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BIOMARKERS TO PREDICT ORAL SQUAMOUS CELL CARCINOMA IN PRECANCEROUS STAGES

BIOMARCADORES PARA PREVISÃO DE CARCINOMA ESPINOCELULAR BUCAL EM LESÕES CANCERIZÁVEIS

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There have been many attempts to establish biomarkers in order to determine the susceptibility of some normal human tissues to undergo malignant change. However, definitive markers for oral squamous cell carcinoma have not been yet achieved. Amongst some of the most promising molecular biomarkers, there is a respectful amount of literature produced on p53, both in human tissue fluids and in biopsies of potentially malignant lesions, 3p gene deletions, and, most recently, image-based ploidy analysis of tissue specimens. In spite of the experimental character and speculative results of all those novel techniques, the image-based ploidy analysis appears to be the most sensitive and reliable method to predict malignant transformation in potentially malignant lesions of the oral mucosa.

UNITERMS: Oral premalignancy; Tumour markers; Squamous cell carcinoma; Image-based ploidy; Cancer prevention.

INTRODUCTION

There have been numerous attempts to establish or even develop tumour markers to determine the susceptibility of normal tissues to transform into cancer. The literature on this subject is extremely vast, however definitive surrogate markers for squamous cell carcinoma of the mouth are still lacking [29].

Current predictive indicators can be subdivided into morphologic (clinicopathological)and molecular (serologic/salivary and genomic).

The present is a brief review on the most common and/or promising biomarkers to predict oral carcinogenesis or even probable malignant transformation in potentially malignant lesions.

Serologic Markers

Main serologic markers include p53 antibodies and fragments of cytokeratin 19 [25]. p53 antibodies can be found in some tissue fluids of cancer patients with a specificity of 96% and a sensitivity of about 30% [20]. Although few studies involving head and neck patients have been reported, recent investigations on oral carcinoma cases have indeed demonstrated p53 antibodies in both sera [6] and saliva [23, 31]. Interestingly, serum p53 antibodies were also detected by enzyme-linked immunosorbent assay (ELISA) in 5% of heavy smokers with no oral lesions [18]. The clinical significance of p53 as both serum and saliva markers remains to be explored and follow-up studies are awaited. Serum-soluble

fragments of cytokeratin 19 can be measured in terms of CYFRA levels and have been reported to be significantly higher in patients with squamous cell carcinoma of the head and neck when compared to healthy controls [13]. Although this marker has shown 95% specificity and 60% sensitivity for head and neck squamous cell carcinoma at a cut-off value of 2.2ng/mL, significantly lower CYFRA levels were detected in both smaller and earlier-staged tumours, reducing its potential diagnostic use.

Morphologic markers

For many years, a few lesions and conditions of the oral mucosa have been regarded as potentially malignant. Although there is no consensus on whether most of such lesions and conditions are really potentially malignant, tobacco associated and idiopathic keratosis of the oral mucosa (leukoplakia) appears to be accepted worldwide as a lesion in which cancer is more likely to occur [1]. Oral premalignancies are traditionally classified as leukoplakia, erythroleukoplakia, erythroplakia, and distinguished from precancerous conditions. Major attention is focused on leukoplakia due its incidence and potential to malignant transformation. Although the incidence of oral keratosis (leukoplakia) is practically difficult to ascertain, it surely exceeds the incidence of oral cancer [26]. Rates of malignant change vary partly according to the population, gender and tobacco habits. Studies in India have reported malignant transformation rates ranging from 0.06 to 0.3 [7, 19], whilst in Sweden these proportions increased up to 3.6% [2]. On the basis of the lowest reported annual transformation rate, patients with smoking and idiopathic oral keratosis carry a 5-fold higher risk of developing oral cancer than controls [2].

Erythroplakias are a lot rarer than leukoplakias and carry an over 90% risk of malignant change, in which case their use as a predictive marker would not be appropriate [12]. Histological grading of epithelial dysplasia was originally believed to be helpful in the prediction of malignant transformation. The WHO have graded it as mild, moderate, or severe, according to the importance of cellular atypia and to the thickness of the dysplastic layers compared with the total epithelial height [11]. Nonetheless, there are no current objective methods for grading dysplasia that give consistent and reproducible results [16]. Studies involving experienced oral pathologists have reported a strong interobserver discrepancy in the evaluation of both the presence and degree of epithelial dysplasia. However, it is still recommended that a statement on the presence or absence of dysplasia in leukoplakia, as well as an assessment of its severity be included in the histological report [3].

Specific genes

Alterations in p53 tumour-supressor gene constitute the most common genetic aberration in a broad spectrum of human tumours [8, 9]. p53 has also become a celebrity amongst the other tumour-related genes given the vast number of publications reporting its correlation with head and neck carcinoma. The distribution and nature of p53 mutations in 793 head and neck cancers described in the literature were analyzed using data from the International Agency for Research on Cancer p53 mutation database. An examination of entries from studies reported on oral, oropharyngeal and other head and neck cancers does not display distinct mutation patterns for various sites examined, but a detailed comparison of mutations of oral and oropharyngeal sites suggest that the pattern of mutations is more complex and different from classical tobacco-associated cancers such as lung, larynx, and the bladder [25].

In potentially malignant lesions of the oral cavity, leukoplakia has particularly been reported with a wide variation of p53 protein expression and a meta-analysis suggests that p53 can be detected in at least half of these lesions [28, 30]. This has lead many authors to erroneously conclude that p53 overexpression is an early event in the pathogenesis of oral carcinoma [33], or to suggest a potential use of p53 as a biomarker to assess the risk status of leukoplakia to identify those lesions with an increased risk of converting to the malignant phenotype [10]. Although p53 protein overexpression is commonly reported, p53 mutations are uncommon in preinvasive lesions [4]. The exception appears to be erythroplakia lesions [17], with 46% of these cases harbouring a p53 mutation, a frequency significantly higher than that reported for leukoplakia. Proliferative verrucous leukoplakia, with a high risk of malignancy that approaches 100%, also demonstrated universal presence of p53 protein in all sequential biopsies taken from 10 subjects in a London study who all developed carcinoma [27].

Chromosomal losses and gains

The advances in cytogenetic methods, restriction fragment length polymorphism (RFLP), and molecular studies with highly polymorphic microsatellite markers have allowed the identification of allelic imbalances or loss of hetorozygosity

(LOH), which may contain putative tumour supressor genes in oral, oropharyngeal, and other head and neck cancers [25].

Following the pioneer studies by Partridge and colleagues [14], deletions in the long arm of chromosome 3 in oral cancer has now been highlighted as a very common event, occurring in approximately 70% of all SCC [15]. Furthermore, it was shown that allelic imbalance or LOH in 3p were present in dysplastic mucosa but not in normal mucosa and, therefore, an early event in the process of carcinogenesis [5]. In potential malignancies, deletions of 3p were rather frequent, especially when dysplasias recurred, or when SCC developed. 3p deletions thus might serve as a biomarker to identify lesions that may relapse or even undergo malignant change [32]. Moreover, it has been demonstrated that if one introduced an intact human chromosome 3 into different tumorigenic cell lines, tumourigenicity would be suppressed in each cell line, with significant decrease in in vitro growth rate and morphologic changes [24].

Loss of loci in chromosome 9p and amplification of 11q have also been related to SCC of head and neck. However, no reports have been found in potentially malignant lesions. Both have shown striking prognostic value for cancer rather than predictive of development of cancer.

Image-based ploidy analysis

Very recently image-based ploidy analysis has been shown highly effective to predict malignant transformation with high sensitivity and specificity and can be applied routinely to biopsy samples in a hospital setting.

Cumulative evidence points to abnormalities in the number of chromosomes (aneuploidy) as a cause rather than a consequence of malignant transformation [21]. Notwithstading this, considering that cancer and genetic instability are almost synonyms, mutations in genes that control segregation of chromosomes during mitosis and/or centrosomal aberrations are critical to determine malignant change. As a matter of fact, aberrant chromosomal segregation occur exclusively in aneuploid tumour-cell lines, causing, therefore, aberration of DNA content (ploidy) to play an essential role on carcinogenesis [21, 22].

A Norwegian study has recently revealed what appears to be the first successful attempt to establish a methodology for prognostication of potentially malignant lesions of the oral mucosa. One hundred and fifty patients with an initial diagnosis of leukoplakia had their lesions analyzed by image-based ploidy and were followed up for 9 years. Out of the 27 aneuploid lesions, 21 underwent malignant transformation. From the 20 tetraploid lesions, 12 changed into the malignant phenotype, whilst only 3 out of the 103 diploid lesions became malignant [21]. Although DNA ploidy measurements by image cytometry is a very crude method, with a coefficient of variation of 3-5%, which translates to 1-2 chromosomes per nucleus, the long follow-up time presented in this study supports the notion that DNA content is a highly sensitive and a specific marker for predicting the subsequent occurrence of an oral squamous cell carcinoma [22].

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RESUMO

Inúmeras tentativas têm sido feitas para estabelecer ou mesmo desenvolver marcadores para determinar a susceptibilidade de tecidos histologicamente normais sofrerem transformação maligna. A literatura é extremamente vasta no assunto, muito embora um marcador definitivo para o carcinoma de boca ainda não tenha sido estabelecido. Os indicadores atuais podem ser subdivididos em morfológicos (clinico-patológico) e moleculares (serológicos/salivares e genômicos). Dentre eles, destacam-se o p53 tanto no soro como no sítio da lesão, a deleção de porções do gene 3p e, mais recentemente, a análise de ploidia baseada em imagem. Embora todos sejam cientificamente objetos de muita pesquisa e resultados especulatórios, a ploidia baseada em imagem parece ser o método mais sensível e confiável para prever transformação maligna em lesões cancerizáveis da mucosa bucal.

UNITERMOS: Lesões cancerizáveis; Marcadores tumorais; Carcinoma espino-celular; Análise de ploidia por imagem; Câncer, prevenção.

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