcinoma.

Cytokeratins/keratins

There may be variations in keratin expression compared to that in normal tissue, depending on the degree of differentiation of epithelial tumors.

Keratins as tumor markers have two main applications:

(i) In distinguishing epithelial from non-epithelial tumors and

(ii) in distinguishing the type of epithelial tumor.

The degree of keratin expression in tumors is remarkably high. Therefore, keratin is also a reliable marker for the following:[11]

- Undifferentiated and anaplastic carcinomas
- Disparately growing infiltrating carcinoma cells
- Metastasizing single carcinoma cells in suspension

CYFRA 21-1

CYFRA 21-1 is an antigenic determinant present in 40 kD protein, the cytokeratin 19. This antigen is expressed in normal, simple epithelium, as well as in proliferating epithelium. Cyfra 21-1 is used as a tumor marker for non-small cell lung cancer (NSCLC), such as SCC, adenocarcinoma, and large cell carcinomas. Both Cyfra 21-1 and CA 19-9 have improved the sensitivity for the detection of adenocarcinoma for lung.[12]

Endothelins

Endothelins are 21-amino acid vasoconstricting peptides produced primarily in the endothelium, and have a key role in vascular homeostasis. Salivary endothelin-1 (ET-1) could be a good biomarker for OSCC development in OLP patients regardless of the degree of OLP disease activity. However, it appeared not to be a good biomarker for detecting recurrence of OSCC in patients in remission.[13]

Glutathione

Glutathione (GSH) concentrations in human epidermoid carcinoma tissues were measured by high-performance liquid chromatography. The GSH content of epidermoid carcinoma intratumor tissue specimens was significantly higher than that in adjacent non-tumor tissue parts. Tissue GSH levels were not correlated with the age of the patients or tumor size.[14]

Interleukin-1α

In a study, the authors analyzed and compared the level of tumor necrosis factor alpha (TNF-α), interleukin (IL)-1α, IL-6, and IL-8 in whole unstimulated saliva among the OLP patients with dysplasia and individuals of control group and OSCC. In moderate and severe dysplasia, the level of each cytokine was significantly higher than in control. In moderate dysplasia, TNF-α and IL-1α levels were significantly increased without being different from that in OSCC, but IL-6 and IL-8 were detected at a concentration significantly lower than in OSCC. In severe dysplasia, the level of TNF-α was also not significantly different from that in OSCC, and the levels of IL-1α, IL-6, and IL-8 were still significantly lower than those in OSCC.[15]

IL-2β

Altered cytokine responsiveness is tightly associated with the development of oral cancer. While in normal cells, stimulation with proinflammatory cytokines leads to growth inhibition, in

oral cancer cells stimulation with proinflammatory cytokines leads to upregulation of positive cell cycle regulators such as nuclear factor kappa B (NF-κB), signal transducer and activator of transcription (STAT), and mitogen-activated protein kinase/extracellular signal-regulated (ERK) pathway. The most obvious example is IL-1β whose values in saliva were the highest of all the studied cytokines, while in serum its values were below the level of detection. It is shown that patients with oral cancer have significantly higher concentrations of salivary IL-1β and IL-6 compared to patients with leukoplakia and healthy individuals. Increase in the concentrations of proinflammatory cytokines in saliva might reflect the development of oral cancer from oral leukoplakia.[16]

IL-6

Chronic inflammation constitutes one of the key risk factors for OSCC. Studies indicate that IL-6–induced inflammation promotes tumorigenesis in the oral cavity by altering global LINE-1 hypomethylation. In addition, concurrent hypermethylation of multiple tumor suppressor genes by IL-6 suggests that epigenetic gene silencing may be an important consequence of chronic inflammation in the oral cavity.[17]

IL-8

IL-8 is an angiogenic chemokine with a high expression level in the tumor tissues. This plays important roles in developing many human malignancies including OSCC. Liu *et al.* at Chung Shan Medical University, Taiwan examined the association of IL-8 gene polymorphisms with the susceptibility and clinicopathological characteristics of OSCC. Their results suggested that combination of IL-8 gene polymorphisms and environmental carcinogens might be highly related to the risk of oral cancer.[18]

Mac-2 binding protein

Mac-2 binding protein (Mac-2 BP), also known as 90K, is a highly glycosylated, secreted protein extensively studied in human cancer, which binds galectin-1, galectin-3, and galectin-7. High expression levels of 90K are associated with a shorter survival, the occurrence of metastasis, or a reduced response to chemotherapy in patients with different types of malignancy.[19]

SCC antigen

SCC antigen, a 48 kD protein, is purified from uterine cervix. The antigen concentration is elevated in SCCs of head and neck, lung, esophagus, and anal canal. The highest concentration of SCC antigen is found in patients with metastases.[20]

S100 calcium-binding protein

S100 proteins comprise a family of low molecular weight proteins found in vertebrates, and are characterized by two calcium-binding sites of the helix-loop-helix (EF-handtype) conformation.

S100A9, a member of the S100 calcium-binding protein family, has been shown to be down-regulated in different types of SCCs and up-regulated in some adenocarcinomas. S100A9 is a novel p53 target gene that can induce cellular apoptosis, in a partly p53-dependent manner, and mediate p53-induced apoptosis.[21]

Tissue polypeptide antigen

Tissue polypeptide antigen (TPA), regarded as a marker of cell proliferation, is a mixture of fragments containing relatively stable a-helical rod domains of simple epithelium-type cytokeratins. These fragments are probably released during necrosis and lysis of the carcinoma cells. TPA is known to be a sensitive, but nonspecific tumor marker. Thus, TPA should be regarded as a broad-spectrum epithelial tumor marker and not as a specific molecular marker for epithelial neoplasms.[22]

Tumor suppressor gene P53

P53, a 53 kD nuclear phosphoprotein, functions as a tumor suppressor by inhibiting cell proliferation. P53 plays a dominant role in cellular apoptosis. P53 gene mutations are reported in approximately 50% of all types of cancers. P53 gene mutations are reported to occur commonly in primary breast, colon, ovarian, lung, and esophageal carcinomas.[23]

Telomerase

Genetic damage affects many chromosomes and genes, and it is the accumulation of these changes that appears to lead to carcinoma. Telomere maintenance by telomerase or, in its absence, alternative lengthening of telomeres protects this acquired altered genetic information ensuring immortality without losing eukaryotic linear DNA; when this does not occur, DNA is lost and end-replication problems arise. Telomerase is reactivated in 80-90% of cancers, and can be used as a target for anticancer therapy and to develop better diagnostic and prognostic markers.[24]

TNF-α

The possible correlation of TNF-α and -β genes with the risk of oral cancer was investigated in a study. The functional polymorphisms TNF-α and TNF-β, which affect gene expression, were investigated by restriction fragment length polymorphism analysis. The frequencies of high-expression A2 TNF-α allele and high expression B1 TNF-β allele were significantly increased in cancer patients compared to control.[25]

α-Amylase

The activity and isoenzyme profile of lactate dehydrogenase (LDH), alkaline and acid phosphatase were studied in tumors of the tongue, cheek, oral floor, soft palate and

palatine tonsils, leukoplakia, and in the oral mucosa, and then compared with those of corresponding sites in healthy subjects, which helped to develop tests for early detection, monitoring, and prognosis of oral cancer. A significant increase in the activity of a-amylase and acid phosphatase was observed in the saliva of oral cancer patients (86-96%).[26]

METHODS FOR EVALUATING TUMOR MARKERS

To evaluate the usefulness of a tumor marker, it is necessary to:

- Find reference values,
- calculate predictive values,
- evaluate distribution of marker values, and
- determine the role of these values in disease management.

Reference values

For testing with relatively specific applications, such as the use of tumor marker in the diagnosis and management of cancer, a decision level may be more appropriate than the upper limit of the normal populations. The decision level can be determined using a predictive value model.[27]

Predictive value model

A useful approach to evaluate multiple tests for the same analyte or multiple markers for the same type of cancer is the receiver operating characteristic (ROC) curve. By superimposing ROC curves of several markers, one can select the most predictive markers.[28]

Distribution of marker values

Application of predictive value model is difficult for analytes that are not diagnostic for a single disease. Most tumor markers are elevated in more than one disease condition. When using predictive value model, it is necessary to select a population that includes disease and non-disease groups.

FUTURE PROSPECTS

- Selected reaction monitoring (SRM) has been introduced as a mass spectrometry based method that can be exploited to specifically select and quantify promising protein biomarkers in serum or tissue.
- Most recent head and neck tumor proteomics studies make use of fresh frozen tissue material that was obtained from patients through surgery. A vast amount of formalin fixed, paraffin embedded tissues from tumors in pathology archives can be used in recovery of proteins.
- An upcoming treatment modality for HNSCC is the targeted therapy using biological agents (e.g. monoclonal antibodies like cetuximab).

CONCLUSION

Tumor markers cannot be used as primary modalities for the diagnosis of cancer. Many tumor markers are discovered every year, but all of them are not useful as they are not validated for clinical use. This article attempts to bring together a majority of tumor markers that can be of importance in dealing with the diagnosis of primary cases of OSCC and also in recurrence cases. With the evolving understanding of the protein, genetic, and molecular basis regarding the development and progression of OSCC, there is an existing body of work trying to determine whether such abnormalities can predict the clinical behavior of various head and neck tumors. Studies have to be conducted rigorously to derive useful information. Nevertheless, the role of such molecular markers and the possibility to exploit them for therapeutic gain is already at the horizon.

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