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Evaluation of Argyrophilic nucleolar organizer regions (AgNORs) score in the diagnostic and prognostic assessment of canine tumors

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Abstract

Argyrophilic nucleolar organized region (AgNOR) stains proteins associated with the nucleolar organized region and corresponds to cell proliferation. AgNOR is widely distributed in the nucleus and is more abundant in malignant cells than in non-malignant cells. In this study, a total of 12 different tumor samples were collected and stained with AgNOR staining technology. The average AgNOR numbers in malignant and non-malignant cells and the distribution of AgNOR dots in the cells were calculated and compared. The number and distribution of AgNOR were higher in malignant tumors than in normal tumors. We believe that AgNOR staining is a simple, rapid and reliable method for assessing cell proliferation and malignancy.

Keywords: Tumor, canine, cytology, nucleolar organizing regions

Introduction

The nucleolar regulatory region (NOR) is a piece of DNA that encodes the transcription of ribosomal RNA (rRNA). rRNA is responsible for the production of many proteins in the cell. Protein synthesis is an important step in cell development. Therefore, a relationship between NOR and cell proliferation has been suggested [1]. NOR can be selectively detected by silver staining in routine histological and cytological smears and can be useful in differentiating benign and malignant tumors [2]. This study shows that AgNOR can be used as a simple and inexpensive method to distinguish benign and malignant tumors.

Materials and Methods

Tumor samples

Samples of different canine tumors were collected from Teaching Veterinary Clinical Complex, Madras Veterinary College, Chennai.

Cytology and Histopathology

The Fine Needle Aspiration cytology (FNAC) smears from tumor suspected cases were collected and stained by Leishman- Giemsa staining technique [3]. A piece of tumor suspected tissue was collected for histopathology in 10% neutral buffered formalin and processed and stained by H&E stains according to the standard protocol [3].

Argyrophilic nucleolar organizer regions (AgNORs) staining technique

The duplicate cytology smears and paraffin embedded sections were stained for interphase NOR's by the standard technique [4].

Statistical analysis

Mean AgNOR count and standard deviation were calculated by counting the cells having five or more AgNOR dots per nucleus, in 100 Nuclei. Unpaired 't' test was applied to find the probability between the means of different groups.

Result and Discussion

In this study, 12 different canine tumor samples and FNAC smears were collected from Teaching Veterinary Clinical Complex, Madras Veterinary College, Chennai.

On FNAC smears (Figure 1) and histopathology slides (Figure 2), AgNOR appears as dark brown to black spots in the nucleus with a golden-brown background, usually >1 micron in size. Table 1 presents the AgNOR score per nucleus

(mAgNOR) for each case.

Figures

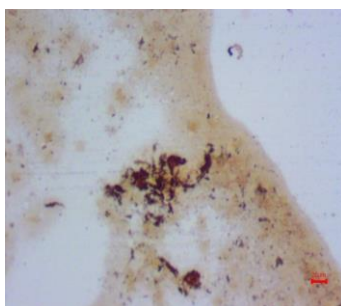


Fig 1: Mast cell tumor- FNAC- AgNOR stain

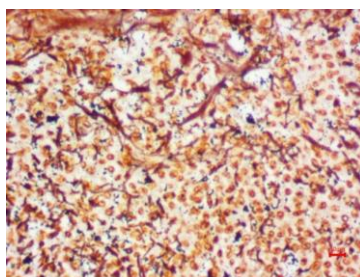


Fig 2: Mast cell tumor- HP- AgNOR stain

Table 1: mAgNOR Scores

S. No	Case no.	Cytological diagnosis	Histopathology diagnosis (H&E)	Mean AgNOR scores	
				FNA smear	Tissue sections
1.	129079	Cutaneous plasma cell tumor	Cutaneous plasma cell tumor	5.5	5
2.	146484	Mast cell tumor	Mast cell tumor	6	4.5
3.	145814	Lipoma	Lipoma	4	2
4.	146278	Fibroleiomyoma	Fibroleiomyoma	4.5	3.5
5.	146783	Sweat gland cystadenoma	Sweat gland cystadenoma	5	3
6.	146631	Mammary tumor	Tubular adenocarcinoma	8	7
7.	147111	Perianal gland adenoma	Perianal gland adenoma	3	2.5
8.	148315	Mammary tumor	Secretory adenocarcinoma	7.5	7
9.	149471	Fibrosarcoma	Fibrosarcoma	6	6
10.	153758	Mammary tumor	Mixed mammary gland tumor	8	6.5
11.	155057	Papilloma	Papilloma	4.5	3.5
12.	155057	Squamous cell carcinoma	Squamous cell carcinoma	5.5	5

Mean mAgNOR score: 5.63, S.D 1.58 (FNA smears); 4.625, S.D 1.74 (Tissue sections). t-stat = 1.483 ($p < 0.05$) [unpaired t test].

For benign tumors, higher mAgNOR counts were observed on FNA smears than the corresponding tissue sections. This increase in count might be attributed to the fact that the cells on smears are in monolayers and air-drying displays the structure of the nucleus better as the cells are flattened making counting easy [2].

The difference in mAgNOR scores between the benign and malignant lesions on aspiration smears as well as tissue sections was statistically highly significant ($p < 0.001$). A broad range of overlap (4.0 to 8.0 on smears and 2.0 to 7.0 on sections) in mAgNOR scores of benign and malignant lesions was observed, thus precluding the use of AgNOR scoring as an independent, sole diagnostic criterion. The overlap of scores between cytology and histology sections could be accounted for by the choice of the fixative used, length of incubation in silver nitrate solution, subjective impression of dot distinction and subjective variation in counting technique [5]. The heterogeneity of tumors in terms of cell growth and section thickness will further increase inconsistencies in

AgNOR counts [6]. Benign tumors have lower mAgNOR scores than malignant tumors. Most studies agree with the observation that AgNOR counts are higher in malignant cases compared to benign cases [7, 8].

Conclusion

In the present study, AgNOR scores in the cytology smears in benign and malignant tumors were correlated with histopathology sections and found that AgNOR counts in malignant tumors were higher and bigger as compared to benign tumors. Therefore, we can conclude that the morphology and distribution of AgNOR in the nucleus when used together with the mAgNOR score might be useful in histopathological diagnosis, prognosis and patient survival.

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