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Correlation between mucoepidermoid carcinoma grade and AgNOR count

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Abstract: Mucoepidermoid carcinoma (MEC) is a malignant glandular epithelial neoplasm having an unpredictable behavior and a tendency to recur. Numerous parameters have been assessed to predict the outcome of this lesion, but have been deemed inadequate, with the exception of tumor stage and grade. In the present study, we attempted to correlate the proliferative activity of MEC with its histopathological grade, using argyrophillic nuclear organizer region (AgNOR) count. Thirty cases of MEC were included in the study. All the slides were stained using hematoxylin and eosin and silver nitrate techniques. Counting was performed at a magnification of ×1,000 with an oil-immersion lens. Positive correlations were seen between AgNOR count and MEC grade ($P < 0.05$), with AgNOR count increasing in proportion with tumor grade. The AgNOR count in various grades of MEC indicates a relative progression in the proliferative activity of this tumor. This index is positively correlated with tumor grade, although there are some exceptions. The utility of AgNOR count in predicting the prognosis of MEC can be considered of importance; however, further assessment, such as survival studies, is necessary. (J Oral Sci 52, 275-279, 2010)

Keywords: mucoepidermoid carcinoma; AgNOR count; proliferative activity.

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Introduction

Mucoepidermoid carcinoma (MEC) is a malignant glandular epithelial neoplasm characterized by mucous, intermediate and epidermoid cells, with columnar, clear cells and oncocytoid features (1). MEC of salivary glands has been traditionally classified with neoplasms of intermediate grade malignancy (2); however, the current consensus considers them to be true carcinomas with an unpredictable course, showing a favorable outcome after surgery in some cases, and a lethal course in others. Almost all cases show a long clinical course with a tendency to recur (3). Thus, histopathological examination plays a key role in predicting prognosis, and findings such as large tumor size, lack of differentiation and the presence of neoplastic emboli have tended to signify poor prognosis (4,5).

Due to its simplicity and sensitivity, its rapid and convenient nature (6-11), and its applicability to paraffin sections, argyrophillic nuclear organizer region (AgNOR) analysis was used in the present study. Nucleolar organizer regions (NORs) are loops of DNA that encode ribosomal RNA and are considered to be important in the synthesis of proteins. NORs can be selectively stained using a silver colloid technique and are visualized as black dots under a light microscope (12). When compared to normal or premalignant lesions, the number of AgNORs in malignant lesions is higher (10), and amongst malignant melanoma and nevocellualr nevi, the count for malignant melanoma is higher. Other authors have used AgNOR count for the histological distinction of breast carcinoma grades (13), benign and malignant pleural mesothelial cells (14), different grades of renal cell carcinoma (15), and certain

salivary gland tumors (16). This shows that AgNOR count is useful for differentiating benign from malignant tumors (17). Previous studies on AgNOR count in benign and malignant salivary gland tumors have been performed, and they demonstrated that NORs can be used as a criterion for differentiating benign salivary gland tumors from malignant tumors (18).

The grading system for MEC reported by Seifert et al., which is based on proportion of cells in a given section, is less reliable than AgNOR count in MEC in the context of MEC outcome (6). Auclair et al. described a three-tiered grading system based on various histopathological features (Table 1). This grading is based on specific parameters and is universally accepted when compared to Seifert's grading system. In the present study, we correlated AgNOR counts with the different MEC grades in the system described by Auclair et al. (19) in order to determine the value of Auclair's grading system in establishing MEC outcome.

Table 1 Histopathological features for grading of mucoepidermoid carcinoma, Auclair et al. (19)

Histopathologic Feature	Point Value
Cystic component < 20%	2
Neural invasion	2
Necrosis	3
4 or more mitoses/10 hpf	3
Anaplasia	4
Tumor Grade	**Point Score**
Low	0 – 4
Intermediate	5 – 6
High	7 or more

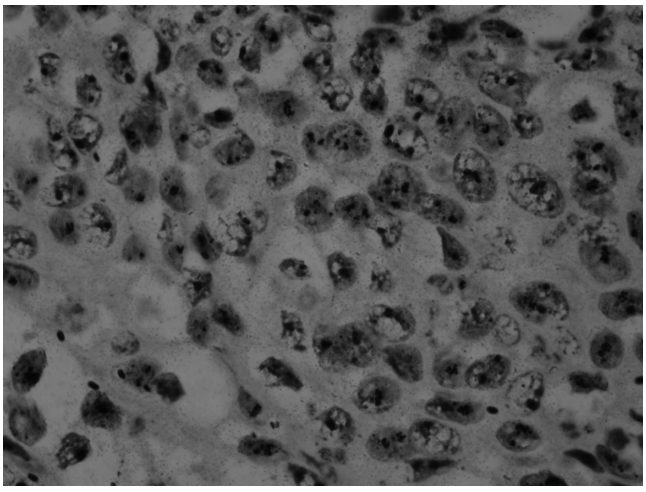

Fig. 1 Round argyrophilic granules in nuclei of tumor cells (AgNOR staining; ×1,000).

Materials and Methods

A retrospective study was undertaken of 30 cases from three institutes (10 cases each; Department of Oral Pathology, Sharad Pawar Dental College, Wardha; Department of Oral Pathology, Modern Dental College and Research Center, Indore; and Department of Pathology, MGIMS, Sewagram). In all cases, we evaluated NORs in tumor cells (AgNOR count) using a semi-quantitative method and correlated this data with the three-tiered grading system for MEC (19). Clinical details for each case including data on primary diagnosis were recorded.

Tumors were graded using a three-tiered system based on hematoxylin and eosin-stained sections (Table 1) into low, intermediate and high grades. The AgNOR staining technique was applied to all paraffin-embedded specimens, with some modifications to the method reported by Ploton et al. (12). The working solution consisted of two parts of a 50% silver nitrate solution and one part of a mixture of 2% gelatin and 1% formic acid, prepared immediately before the staining procedure. Sections were hydrated in decreasing concentrations of alcohol, rinsed in distilled water, and subsequently incubated in freshly prepared working solution for 30 min at room temperature in the dark. Sections were then washed in distilled water for 1 min, dehydrated in increasing concentrations of alcohol, cleared and mounted in Disterine Plasticiser Xylene (DPX) medium. NORs were counted in tumor cells, appearing as brownish black intranuclear dots on a pale yellow background.

The NORs thus detected indicate the quantity of protein quantity bound to juxtanucleolar ribosomal DNA. AgNOR counts were obtained using a semi-quantitative method; 100 nuclei from each case were randomly examined, silver-stained dots were counted using a ×1,000 oil-immersion lens, and the mean number of AgNORs for each epidermoid cell type was determined.

Results

The grade-wise distribution of MEC was as follows: low grade, 16 cases; intermediate grade, 8 cases; and high grade, 6 cases (Table 2). AgNORs are histologically characterized as granules easily distinguished from nucleoli (Fig. 1) because of their high levels of argyrophilic staining. Mean AgNOR counts were determined for all grades of tumors and the data was analyzed by one way analysis of variance (ANOVA) and values were tabulated (Table 3).

Correlations between AgNOR count and tumor grade (Table 3)

Significant differences were seen between AgNOR counts in different grades of MEC by Tukey-Kramer's

Table 2 Comparison of AgNOR count and different grades of mucoepidermoid carcinoma

Case No.	Age	Sex	Grade	Site	AgNOR count
1.	48	M	Low	Palate	1.34
2.	67	F	Low	Parotid Gland	1.37
3.	53	F	Low	Palate	1.50
4.	42	M	Intermediate	Parotid Gland	2.52
5.	39	F	Low	Submandibular Gland	1.62
6.	55	M	Intermediate	Parotid Gland	2.57
7.	36	F	Low	Palate	1.61
8.	46	M	High	Palate	3.35
9.	59	M	Intermediate	Palate	2.89
10.	60	M	Low	Parotid Gland	1.58
11.	48	M	Intermediate	Palate	3.08
12.	57	F	Low	Palate	1.00
13.	52	F	Intermediate	Palate	2.13
14.	33	M	High	Submandibular Gland	4.47
15.	65	F	Low	Palate	1.38
16.	56	M	High	Palate	3.03
17.	50	F	Low	Palate	1.53
18.	38	M	Low	Submandibular Gland	1.30
19.	70	F	Intermediate	Palate	2.61
20.	47	M	High	Palate	3.58
21.	60	M	Low	Parotid Gland	1.52
22.	57	M	High	Palate	3.72
23.	53	M	Low	Palate	1.48
24.	68	F	High	Parotid Gland	4.73
25.	51	M	Intermediate	Submandibular Gland	2.37
26.	54	M	Low	Palate	1.11
27.	67	M	Low	Palate	1.08
28.	59	F	Low	Parotid Gland	1.16
29.	58	M	Intermediate	Palate	2.82
30.	64	M	Low	Parotid Gland	1.13

Table 3 Results of Tukey-Kramer Multiple Comparisons Test for Different Grades of Mucoepidermoid Carcinoma

Grade of MEC	Mean AgNOR count ± Standard Deviation	Comparison	*P*-value
Low	1.35 ± 0.20	Low vs Intermediate	$P < 0.001$
Intermediate	2.62 ± 0.30	Low vs High	$P < 0.001$
High	3.81 ± 0.65	Intermediate vs High	$P < 0.001$

multiple comparisons test. All three grades significantly differed in mean AgNOR count ($P < 0.001$); high AgNOR counts were seen in high-grade tumors and low counts were seen in low-grade tumors (Table 3). However, there were some exceptions; in Case 11, an intermediate-grade tumor had a relatively high AgNOR count (3.08), while in Case 16, a high-grade tumor had a relatively low AgNOR count (3.03).

Discussion

In rapidly proliferating cells, chromosomal and AgNOR distribution remains disorganized with the resultant formation of multiple, small and widely dispersed nucleoli. Actively proliferating cells have impaired nucleolar association and therefore exhibit a higher AgNOR count, regardless of the ploidy state of the cell (20).

The importance of tumor grade and stage in establishing the proliferative activity of salivary mucoepidermoid

tumors is widely accepted (21). Using the new classification (19) based upon the degree of glandular differentiation and upon the percentage of mucous cells, the most differentiated tumors generally have a lower proliferative activity, the intermediate group of predominantly non-mucous cells generally has relatively high proliferative activity, and the high-grade group has markedly increased proliferative activity. Undifferentiated carcinomas are often complicated by metastases and have a lethal outcome. However, histologic type is not necessarily correlated with clinical aggressiveness and the prognosis of MEC thus remains unpredictable (6).

Hence, we retrospectively analyzed a series of 30 MEC using the silver nitrate staining technique, and used the counts as a measure of proliferative activity and as a probable marker of outcome. This method has been used with success in various cancers (22), such as tumors of the stomach (23), renal carcinoma (24), urothelial carcinoma (25), lung carcinoma (26), central nervous system tumors (27), and salivary tumors (6,9,28,29).

In previous studies of AgNOR in salivary gland tumors by Morgan et al. (9) and Van-Heerden (7), the number of MEC cases they included was low, and comparisons according to grade were not performed. In a study by Adeyemi et al., (29) 7 salivary gland tumors were studied, and there were no cases of intermediate grade (1 high-grade case, and 6 low-grade cases), and thus, comparisons between grades were not possible. In a study by Chommette et al. (6), the cases were divided based on outcome (poor outcome and good outcome), rather than by MEC grade. In a study by Alaeddini et al. (28), AgNOR counts were found to be correlated with MEC grade, and the results were in agreement with those of the present study. Thus, AgNOR counts can apparently be used as an indirect predictor of MEC outcome.

AgNOR count, based on the mean number of argyrophilic granules found in the nuclei of tumor cells, is a relative measure of proliferative activity of MEC. Low counts (mean count, 1.36) were seen in low-grade cases and higher counts were seen in (mean count, 3.82) high-grade cases. This index is thus positively correlated with tumor grade, although there were some exceptions. The utility of AgNOR count and the predictability of MEC prognosis are considered to be important, although the present results require further assessment, such as conducting survival studies.

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