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The flow cytometric analysis of premalignant and malignant lesions in head and neck squamous cell carcinoma

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Summary We attempted to identify the molecular mechanisms involved in Head and Neck Squamous Cell Carcinoma (HNSCC) pathogenesis by measuring the nuclear DNA content (ploidy) in premalignant (potentially malignant) and malignant patients as compared to normal controls, and to determine whether DNA ploidy could be used to predict the clinical outcome. From March 2001 to December 2003, the analysis was carried out in a set of 41 patients with premalignant lesions and 79 suffering from squamous cell carcinoma of laryngeal, oesophageal, nasopharyngeal, nasal and oral lesions and 50 controls. Representative samples were taken by punch biopsy and processed using standard formol-paraffin technique for histopathological examination. Fifty micrometer thick sections of paraffin-embedded tissues were analyzed to detect the DNA content by image cytometry. Of the potentially malignant patients, 46% had diploid lesions, 37% had tetraploid lesions and 17% had aneuploid lesions. While of the patients with cancer, 90% had aneuploid lesions, 10% had diploid lesions and none had tetraploid lesions. DNA diploidy tended to occur earlier in the progression from premalignant to malignant lesions and this helps us early detection of HNSCC by DNA from lesions in high risk groups and examination of its ploidy. Knowledge of tumor cell ploidy by DNA image cytometry may facilitate the evaluation of malignant and premalignant lesions in HNSCC. The present findings are promising to supplement clinical and histopathological parameters in evaluating prognosis and to demonstrate methods that are readily applicable for routine diagnostic work.

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Introduction

Approximately 3% of malignant tumors originate in the oral cavity. The majority of which correspond to squamous cell carcinomas (SCCs).[1] It is the sixth most prevalent cancer in the world with a global yearly incidence of 500,000[2] and it is an aggressive malignant neoplasm. The prognosis for patients with this pathology depends on the size, infiltration and location of the lesion, presence or absence of metastatic spread, and to a certain degree the differentiation of the tumor[3].

Oral leukoplakia/erythroplakia has been considered a premalignant condition because its presence places the individual at increased cancer risk. However, the overall incidence of cancer development in subjects with oral leukoplakia is in the range of 4.4—17.5% in different studies.[4] The evidence that oral leukoplakias are pre-malignant mainly derived from follow-up studies, mostly obtained on hospital-based observations.

While, between 5% and 15% of oral white patches are classified histologically as dysplasia, 15—20% develop into carcinoma.[5] A recent study of 150 patients with oral leukoplakia and another of 37 patients with oral erythroplakia histologically typed as dysplasia convincingly showed that histological grading into mild, moderate, and severe epithelial dysplasia had no significant value in predicting malignant development.[6,7]

When histological evidence of moderate to severe dysplasia is present, the incidence of cancer development substantially increases to 36%. White patches (leukoplakia) of the oral cavity have a well-documented potential to develop into SCC,[4] and when this occurs, the odds of surviving more than five years are poor. Although histological evidence of epithelial dysplasia is still the most important predicator for cancer risk, epithelial histological appearance is not always predictive of individual patient outcome; e.g., the cancer risk is still significant in subjects with hyperplasia and mild dysplasia, and many individuals with severe dysplasia show persistent stability for many years. Thus, there is considerable uncertainty as to whether or not all clinically detectable lesions characterized as precursors will eventually develop into carcinoma.

Premalignant conditions showing abnormal DNA content are at high risk of transformation. The ability to subject histological material to genetic analysis has provided new insight into the origin of multiple preneoplastic and neoplastic lesions arising in the oral cavity.

In the current study, it is possible to use premalignant and malignant cells for analyzing the DNA patterns and ploidy in cytophotometry. Tumor ploidy, measured through flow cytometry, seems to be another prognostic variable. The technique combines knowledge acquired from DNA contents analysis,[3,8,9] the development of fluorochromes and computer data processing, which are used to establish a series of cellular parameters (Fig. 1). It is an objective and automated method for measuring cellular parameters and thus helps in tumor diagnosis, measuring the DNA content and therefore reflecting aneuploidy[10] being a highly sensitive and specific parameter. The DNA content also relates to the prognosis,[11—16] in such a way that a DNA index (DNA content of G1 phase tumor cells/DNA content of G1 phase euploid cells) other than 1 is accompanied by a worse prognosis. Likewise, this technique can be applied to the treatment of patients, since in chemotherapy and radiotherapy the response to treatment can be related to the alterations produced in the cell cycle.[17] Previous studies of the prognostic value of DNA quantitation in premalignant oral lesions included fewer than 25 patients with at least five years of follow-up.[18—20]

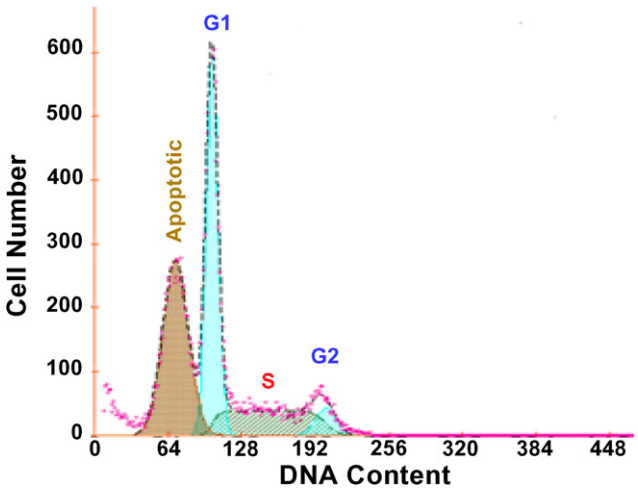

Figure 1 The profile of four cell populations with their corresponding DNA contents and/or aneuploidy during the normal cell cycle measured by image flow cytometry.

Terminology and definitions

The WHO has accepted the latest international attempt on terminology and definitions and there is general agreement that a pre-cancerous and/or premalignant lesion is defined as ''a morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart'', whereas a pre-cancerous and/or premalignant condition is defined as ''a generalized state associated with a significantly increased risk of cancer''. The latter definition signifies that the cancer can arise in any part of the oral cavity and not necessarily in a pre-existing lesion.[21] The designations 'pre-cancer', 'pre-cancerous', 'pre-malignant', and 'precursors' will be used synonymously throughout this study for diseases with a malignant potential.[22]

Materials and methods

The present study included 120 individuals who were admitted to the Ear, Nose and Throat department, Sohag University Hospital in the period between 2001 and 2003. Individuals with precancerous lesions involved larynx, pharynx, oral cavity, and nose. SCC included larynx, pharynx, oesophagus, and nasopharynx. While the reference subjects (controls) included 50 individuals.

Seventy nine tissue samples of HNSCC and 41 precancerous tissue samples were collected from the patients before treatment. Samples were immediately frozen and stored at −80 °C. All specimens subjected to the histopathological examination for diagnosis and grading.