

SERUM TUMOR MARKERS IN ORAL CANCER - A BRIEF REVIEW

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ABSTRACT

Oral squamous cell carcinoma (OSCC) is the most common malignant tumour in the oral and maxillofacial region. It is the sixth most common malignancy and is a major cause of cancer morbidity and mortality. OSCC accounts for 95% of malignant lesions of the mouth and is a major problem worldwide. The relative prevalence of oral SCC is 3-5% of all cancer. Serum tumor markers are defined as proteins with carbohydrate or lipid domains that are found circulating in blood and/or various other body fluids. Their appearance and changing concentrations are associated with the development and growth of malignant tumors. Serum or biochemical tumor markers constitute a variety of heterogenous substances that show quantitative changes during tumor development. These serum tumor markers have been used as prognostic markers for tumor recurrence or metastasis, e.g., CEA, SCCAg, Cyfra 21-1, TPS, etc. The purpose of this article is an attempt to review various serum tumor markers role in diagnosis and prognosis of oral cancer

Key words: Oral squamous cell carcinoma, Serum tumor marker, Squamous cell carcinoma antigen, Carcinoembryonic antigen, Tissue polypeptide antigen, Tissue polypeptide specific antigen, Cyfra 21-1

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INTRODUCTION:

The term 'cancer' has originated from Greek word Karkinos, a crab, referring to an irregular jagged shape often assumed due to local spread of carcinoma.^[1] Oral cancer is an epithelial neoplasia generally beginning as a focal clonal overgrowth of altered stem cells near the basement membrane, expanding upward and laterally, replacing the normal epithelium. The neoplastic process is a beginning with normal epithelium progressing through hyperplasia to dysplasia to carcinoma in situ and invasive carcinoma.^[2] The term 'oral cancer' includes a diverse group of tumors arising from the oral cavity, usually includes cancers of the lip, tongue, pharynx and oral cavity.^[3]

The first known attempt to find markers for malignancy was made 2000 years ago and is described in an Egyptian papyrus, where breast cancer was distinguished from mastitis. Incidentally first tumor marker in modern medicine was identified by Bence – Jones in 1846, who detected heat precipitates in samples of acidified urine from patients suffering from “Mollities ossium”. In 1965 Gold et al, isolated glycoprotein molecule from specimens of human colonic cancer and thus discovered first “tumor antigen” later identified as carcino-embryonic antigen.^[4]

“A tumour marker is a substance present in or produced by a tumor or by the tumor's host in response to the tumor's presence that can be used to differentiate a tumor from normal tissue or to determine the presence of a tumor based on measurement in the blood or secretions”.^[5] There are different types of molecular tumor markers including DNA, mRNA, proteins, antigens, or hormones measured quantitatively and/or qualitatively by appropriate assays. Tumor marker assays comprise immunohistochemical (IHC) test, quantitative immunoassays like radioimmunoassay or enzyme linked immunosorbent assay, polymerase chain reaction (PCR), western or northern blot and more recently microarrays (genomic and proteinomic) and mass spectrometry. Tumor markers could identify a disease process, a specific tissue or patient's characteristics and help in establishing the severity and extend of the disease.^[6]

An ideal tumor marker is an abnormality which is specific for particular types of malignancy.^[7] The tumor markers may be useful for the four following clinical purposes:

- ❖ Screening a healthy population for the presence of cancer or for detecting a group at a higher risk for developing a cancer.
- ❖ Making a diagnosis of cancer: a diagnostic tumor marker is a marker that will aid in detection of malignant disease in an individual.
- ❖ Determining the prognosis in a patient with cancer. This would provide to the clinician a tool for early prediction of tumor recurrence, progression and development of metastases, following the initial surgical removal of the cancer but without administration of adjuvant therapy.
- ❖ Monitoring efficacy of antitumoral treatment: tumor markers may predict how the patient is going to respond to a given therapy which includes surgery, radiation, chemotherapy or more recently targeted treatments.^[6]

Squamous cell carcinoma antigen (SCC)

Squamous cell carcinoma antigen, a serpin associated with squamous cell carcinomas of different organs, comprises two nearly identical, approximately 45 kDa proteins, SCC-1 and SCC-2, which possess unique proteinase inhibitory properties. SCC-1 and SCC-2 reside in the cytosol of squamous cells, and their presence in the sera of patients with advanced squamous cell carcinomas is mainly due to a passive release rather than an active secretory process into the circulation. Elevated serum SCC has been detected in patients with squamous cell carcinoma of the esophagus, lung, head and neck, anal canal and uterine cervix.^[8]

Carcinoembryonic antigen (CEA)

CEA is a protein found in many types of cells but associated with tumors and the developing fetus. This antigen was first discovered in patients with adenocarcinoma of the colon in 1965. It is a complex glycoprotein of molecular weight 180 kDa that is associated with the plasma membrane of tumor cells, from which it may be released into the blood. CEA represents a heterogeneous group of molecular

species that consist of single polypeptide chains with varying carbohydrate components. The ratio of protein to carbohydrate varies from 1:1 to 1:5 in CEA molecules from different tumors. CEA is metabolized primarily by the liver with a circulating half-life that ranges from one to eight days. CEA was first identified in colon cancer; an abnormal CEA blood level is specific neither for colon cancer nor for malignancy in general. Elevated CEA levels are found in a variety of cancers other than colonic, such as head and neck breast, lung, pancreas, stomach, and ovary.^[6]

Serum Cytokeratin Fragments

In circulation, CK are detected either as partially degraded single protein fragments, as small complexes, or as large polymeric protein complexes. It has been reported that epitopes for CK 8 are located at amino acid (aa) residues 340–365, for CK18 at, 270–429 and for CK19 at, 311–367.^[9] The half life of the CK fragments in circulation is about 10-15 h, depending upon the size of the fragment. The process that cause the release of soluble CK fragments into the circulation have not been completely elucidated but appear to involve multiple pathways including proteolytic degradation of CK in dying cells, abnormal mitosis, spillover of monomeric CK polypeptides from proliferating cells, apoptosis, etc. These CK fragments can be detected in a number of body fluids including blood, urine, cystic fluid, ascites, pleural effusions, and CSF after their release from tumor cells. It was also reported that, in normal, apparently healthy individuals, the level of CK in the circulation is low and it rises significantly in patients with carcinomas.^[11]

Serum CK fragments as tumor markers

Cytokeratin fragments in serum, offer a simple, minimally invasive, cheap, and reliable tool for more efficient management of cancer. As described earlier, TPA, TPS, and Cyfra21-1 are being mainly used as prognostic markers. The levels of these CK fragments in serum can be quantified using various commercially available specific serological assays.^[9]

The clinical value of determining soluble CK protein fragments in body fluids lies in the early detection of recurrence and the fast assessment of the efficacy of response to therapy in carcinomas. In addition

to this, other serum tumor markers have also been examined for their value in the management of various malignancies. To list a few in HNSCC, SCCAg and CEA, in lung cancer, CEA, SCCAg, Neuron specific enolase(NSE), progastrin releasing peptide (ProGRP), in breast cancer, CA15-3, in gastrointestinal cancer CEA, CA-242, in cervix cancer CA 125, have been examined.^[10]

Tissue polypeptide antigen (TPA)

Tissue polypeptide antigen (TPA) is a heterogeneous combination of molecules of molecular weight between 20-45 kDa. It was first defined as a tumor associated antigen in 1957 by Bjorklund.^[12] This is one of the oldest tumor markers in use. It has been shown that TPA is immunologically related to a mixture of non-epidermal CK, like CK 8, 18, and 19. TPA is produced during the S and G2 phases of cell cycle. It is secreted into the circulation during and immediately after mitosis, it has been shown that the concentration of the antigen is higher in the tumor tissues and in the serum of cancer patients as compared to normal tissues or normal serum, respectively. Due to its broad specificity, TPA is not being used frequently as a tumor marker in recent years.^[13]

Tissue polypeptide specific antigen (TPS)

The assay for tissue polypeptide-specific antigen (TPS) detects soluble fragments of cytokeratin 18, an acid cytokeratin protein present in epithelial cells.^[14] Tissue polypeptide specific antigen was identified long ago in human carcinomas and cell lines by the using of antibodies directed toward insoluble tumor material. These antibodies have been shown to stain cytoskeletal intermediate filaments in HeLa cells. It is a specific cytokeratin-based assay, which detects a defined epitope structure located on the rod domain within aminoacid (aa) residues 322-342 of human CK 18 using M3 monoclonal antibody.^[10]

Cyfra 21-1

This marker is recognized by two monoclonal antibodies against fragments of CK 19 in the serum. CK 19 is a type I CK which is released into the serum as soluble fragments. CK 19 is a 40 protein sequence. The epitopes of the two antibodies were determined to be within helix 2B of the rod domain of CK 19, the

epitope sequences lie within the a.a sequence 311-335 for the catcher antibody Ks 19.1 and within 346-367 for the detector BM 19.21. These sequences are unique as could be confirmed from sequence database. Both these antibodies raised by immunization of mice with MCF-7 cells. It is a cytoplasmatic protein which forms the intermediate filament cytoskeleton within epithelial cells. The cytokeratins appear to be distributed in the various epithelia, according to the cell differentiation. During the malignant transformation, the epithelial cells appear to contain the same cytokeratins as do normal cells.

In vitro cleavage of CK19 protein has been reported by to occur through spontaneous caspase 3 activity, resulting in the release of Cyfra 21-1 into the supernatants of cancer cell lines. The elevation of extracellular Cyfra 21-1 concomitantly with significant increase of intracellular Cyfra 21-1 during apoptosis; furthermore, the cell dying by caspase independent death in the presence of the Z-VAD caspase inhibitor did not release measurable Cyfra 21-1. So, the release of Cyfra 21-1 has been suggested to occur in cells during intermediate stage of apoptosis, as a consequence of caspase activation, then into the extracellular space.^[15]

Cyfra 21-1, as reported before, is a soluble fragment of cytokeratin 19. The assumption is that Cyfra 21-1 is released into the bloodstream during cell death, and therefore its level correlates very well with the tumour mass, or more specifically with the necrosis in the tumour, which is a function of the tumour mass. The finding that Cyfra 21-1 levels may be an independent marker and the preferred prognostic factor in head and neck cancer may indicate that this marker reflects tumour mass more accurately than it does the stage of the disease as expressed by the TNM. This findings may also have a therapeutic implication, as the tumour mass is one of the main parameters in deciding a therapeutic regimen.^[16] The detection of soluble K19 fragments in the serum released by carcinoma cells by the Cyfra 21-1 assay has found broad clinical application as a marker to monitor treatment and evaluate response to therapy and has proven particularly useful in the case of squamous cell carcinomas of the lung.^[5]

Conclusion:

Serum tumor markers have the potential to be valuable tools for diagnosis, prognosis, and treatment monitoring of different cancers. Their clinical utility has been demonstrated in lung and breast cancer and to some extent in head and neck cancers. It is apparent that these markers may also prove useful in predicting the risk of recurrence and/or involvement of regional lymph node metastasis in human oral cancers.

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