

EVALUATION OF NUCLEOLAR ORGANIZER REGIONS IN MAXILLARY OSTEOSARCOMA

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ABSTRACT

Maxillary osteosarcomas are a relatively frequent malignant tumor of the oral cavity. Similarly to other skeletal osteosarcomas, they exhibit different cellular differentiation patterns, i.e. chondroblastic, osteoblastic, or fibroblastic. Although their histological features resemble those of osteosarcomas of the long bones, their pattern of evolution usually differs.

Morphometric variations in silver stained Nucleolar Organizer Regions (AgNOR) have proved of value to study the biology of several tumors. However, information on the analysis of AgNOR in maxillary tumors is scarce. The aim of the present study was to analyze the variations of different morphological parameters related to AgNOR in a series of 32 cases of maxillary osteosarcoma. In each case we analyzed 100 nuclei corresponding to the prevalent cellular differentiation type, selecting the most aggressive area. We employed software previously developed at our laboratory that yields information on different AgNOR-related parameters. The results were com-

pared with those previously reported in a study on 12 cases of osteosarcoma of long bones. Six cases of oral mucosa squamous cell carcinoma were also included for comparative purposes.

Single AgNOR volume proved to be the most discriminatory and informative parameter. The value of single AgNOR volume was considerably lower in mandible osteosarcomas than in osteosarcomas of the upper maxilla ($p=0.02$). The values were significantly lower in maxillary osteosarcomas than in long bone osteosarcomas and in oral carcinomas. This finding would suggest a slower rate of cell activity in maxillary osteosarcomas, associated in turn to its known lower degree of aggressiveness. The present results suggest that the analysis of AgNOR is a valuable and easily applicable marker to determine the degree of malignancy and biology of maxillary osteosarcomas.

Key words: maxillary osteosarcoma, nucleolar organizer regions, malignancy biomarkers.

EVALUACIÓN DE LAS REGIONES ORGANIZADORAS DEL NUCLEOLO EN OSTEOSARCOMAS DE MAXILAR

RESUMEN

Los osteosarcomas de maxilares son entidades relativamente frecuentes entre los tumores malignos de la cavidad bucal. Al igual que los osteosarcomas de otras localizaciones del esqueleto pueden presentar diferentes patrones de diferenciación celular (condroblástico, osteoblástico o fibroblástico). Si bien sus características histológicas son similares, tienen generalmente un comportamiento evolutivo diferente al de los huesos largos.

Las variaciones morfométricas de las regiones organizadoras de nucleolo identificadas por impregnación argéntica (AgNOR) han demostrado ser marcadores útiles para el estudio de la biología de diversas entidades tumorales, pero hay muy escasa información de su análisis en tumores de los huesos maxilares. El objetivo de este trabajo fue analizar las variaciones de diferentes parámetros morfológicos de las AgNOR en una serie de 32 casos de osteosarcomas de maxilar. En cada caso se analizaron 100 núcleos en el patrón de diferenciación celular predominante seleccionando la zona de mayor agresividad. Se utilizó un programa que aporta información sobre diferentes

parámetros de AgNOR, desarrollado previamente en nuestro laboratorio. Los resultados se compararon con los obtenidos previamente en 12 casos de osteosarcomas de huesos largos. Se incluyeron también para la comparación 6 casos de carcinomas de células escamosas de la mucosa bucal.

El parámetro más indicativo resultó ser el volumen individual de las AgNOR. Este parámetro en los osteosarcomas con localización mandibular fue considerablemente menor que aquellos localizados en maxilar superior ($p=0.02$). En los osteosarcomas de maxilar los valores fueron significativamente menores que en los de huesos largos y en los carcinomas bucales. Ello podría ser indicativo de una menor actividad celular, a su vez asociada a su reconocida menor agresividad. Estos resultados sugieren que el análisis de AgNOR podría ser considerado como un marcador de utilidad y de fácil aplicación para determinar el grado de malignidad en osteosarcomas de maxilar y estimar su comportamiento biológico.

Palabras clave: osteosarcoma de maxilar, regiones organizadoras del nucleolo, biomarcadores de malignidad.

INTRODUCTION

Nucleolar Organizer Regions (NORs) are regions of DNA that contain genes that encode ribosomal RNA. Transcriptionally active NORs are associated to specific argyrophilic proteins that are detected by silver staining (AgNOR) (1-4). Variations in AgNOR thus reflect changes in protein synthesis. Numerous studies have shown that an increase in the number and/or volume of AgNOR is associated to an increase in cell activity, be it in terms of proliferation, differentiation or secretory activity (5-7). The intense metabolic activity of malignant cells is also evidenced by variations in AgNOR. Within this context, the quantitative evaluation of variations in AgNOR has proved to be an efficient marker of the degree of malignancy of a tumor (8-10).

Image analysis of AgNOR has shown that the evaluation of shape and volume-related parameters can be more discriminatory and informative than the sole evaluation of number (11, 12). Our laboratory demonstrated the value of AgNOR morphometry in the early detection of radioinduced changes and of alterations associated to chemical carcinogenesis and as a marker of field cancerization in human oral mucosa (13- 16). In all of these cases, AgNOR alterations were observed in the epithelia. However, routine hematoxylin-eosin stained histological preparations did not reveal any unusual microscopic epithelial features.

In the field of bone pathology, the evaluation of AgNORs has been applied to the diagnosis and prognosis of different entities (17- 20) and to the assessment of the response of malignant bone tumors to therapy (21, 22).

We previously reported changes in AgNOR morphology associated to biological behavior in central and parosteal osteosarcomas of long bones (23).

Maxillary osteosarcoma is a variant that represents 5-13% of all skeletal osteosarcomas (24, 25). To date, only 2 reports are available in the literature on the quantitative evaluation of AgNOR in maxillary osteosarcomas. Afolabi et al. (21) evaluated the number of AgNOR in the nuclei of normal bone tissue, of benign and malignant maxillary bone tumors, including a set of twenty-three maxillary osteosarcomas. In two cases, AgNOR were evaluated before and after chemotherapy. These authors reported significant differences in the number of AgNOR between the groups under study, with higher values in malignant tumors. The number of

AgNOR decreased significantly as a result of the cytostatic treatment.

Eslami et al. (26) evaluated the number of AgNOR in 14 cases of maxillary osteosarcoma (well differentiated and poorly differentiated), ossifying fibroma and fibrous dysplasia. These authors reported the value of AgNOR in differential diagnosis between these lesions.

The aim of the present study was to evaluate a series of 32 cases of maxillary osteosarcoma employing image analysis of AgNOR. The results were compared to those obtained with a similar technique in long bone osteosarcomas (23) and squamous cell carcinomas (the most frequent malignant tumor of the oral cavity) (5).

MATERIALS AND METHODS

Thirty-two cases of maxillary osteosarcoma were included in the study.

Eighteen cases were localized in the mandible and 14 cases were localized in the upper maxilla.

The prevalent histological differentiation patterns were osteoblastic (15 cases), chondroblastic (14 cases) and fibroblastic (3 cases) (Fig. 1).

Archival paraffin blocks were sectioned and silver stained in keeping with the technique of Howell et al. (4) modified by our laboratory (27). Briefly, sections were immersed in a solution of 7% nitric acid for 5 min. The acid was then removed by washing in running water. The AgNOR staining solution was prepared by mixing 1:2 volumes of 2% aqueous solution of gelatine and 50% aqueous silver nitrate solution. Sections were stained under safe light conditions during 40- 45 minutes. After washing, sections were dehydrated and mounted.

The morphometric evaluation of AgNOR was performed within the fields of the prevalent cellular differentiation pattern, selecting the area with the highest degree of histological aggressiveness in keeping with Broders' criteria (28).

We obtained images within the selected area to reach a total of one hundred nuclei with an x100 objective, 1.3 aperture, employing a Zeiss MPM800 microscope. Image analysis was performed with software developed ad hoc by our laboratory (5) to yield information on number and morphology of AgNOR in terms of the following parameters:

- Nuclear volume;
- Single AgNOR volume;
- Total volume of AgNOR per nucleus;

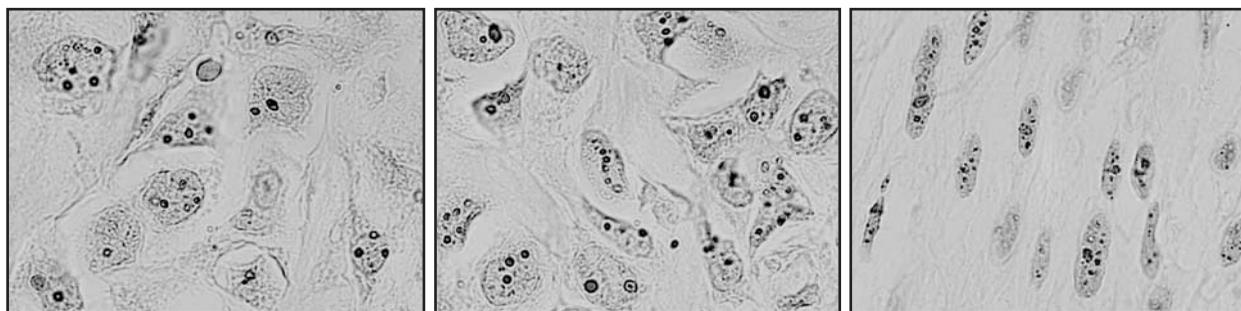


Fig. 1: Microphotographs of silver stained histological sections showing different differentiation patterns of maxillary osteosarcomas. Left to right: chondroblastic, osteoblastic and fibroblastic. Orig. Mag. X1000. AgNORs are larger, more numerous and irregular in the chondroblastic pattern. Overall, the osteoblastic pattern exhibited an appearance that was intermediate between the chondroblastic and osteoblastic patterns.

- Proportion of nuclear volume occupied by AgNOR;
- Number of AgNOR per nucleus;
- Nuclear contour index;
- AgNOR contour index.

The data were compared to previous data reported by our laboratory on AgNOR of central or conventional and parosteal or peripheral osteosarcomas (23) of long bones and in semi-differentiated squamous cell carcinomas of oral mucosa (5).

Statistical analysis of the data was performed by ANOVA.

RESULTS

All the nuclei of the cases of maxillary osteosarcoma under study, even those that had been previously demineralized in 7% nitric acid, exhibited silver staining for NOR.

AgNOR volume was smaller in mandible osteosarcomas than in upper maxilla osteosarcomas. Single AgNOR volume was the most informative parameter. Nuclear volume and the parameters related to

number and shape of AgNOR did not exhibit statistically significant differences (Table 1).

All AgNOR parameters exhibited higher values in the chondroblastic pattern than in the osteoblastic and fibroblastic patterns (Fig. 1). However, these differences did not reach statistical significance (Table 2).

A marked increase in the number of AgNOR with a substantial concomitant reduction in single AgNOR volume was observed in maxillary osteosarcomas compared to osteosarcomas (conventional and parosteal) of long bones and to semi-differentiated squamous cell carcinomas of the oral mucosa (Table 3).

DISCUSSION

The value of the histochemical demonstration of AgNOR for the study of tumor biology and its efficacy as a marker of aggressiveness vary with the different types of neoplasms (6-10). Originally, AgNOR counting by direct light microscopy observation was the parameter most employed (1). The

TABLE 1. AgNOR-related parameters in osteosarcomas of the mandible and upper maxilla. Values are expressed in image pixels. 1 pixel = 0.009 μ .

Localization	Mandible		Upper maxilla
Number of cases	18		14
Nuclear volume	4456.35 \pm 2125.95	>	4415.13 \pm 1831.23
Single AgNOR volume*	33.98 \pm 11.28	<	49.26 \pm 25.45
Total AgNOR volume per nucleus	427.94 \pm 237.34	<	530.16 \pm 206.22
Proportion of nuclear volume occupied by AgNOR	0.10 \pm 0.04	<	0.13 \pm 0.06
Number of AgNOR per nucleus	13.31 \pm 7.55	>	12.39 \pm 5.74
Nuclear contour index	0.07 \pm 0.01	=	0.07 \pm 0.02
AgNOR contour index	0.63 \pm 0.12	>	0.55 \pm 0.15

* Statistically significant difference (ANOVA), p: 0.029.

TABLE 2. AgNOR-related parameters for different differentiation patterns. Values are expressed in image pixels. 1 pixel = 0.009 μ .

Histological pattern	Chondroblastic	Osteoblastic	Fibroblastic
Number of cases	14	15	3
Nuclear volume	4232.59 \pm 2345.27	4822.64 \pm 1873.34	3132.96 \pm 175.41
Single AgNOR volume*	42.7 \pm 23.6	38.70 \pm 16.25	24.57 \pm 4.53
Total AgNOR volume per nucleus	514.42 \pm 211.15	459.52 \pm 234.96	260.40 \pm 80.35
Proportion of nuclear volume occupied by AgNOR	0.13 \pm 0.05	0.10 \pm 0.05	0.08 \pm 0.02
Number of AgNOR per nucleus	13.94 \pm 6.97	12.97 \pm 7.49	10.75 \pm 3.09
Nuclear contour index	0.08 \pm 0.01	0.07 \pm 0.02	0.08 \pm 0.00
AgNOR contour index	0.60 \pm 0.15	0.58 \pm 0.12	0.73 \pm 0.08

TABLE 3. Volume and number of AgNOR in osteosarcomas (OS) of the maxilla and long bones and in oral mucosa semi-differentiated squamous cell carcinomas (SCC). Values are expressed in μ m².

	Maxillary OS (1)	Long Bones OS (2)		Oral SCC (3)
		Parosteal	Central	
N Cases	32	6	6	6
Single Ag NOR vol μ 2	0.4 \pm 0.2 *	1.1 \pm 0.35	0.8 \pm 0.2 #	1.4 \pm 0.5
Total Ag NOR vol per nucleus μ 2	4.2 \pm 2	1.85 \pm 0.5	2 \pm 0.4	10.8 \pm 4.2 **
N Ag NOR	13 \pm 7	1.75 \pm 0.2 ***	2.4 \pm 0.4 ***	8 \pm 2.4

Test Bonferroni: Single AgNOR volume. * p <0.05 (2,3)

p <0.05 (3)

Total AgNOR volume per nucleus. ** p <0.05 (1,2)

N° AgNOR. *** p <0.05 (1)

(1) Present study of maxillary osteosarcoma.

(2) Schwint et al. Clin Orth Relat Res 327: 253- 258, 1996.

(3) Cabrini et al. J Oral Pathol Med 21: 275-279, 1992.

evaluation of other end points was made possible by image analysis (11, 12). Total AgNOR volume per nucleus was recognized as the most contributory parameter in terms of diagnosis and prognostic evaluation. Employing software that affords information on numerous AgNOR-related parameters, we showed that the discriminatory value of each of these parameters differs for each model (13-16). In the present study, the value of single AgNOR volume was lower in mandible than in the upper maxilla osteosarcomas. However, there are no unequivocal reports in the literature on differences in the evolution of osteosarcomas in terms of these different tumor sites. Future studies are warranted

to examine the actual value of this marker in this sense.

The different cellular patterns did not exhibit statistically significant differences in AgNOR expression. However, the values of all the AgNOR-related parameters were higher for the chondroblastic pattern. If this trend were confirmed, it would reflect higher cellular activity, conceivably associated to the synthesis of the chondroid matrix. This difference could also be associated to differences in degree of malignancy and biological behavior. However, several studies (29, 30) report that this cellular pattern is associated to a better prognosis and longer survival time than the rest of the variants.

Maxillary osteosarcomas differ from the rest of the skeletal osteosarcomas in terms of biological behavior. Maxillary osteosarcoma is considered a variant of skeletal osteosarcoma and differs from those localized in long bones. It exhibits a predominantly chondroblastic differentiation, presents in older patients (in the third and fourth decades of life), survival time is longer, metastases are rare and occur at later stages in the disease and death generally occurs as a result of local invasion (24-25, 29, 30).

No data are available in the literature to explain these differences in terms of tumor biology.

The comparative analysis of the expression of AgNOR revealed that single AgNOR volume is significantly lower in maxillary osteosarcomas than in long bone osteosarcomas (23) and in squamous cell carcinomas (5), a malignant, very aggressive oral tumor. This finding would indicate that single

AgNOR volume would be the marker with the greatest prognostic value. It is noteworthy that this was the only parameter that exhibited statistically significant differences between mandible and upper maxilla osteosarcomas.

The AgNOR staining technique is simple, inexpensive and can be used in demineralized samples. The results of the present study suggest that this technique could be applied routinely in sections adjacent to those stained with hematoxylin-eosin. The mere observation of single AgNOR size would contribute to the assessment of individual cases. Furthermore, image analysis with software to evaluate multiple parameters in significant series of cases of known evolution would provide insight into the biological behavior of maxillary osteosarcomas in terms of the prevalent pattern of cellular differentiation and localization.

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