

CYFRA 21-1: AN OVERVIEW

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Abstract

Cytokeratins are group of intermediate filament proteins and based on the tissue expression, cytokeratins are classified into simple epithelial cytokeratin and stratified epithelial cytokeratins. CK expression pattern in the malignant cells are usually retained from the cell of origin, and therefore CK are being used in tumor typing. Cyfra 21-1 is a soluble fragment of cytokeratin 19 is an acid type cytokeratin, with a molecular weight of 40,000d. 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 aim of this article is to review on cyfra 21-1 and its role as a diagnostic and prognostic marker.

Keywords: Cytokeratins, Intermediate filaments, cyfra

Introduction:

Amongst the three cytoskeletal systems found in eukaryotic cells, the intermediate filament (IF) protein family is most complex. Depending on their polymerization properties and tissue specificity they are divided into six subtypes. Intermediate filaments of type I and type II are cytokeratins¹. Cytokeratins are also classified based on the expression as simple epithelial cytokeratin and stratified squamous cytokeratin. CK like all other IF demonstrate high resistance to detergent action and to high and low ionic salt concentrations². Cytokeratins are mainly involved in the protection of epithelial cells from mechanical and non-mechanical stresses that resulting in cell death. Other emerging functions include roles in cell signaling, the stress response, apoptosis, and other tissue specific functions.

Cytokeratins

CK make up the largest subgroup of IF proteins and represent the most abundant proteins in epithelial cells. Their expression is site

specific and differentiation dependent. The epithelial CK are closely related-both biochemically and immunologically. At present, more than 60 CK genes have been identified from the human genome sequence; of them 54 are functional genes. CK are sub-grouped into type I (40- 56.5 kDa) and type II (53-67 kDa) CK. Type I are acidic while Type II are basic CK¹.

Depending on their tissue expression pattern, they have been grouped into simple epithelia specific CK (CK7, 8, 18, 19, 20) and stratified epithelia specific CK (CK 4, 5, 13, 14, etc.). The most abundant epithelial CK are CK 8, 18, 19. Protein structures of CK consist of a central alpha helical rod domain, flanked on either side by amino terminal (head) domain and carboxy terminal (tail) domain the alpha helical rod domain is a highly conserved region amongst all IF, while the head and the tail domains impart differential characteristics like molecular weights, isoelectric point, and antigenicity². The type I keratin K19 is the smallest keratin and is exceptional since it

widely lacks the non- α -helical tail domain typical for all other keratins. So it is also called as tail-less intermediate filament protein³.

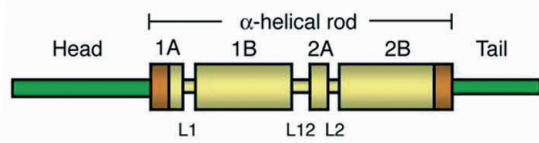


Fig 1: protein structure of cytokeratin intermediate filament

The primary function of CK is to protect epithelial cell from mechanical and non-mechanical stresses that resulting in cell death. Other emerging functions include roles in cell signaling, the stress response, apoptosis, and other tissue specific functions. The involvement of CK in a number of human diseases is now established⁴. Cytokeratins undergo several post-translational modifications these modifications influence the biological activity of the filaments resulting in increased solubility and filament reorganization².

CK expression pattern in the malignant cells is usually retained from the cell of origin, and therefore CK are being used in tumor typing⁵. Simple epithelia specific CK 8, 18 and 19 are normally not expressed in oral tissues, however, they are expressed in oral SCC. Aberrant expression of CK 8 and 18 is the most common change in human oral cancer. Ck 8 and 18 expression has also been correlated with invasiveness of the tumor margin and poor prognosis of human oral SCC⁶.

Cytokeratin deposition has been reported to occur in the necrotic regions intratumorally because of increased proteolytic activity in these cells. Another consequence of the increased proteolytic activity in tumor cells is the appearance CK fragments in the sera of cancer patients². The three most frequently used CK which are being evaluated as serum markers for their utility in clinical applications are tissue polypeptide antigen (TPA), tissue polypeptide specific antigen (TPS), and cytokeratin

fragments 21-1 (Cyfra 21-1). Assays for TPA measure CK 8, 18, and 19 and assays for TPS and Cyfra 21-1 are more specific and measure CK 18 and CK 19 levels, respectively.⁷

Cyfra 21-1

Cyfra 21-1 is a soluble fragments of cytokeratin 19 is an acid type cytokeratin, with a molecular weight of 40,000d¹. This marker is recognized by two monoclonal antibodies against fragments of CK 19 in the serum. 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.

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. Apoptosis results in fragmentation of cells into apoptotic bodies which are engulfed by neighboring cells and macrophages. Apoptotic bodies that are not engulfed by macrophages will disintegrate ('secondary necrosis') and their contents may subsequently reach the circulation⁸.

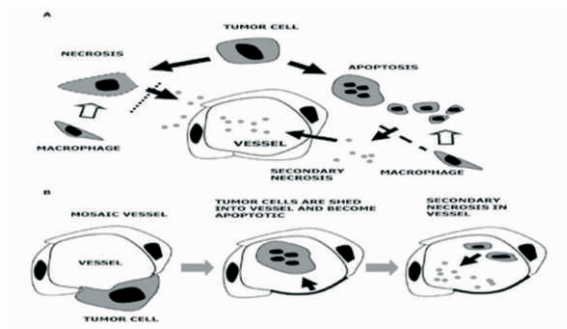


Fig 2: schematic diagram shows cytokeratin fragments in circulation

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 regimen⁹. 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 therapy¹⁰.

Cyfra 21-1 in diagnosis, prognosis and surveillance of cancers

Ilana Doweck et al (1995)¹¹ measured Cyfra 21-1 (cytokeratin fraction 21-1) in oral squamous cell carcinoma patients and found Cyfra 21-1 levels were in good correlation with the tumor stage expressed by the tumor size and the lymphatic spread (N) and were inversely correlated with histologic grades.

Kurokawa et al (1997)¹² found 90% accuracy for cyfra21-1 and concluded Cyfra may be useful for the screening of patients with suspected oral squamous cell carcinoma.

Yen et al (1998)¹³ assessed clinical value of CYFRA for squamous cell carcinoma of the head and neck, and found diagnostic sensitivity of CYFRA was superior especially for nasopharyngeal carcinoma. So CYFRA may be useful in monitoring recurrence of certain types of SCCHN, which are sometimes difficult to detect.

Nagler et al (1999)⁷ examined the early diagnoses and treatment monitoring roles of cyfra 21-1 and found to be significant.

Deng et al (2003)¹⁴ in his study found that Serum levels of CYFRA 21-1 in patients with HNSCC were significantly higher than those of healthy controls and concluded CYFRA 21-1 is valuable not only for diagnosis but also for close monitoring of patients with HNSCC.

Zhong et al (2007)¹⁵ also found there was significant correlation in cyfra level with tumour recurrence and survival rate, the higher the serum Cyfra 21-1; the higher the tumour recurrence rate and lower the survival rate. So he concluded serum Cyfra 21-1 was an independent prognostic factor for OSCC.

Conclusion

Cyfra 21-1 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. However, systematic follow-up studies from defined sub-sites and using antibodies specific to identified CK fragments in circulation are necessary to evaluate their sensitivity and specificity before they can be used as non invasive markers for prognostication, follow-up of therapy, and detection of recurrence and metastases in oral cancer.

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