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Role of AgNORs in Oral Lesions-A Review

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Abstract

The biologic behavior of the oral lesions can be determined by their active phases of cell division, cells entering the cell cycle and their proliferation. AgNORs (argyrophilic nucleolar organizer regions) staining is the one of the cytochemical technique used to evaluate the cell proliferation of oral lesions. Tumor cells in premalignant and malignant conditions usually exhibits a rise in synthesis of abnormal proteins and proportionately there is a increase in the AgNOR material. The aim of this study was to review the concepts of proliferative activity of cells of malignant and premalignant conditions through the AgNORs staining procedure and thereby proving the usefulness of AgNORs in diagnosing the oral lesions.

Keywords: *Nucleolar organizer regions, NOR-associated proteins, Argyrophilic nucleolar organizer regions, proliferative activity, AgNOR dots, quantitative, qualitative.*

Introduction

In a cell, the nucleus plays an important role in the proliferative activity and synthesis of proteins. Nucleolar organizer regions(NORs) are the loops of DNA present in the nucleoli of cells possessing the genes that transcribe for synthesize of rRNA [1]. These Nucleolar organizer regions(NORs) correspond to secondary constrictions of metaphase chromosomes of eukaryotic cells. These Nucleolar organizer regions(NORs) are believed to be the centre for proliferative activity of the cell and these are located on the short arm of acrocentric chromosomes 13, 14, 15, 21, and 22 [2]. Nucleolar organizer regions(NORs) are associated with certain acidic, argyrophilic, non histonic proteins called NOR-associated proteins (NORAPs) [3].

These NOR-associated proteins (NORAPs) are demonstrated by colloidal silver, which makes bond with these proteins and these resultant silver stained reaction products are argyrophilic nucleolar organizer regions (AgNORs). In normal epithelial cells, the AgNORs are tightly packed within the nucleoli and are not identifiable. But in cases of neoplastic cells, nucleolar disaggregation may occur which may result in the dispersion of individual AgNOR. AgNOR correlates with the proliferative rate of the cell, as can be estimated by Ki-67 and the percentages of the S phase cells and the mitotic cells[2].

AgNOR staining procedure has found with many advantages such as simplicity, low cost, ease of use, specificity, good correlation with proliferative markers when compared to other histopathological staining procedures. After silver staining procedure, the AgNORs can be identified as black dots throughout the nucleolar area [1]. NORs reflects protein synthesis and are well known to get increase in their count in cases of premalignant and malignant conditions [3].

There are several studies have proved that AgNOR counts in their quantitative terms can be used to differentiate between benign and malignant lesions [4-7]. Whereas some studies have shown that qualitative representation of AgNOR such as morphological characteristics, shape, size and the pattern of distribution determines the proliferative activity of the cell and thereby used as a marker of malignant or premalignant lesions [8,9]. The interphasic NORS can be clearly

examined at the light microscope level by using a silver reaction which stains the acidic proteins of the NORS (RNA Polymerase 1 upstream binding factor, Topoisomerase 1, Nucleolin, Fibrillin, C23 protein and B23 protein) on routinely used histopathological and cytological tissue samples [10].

ARGYROPHILIC NUCLEOLAR ORGANIZER REGION STAINING PROCEDURE:

According to Crocker and Nar, the biopsy specimens are subjected to routine paraffin sectioning at 4 μm thickness after fixation with 10% formal saline. The sections are then deparaffinized in xylene and hydrated through decreasing grades of ethanol to double distilled deionized water. The sections were then reacted with freshly prepared silver colloidal solution (1 part by volume of 2% gelatin in 1% formic acid and two parts by volume of 50% aqueous silver nitrate solution) in a closed coplin jar for 35 min at room temperature, while ensuring that a dark environment was maintained throughout the reaction time. The silver colloidal solution was washed with double distilled ionized water. The sections were then treated with 5% sodium thiosulphate for 5 minutes and washed in double distilled deionized water, dehydrated through increasing grades of alcohol, cleared in xylene and mounted[11].

ARGYROPHILIC NUCLEOLAR ORGANIZER REGION COUNTING (QUANTITATIVE ASSESSMENT):

One hundred epithelial cells should be counted in each specimen under×100 oil immersion objective of the light microscope following in a zigzag manner. A standardized mean for the enumeration of NORs in the histological sections was proposed by Crocker et al[12]. By careful microscopic examination, AgNOR dots should be counted. While counting, both intranucleolar and extranucleolar dots should be included in the counting regime and where two or more dots were so closely aggregated within a nucleolus, the aggregate should be recorded as one. Whereas in some areas the closely aggregated dots separated by a halo of nucleoplasm, then the dots are counted separately[13].

ARGYROPHILIC NUCLEOLAR ORGANIZER REGION TYPES (QUALITATIVE ASSESSMENT):

Three types of AgNOR products are identified in histological sections according to Warnakulasuriya and Johnson[14]:

• AgNORs-Type I– Single or few large dots within the nucleus representing the nucleolus

• AgNORs Type II– Discrete small dots within the nucleolus

• AgNORs Type III– Fine black dots dispersed throughout the nucleoplasm.

Discussion

The neoplastic cells generally show a marked increase in the synthesis of normal and abnormal products of the cells such as proteins which results in significant rise in AgNOR material. The AgNOR counts increase with increased cell ploidy and with increased transcriptional activity in the stages of active cell proliferative phases[15]. The stage of the cell cycle, the transcriptional and metabolic activity of the cell or the number of NOR-bearing chromosomes determines the variations obtained in the size and number of AgNOR dots. In a rapidly and actively proliferating cell, there is a disorganized distribution of chromosomal and AgNORresulting in the formation of multipleand dispersed nucleoli. In addition, in these cells there is a disorganized nucleolar aggregation resulting in the increased AgNOR count, regardless of the ploidy state of the cell[2].

AgNORs have found greater application in the tumor histopathology in determining the growth potential and malignant potential of tumors, in differentiating between benign and malignant neoplasms, to assess the prognosis[16-19] and also to evaluate the risk of recurrence[20]. In oral pathology, AgNORs play as auseful tool in differentiating between odontogenic cysts and tumors, to distinguish recurrent and nonrecurrent giant cell lesions of the jaws, determining the biologic behaviour of OSMF, differentiating oral squamous cell carcinoma from benign and reactive lesions, in detecting incipient cellular alterations [21-25]. AgNORs have also proved to be useful marker of tumor progression, predict the response of tumor to treatment, and to detect residual viable tumor [26,27].

AgNOR count is useful in determining the differences between various grades of malignancies [3]. There is a study showing increase in the AgNOR count from well differentiated squamous cell carcinoma thoroughly moderately grade and reaches peak in poorly differentiated squamous cell carcinoma.

According to Sandhya Panjeta Gulia et al, their study proved that the mean AgNOR count showed a linear and significantly increasing in nature as the histopathological grade of the tumour increased ($p<0.05$) [2]. According to the study of Khushbhu et al, the Mean AgNOR count increased in the following sequence: i.e OSMF with mild dysplasia, leukoplakia with mild dysplasia, OSMF with moderate dysplasia, leukoplakia with moderate dysplasia and squamous cell carcinoma. In qualitative assessment, most of the sample present with Type II AgNOR pattern while Type III AgNOR pattern was found to be increasing with the increase in the grade of dysplasia [1].

According to the study of Elangovan et al, in a quantitative assessment, there is a difference present between the number of AgNORs counts between the normal mucosa, inflammatory lesions, and carcinomas, but the premalignant lesions show almost equalnumber of AgNOR count to normal mucosa. The number of AgNORs was found to be related to epithelial proliferation. In a qualitative assessment, morphological characteristics determines that there is no difference between normal mucosa and inflammatory lesion were alike, but the premalignant and malignant lesions showed significant differencefrom normal, with a increased degree of AgNOR pleomorphism being observed in carcinomas[3].

Conclusion

On analysing the various studies, there is a standard conclusion remains that AgNOR quantity is directly proportional to the proliferative activity of the cell and reaches its peak in malignancy. Qualitative characteristics of AgNOR is also useful to differentiate between hyperplastic, premalignant, and malignant lesions.AgNOR technique can definitely be used as a supportive tool to the routinely performed haematoxylin and eosin staining and that it will help in the prognosis and the therapeutic decision making in squamous cell carcinomas of the upper aerodigestive tract. In future, more studies need to be carried out to prove the usefulness of AgNOR technique as a supplementary tool in diagnosing the premalignant and malignant lesions, deciding the treatment plan and in evaluating the prognosis of the patients.

Ethical Clearance – Not required since it is a review article

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Conflict of Interest – nil

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