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Review Article

Role of Proliferative Immune Markers in Oral and Maxillofacial Pathologies: A Review

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ABSTRACT:
Immunohistochemistry is important in diagnosis, investigation, and determining the behaviour and pathogenesis of oral tumours. Immunohistochemistry protocols were developed using antibodies tagged with chromogens to identify specific markers. Cell proliferative activity has been extensively investigated in head and neck tumours. Ki-67/MIB-1 immunostaining, tritiated thymidine or bromodeoxyuridine labelling indices, DNA S-phase fraction, proliferating cell nuclear antigen expression, potential doubling time and analysis of the nucleolar organizer region associated proteins (AgNORs) have earlier shown significant correlation with prognosis in many cases of tumours of the oral cavity, salivary glands, pharynx and larynx. Provided that large and homogeneous series are evaluated by standardized methods, cell proliferative activity can still be regarded as an inexpensive and reliable prognostic factor in head and neck tumours.
Keywords Immunohistochemistry, cell proliferative activity, head and neck tumour's, prognosis.

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INTRODUCTION

The application of immunologic research methods to histopathology has been resulted in marked improvement in the microscopic diagnosis of neoplasms. Although histologic analysis of hematoxylin and eosin stained tissue sections remains at the core of the practice of head and neck surgical pathology, immunohistochemistry had become a powerful tool in the armamentarium of the pathologist.[1] It affords a significant advantage in the diagnosis of difficult and equivocal tumors. Immunohistochemistry has also provided insight into tumor histopathogenesis and has contributed to more accurate determination of patient prognosis. Predictable tumor expression of many of the same antigens (a macromolecular protein or polysaccharide that can bind to an antibody molecule) as their cells of origin or normal tissue
counterparts validate the principle of tumor classification by immunohistochemistry.

APPLICATION OF IMMUNO-HISTOCHEMISTRY

Application of immunohistochemistry in distinguishing undifferentiated oral neoplasms of different origins was achieved through the detection of tumor antigens using known antibodies. Thus, immunohistochemistry is important in diagnosis, investigation, and determining the behavior and pathogenesis of oral tumors.[2] Tumor markers are measurable biochemicals that are associated with a malignancy. These markers are either produced by tumor cells (tumor-derived) or by the body in

response to tumor cell (tumor-associated). They are typically substances that are released into the circulation and thus measured in the blood.[3]

TUMOR MARKERS

Tumor markers are not the primary modalities for cancer diagnosis rather they can be used as laboratory test to support the diagnosis.[4] The tumor marker level may also reflect the extent (the stage) of the disease, indicating how quickly the cancer is likely to progress and helping to determine the prognosis (outlook). Rising levels of tumor markers on test results can be but are not always worrisome. Although changes in tumor marker levels may be the cause for alarm, other noncancerous diseases can cause test results to vary. Conditions in the laboratory that process tests may also alter the results. [5] One of the most important uses for tumor markers is to monitor patients being treated for cancer. If the initially raised tumor marker level goes down with treatment, it indicates that the treatment is working and is having a beneficial effect. On the other hand, if the marker level goes up, then the treatment is probably not working and change of treatment should be considered. Some newer tumor markers help to assess how aggressive a cancer is likely to be or even how well it might respond to certain drugs. Markers are also used to detect cancers that recur after initial treatment. Some tumor markers can be useful once treatment has been completed and with no evidence of residual cancer left. These include prostate-specific antigen (for prostate cancer), human chorionic gonadotropin (for gestational trophoblastic tumors and germ cell tumors of ovaries and testicles), and cancer 125 (for epithelial ovarian cancer).[6] Cell proliferation is regarded as one of the most important biological mechanisms in oncogenesis.[7] A survey of the results of many studies has shown that proliferative activity is of high prognostic significance in several types of cancer.

LITERATURE REVIEW

A Medline-based search up to July 2003 selected 6305 reports containing the terms ‘proliferative activity and tumor’; 4373 reports containing the terms ‘proliferative activity’ and ‘tumor diagnosis’, and 1005 containing the terms ‘proliferative activity’ and ‘tumor prognosis’. Cell proliferation has also been extensively investigated in head and neck tumors: up to July 2003, 306 papers have been published on the proliferative activity in tumors of the oral cavity, salivary glands, pharynx and larynx.[8]

CLASSIFICATION

Proliferation markers can be classified into three main categories: (i) growth fraction markers; (ii) markers of specific phases of the cell cycle; and (iii) cell cycle time markers. The growth fraction, i.e. the proportion of the cells committed to the cycle, may be easily assessed by Ki 67 or MIB-1 antibodies, which identify an antigen expressed in G1, S and G2 phases of cycling cells. The M-phase can be evaluated by counting the mitotic figures: this is the oldest and, probably, the most popular way of assessing proliferation, even if strict morphological criteria for the recognition of mitotic figures are required. S-phase fraction (SPF) can be assessed by incorporation techniques, such as the in vivo or in vitro incorporation with tritiated thymidine (TH3) or bromodeoxyuridine (BrdU), which can be regarded as the ‘gold standard’ marker of S-phase cells. SPF can also be detected by static or flow cytometry (FCM) analysis of the DNA, or the immunohistochemical detection of proliferating cell nuclear antigen (PCNA/Cyclin), a nuclear protein involved in DNA synthesis. The very reliable punctuated labeling of PCNA is identical to the labeling pattern obtained with BrdU and is the method of choice of evaluating the S-phase index in histopathology.

CELL CYCLE TIME

Cell cycle time can be evaluated by the potential doubling time (Tpot), a procedure that requires in vivo intravenous BrdU infusion and bivariate FCM, or by the quantification of the argyrophilic proteins associated with the nucleolar organizer regions (AgNORs), loops of DNA which transcribe to ribosomal RNA. AgNOR proteins can be easily detected on routinely fixed and paraffin embedded tissues. The AgNOR quantity is strictly related to the rapidity of cell proliferation: the higher the AgNOR quantity, the shorter the doubling time.[9]

PROLIFERATIVE CHARACTERISTICS

The proliferative characteristics of normal oral epithelia and leukoplakias have been studied previously using mitotic counts and pulse labeling with tritiated thymidine or bromodeoxyuridine. Most of these reports have not specified the site of the oral mucosa studied or have focused on buccal mucosa. Although many studies have considered the deepest three epithelial layers as a single progenitor compartment, others have divided this compartment into the basal and parabasal layers. In addition, a recent report describes the LI of normal oral mucosa using immunohistochemical staining of PCNA. The increased proliferative capacity of leukoplakia has been recognized by several laboratories. Recently, similar conclusions have been reached using immunohistochemically detectable proliferation markers such as PCNA and 10-67. In detailed quantitative study of PCNA expression in head and neck lesions, Shin *et al.* showed a gradual increase in the PCNA LI in all three layers, reaching a maximum in dysplastic epithelia.[10]

DISCUSSION

A large tumor specimen may be classified positive or negative depending on whether the proliferation markers are evaluated only at the most ‘active’ sites (e.g. the invasive tumor front) [11], in randomly selected

areas or in the maximally positive tumor field. There is a difficulty in identifying the neoplastic cells. It is often hard to distinguish by MIB-1 or PCNA immunostaining small neoplastic cells from reactive proliferating lymphocytes in poorly differentiated rhinopharyngeal carcinomas. AgNOR staining is particularly suitable in such cases, since it allows for an easy distinction between neoplastic and non-neoplastic cells, avoiding the need for double (Cytokeratin/MIB-1) staining. Finally, what was indeed measured in most studies reported is not the actual proliferative activity of a tumor, in as much as the mechanisms responsible for proliferative activity are the proportion of cells committed to cycle (growth fraction, or G) and the speed of the cycle, which is inversely proportional to the generation time (T).[7] The tumor growth fraction can be easily assessed on routinely processed tissues by the Ki67 or MIB-1 labeling index, since Ki67 or MIB-1 antibodies recognize an antigen expressed in all cycling cells.[12] On the contrary, evaluation of the speed of cell cycle by the potential doubling time (Tpot) is a rather lengthy procedure and is not suitable for retrospective studies. However, since the AgNOR quantity reflects the rapidity of cell proliferation,[13] and can be detected in routinely fixed and embedded tissues,[14] AgNOR analysis can be regarded as an easy and reliable technique to evaluate the tumor cell doubling time on histological preparations. Indeed, a high cell proliferation, as expressed by the MIB-1 labeling index, was a significant indicator for treatment failure in a large matched-pair study design of recurrent and non-recurrent oral and oropharyngeal carcinomas initially treated with primary surgery combined with curative post-operative radiation.[15] Also, in another large matched-pair study on recurrent and non-recurrent laryngeal carcinomas, homogeneous for site (glottis), stage (T1 and T2) and treatment (transoral laser surgery alone), investigated using well standardized MIB-1 and PCNA staining and scoring, high proliferative activity appeared to be a significant prognostic factor. Lastly, the standardized AgNOR analysis showed the strong independent prognostic value of cell proliferation in a large series of oral squamous cell carcinoma.[11]

CONCLUSION

Many molecular markers are associated with the occurrence, progression, and prognosis of carcinoma. Markers of increased proliferation in oral cancer have been identified and explored for more than a decade. However, markers of cellular proliferation are difficult to interpret as an independent scale for judgment for tumor prognosis, but it does significantly help in assessing the magnitude of tumor spread.

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