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Original Research

Evaluation of AgNOR Counts in Different Types of Canine Mammary Tumors

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Abstract

Seventy-two canine mammary tumors were classified into 20 histologic subtypes which included 63 malignant tumors and 9 benign tumors. AgNOR counts were performed manually in all the tumor tissue sections. AgNORs which appeared as black dots were multiple, small, irregular shaped and arranged discretely or as overlapping dots in malignant tumors while in benign tumors, they were bigger, round and discretely arranged. The mean AgNOR number of mammary tumors ranged from 1.1 to 6.7 and was higher (4.58 ±1.04) in malignant tumors than in benign tumors (1.4±0.36). Among the different histologic subtypes, fibrosarcoma had the highest AgNOR number followed by anaplastic carcinoma, malignant myoepithelioma and the other histologic subtypes.

Keywords: AgNOR, Benign Tumor, Canine Mammary Tumor, Malignant Tumor

Introduction

Mammary tumors are the most common tumors of intact female dogs. Forty to fifty percent of cases are considered malignant, with carcinomas being the most common malignant type. After mastectomy, the main cause of death in dogs with mammary carcinoma is metastatic disease. To make a diagnosis, a number of clinical and paraclinical tests are used because it is essential to find the most aggressive malignant tumor that needs radical surgery and supplementary treatment as early as possible (Hellmen *et al*., 1993). As the range of options for the treatment of patients with mammary tumors widens, it becomes increasingly important that the clinician is provided with accurate prognostic information on which therapeutic decisions are based upon. A variety of efforts have been made to evaluate prognostic factors in mammary gland malignancies, including the use of specific morphologic classifications and staging systems, evaluation of cellular DNA parameters, nucleolar organizer region (NOR) counts, and oncogene expression.

Assessment of cell kinetics is a field of much interest in modern oncology. The study of parameters that reflect the cell-cycle phases of neoplastic cells has been shown to be useful for evaluation of the biologic behaviour of tumors. There has been a wide variety of methods introduced to this area of research, but only a few of them are applicable on formalin fixed, paraffin embedded tissues. Among these, the estimation of growth fraction by means of Argyrophilic Nucleolar Organizer Regions (AgNORs) counting method is very economic and relatively easy to perform, therefore gaining increasing interest for diagnostic and prognostic purpose. AgNORs are silver stained non-histone proteins of chromosomal loops within the nucleolus visualised at the light microscopic level as well-defined black dots. The arrangement of these loops of ribosomal DNA (rDNA) is directly related to proliferative activity of the cell due to the transcription of the ribosomal RNA (rRNA) needed for the assembly of ribosomes. Decondensation of the rDNA loops during higher transcription activity leads to a segregation of the associated proteins. This can be demonstrated at the optical level by an increased number and area of silver stainable intranuclear and intranucleolar particles (AgNORs) (Lohr *et al*., 1997). With this background, the present study was planned to evaluate AgNOR counts in different canine mammary tumors classified based on modified WHO classification (Goldschmidt *et al*., 2011).

Materials and Methods

A total of seventy-two canine mammary tumor cases collected from Veterinary Clinical Complex, NTR college of Veterinary Science, Gannavaram and different Veterinary Hospitals in and around Vijayawada, were included in the study. Mammary tumor samples were obtained by excisional biopsy (mastectomy and nodulectomy) and representative tissue samples were preserved for histopathology in 10% neutral buffered formalin. The tissue samples were processed for histopathological examination by routine paraffin embedding technique and haematoxylin and eosin staining. Histologically, the canine mammary tumors were classified on the basis of criteria proposed by Goldschmidt *et al*. (2011). All duplicate sections were stained for interphase NORs using the technique described by Lohr *et al*. (1997). Sections were examined under oil immersion objective (x 1000 magnification). All the AgNOR dots (black) scattered in the nucleus were counted in 100 consecutive nuclei in each tumor tissue section and mean number of AgNOR dots per nucleus was calculated for each sample.

Results and Discussion

In the present study, 63 cases were classified as malignant and 9 cases as benign tumors (Table 1). Based on Goldschmidt *et al.* (2011) classification, twenty major histologic subtypes of canine mammary tumors were recorded (Table 2). In malignant tumors (Fig. 1), AgNORs appeared as multiple, small, irregular black dots which were arranged either discretely or as overlapping structures (Fig. 2) while, in benign tumors (Fig. 3), they were bigger, round black dots and discretely arranged (Fig. 4). The mean AgNOR number in various histologic subtypes of canine mammary tumors is shown in Table 1 and 2. The mean AgNOR number of mammary tumors ranged from 1.1 to 6.7 and was higher (4.58) in malignant tumors compared to corresponding benign tumors (1.4). Among malignant canine mammary tumors, fibrosarcoma had the highest AgNOR number (6.7) followed by anaplastic carcinoma (5.98), malignant myoepithelioma (5.8), carcinoma and malignant myoepithelioma (5.6), micropapillary invasive carcinoma (5.55), carcinosarcoma (5.48), mucinous carcinoma (5.25), ductal carcinoma (4.8), intraductal papillary carcinoma (4.67), squamous cell carcinoma (4.65), complex carcinoma (4.15), comedocarcinoma and osteosarcoma (4.1 each), carcinoma *in situ* (4), solid carcinoma (3.98), carcinoma arising in a mixed tumor (3.7) and simple carcinoma (3.63). In benign tumors, simple adenoma had the highest AgNOR number of 1.48 followed

by intraductal papillary adenoma (1.3) and myoepithelioma (1.1).

Table 1: AgNOR count in malignant and benign canine mammary tumors

S. No.	Tumor Type	No. of samples	Mean AgNOR Number ± SD
1	Malignant tumors	63	4.58 ± 1.04
2	Benign tumors	9	1.4 ± 0.36

Table 2: AgNOR count in different histologic subtypes of canine mammary tumors

S. No.	Tumor subtype	No. of Samples	Mean AgNOR Number±SD
1	Carcinoma *in situ*	2	4±0.28
2	Simple carcinoma	3	3.63±1.33
3	Micropapillary invasive carcinoma	2	5.55±0.92
4	Solid carcinoma	13	3.98± 0.74
5	Comedocarcinoma	1	4.1
6	Anaplastic carcinoma	4	5.98± 0.33
7	Carcinoma arising in a mixed tumor	3	3.7±0.36
8	Complex carcinoma	10	4.15±0.93
9	Carcinoma and malignant myoepithelioma	2	5.6±0.14
10	Ductal carcinoma	1	4.8
11	Intraductal papillary carcinoma	9	4.67±1.14
12	Squamous cell carcinoma	2	4.65±0.21
13	Mucinous carcinoma	2	5.25±0.21
14	Malignant myoepithelioma	2	5.8±0.85
15	Osteosarcoma	2	4.1±0.14
16	Fibrosarcoma	1	6.7
17	Carcinosarcoma	4	5.48±0.21
18	Simple Adenoma	6	1.48±0.42
19	Intraductal papillary adenoma	2	1.3±0.14
20	Myoepithelioma	1	1.1

In the present study, quantification of AgNORs / cell was done by manual counting method by enumerating the silver stained black dots per cell directly at microscope by focusing through tissue section at x1000 magnification. Many researchers measured AgNOR area along with AgNOR number using computerized morphometric methods (Bostock *et al*., 1992; Lohr *et al*., 1997 and Sarli *et al*., 2002). However, Bundgaard-Andersen *et al*. (2008) compared manual method with computerized morphometric method and reported that morphometry gave less precise results when compared to manual counting of AgNORs because computerized image analysis may not be able to resolve several discrete AgNORs present within a single nucleolus.

The morphological features of AgNORs in the present study are in accordance with the findings of other researchers (Bostock *et al*., 1992; Lohr *et al*., 1997; Sarli *et al*., 2002 and Veena *et al*., 2014). The observation of high AgNOR number in malignant tumors than in benign tumors in the present study is consistent with the finding of other researchers indicating the tendency towards greater number of AgNORs in malignant tumors compared to benign tumors (Bostock *et al*., 1992; Lohr *et al*., 1997; Sarli *et al*., 2002; Veena *et al*., 2014 and Bundgaard-Andersen *et al*., 2008). The quantity of AgNORs is an estimate of the proliferation activity of the cell, as cells replicating fast or cells with a high metabolic level have multiple and prominent nucleoli and hence AgNOR dots, than cells that are less active or in resting phase (Bundgaard-Andersen *et al*., 2008). However, Bundgaard-Andersen *et al*. (2008) reported that the normal mammary gland had higher AgNOR counts compared to benign tumors in few cases and attributed it to the proliferative activity of mammary tissue during metestrus in bitches.

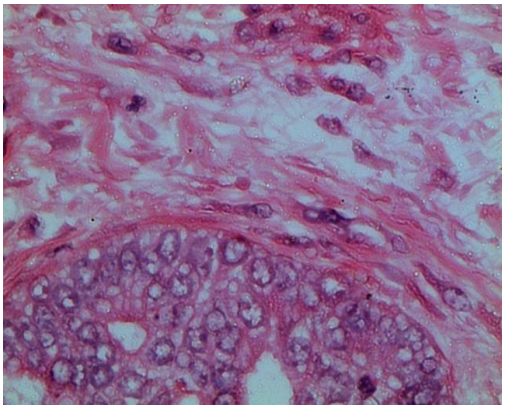

Figure 1: Carcinoma and malignant myoepithelioma, H&E x400

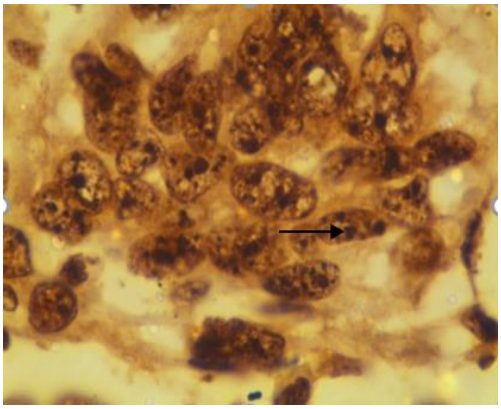

Figure 2: Carcinoma and malignant myoepithelioma showing three to five small and irregular shaped AgNORs (black dots indicated by arrow) scattered in the nuclei. AgNOR stain x1000

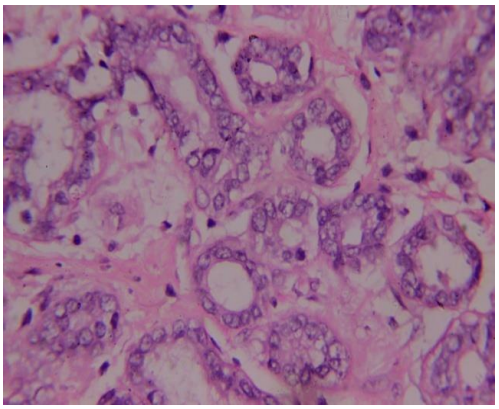

Figure 3: Simple adenoma, H&E x100

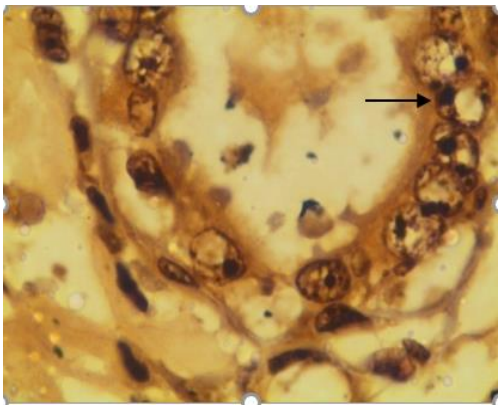

Figure 4: Simple adenoma showing one to two large round AgNORs (black dots indicated by arrow) in the nuclei. AgNOR stain x1000

In present study, among malignant canine mammary tumors, fibrosarcoma had the highest AgNOR number followed by anaplastic carcinoma, malignant myoepithelioma, carcinoma and malignant myoepithelioma, micropapillary invasive carcinoma, carcinosarcoma, mucinous carcinoma and followed by other subtypes while in benign tumors, simple adenoma had the highest AgNOR number. High AgNOR counts were previously reported in anaplastic carcinoma (Lohr *et al*., 1997), tubular adenocarcinoma, spindle cell carcinoma and solid carcinoma (Sarli *et al*., 2002), cystic adenocarcinomas and malignant mixed mammary tumors (Veena *et al*., 2014). The variations in different studies could be attributed to increased inter and intra observer errors due to the more subjective evaluation (Bostock *et al*., 1992 and Lohr *et al*., 1997). One source of variation also lies in enumerating extranucleolar AgNORs which may appear as clustered dots within the nucleoplasm rather than as well defined structures (Crocker *et al*., 1989). Variations in tissue fixation may further decrease the size of the AgNORs compounding the difficulty in differentiating these structures from clusters of cell debris. Another source of variation both among observers and specimens is, nuclear orientation. Further, Johnson *et al*. (1995) stated that AgNORs are more accurately measured in cytologic specimens than in histologic specimens because the entire nucleus of each cell is visible, rather than having sections at an angle to the nuclear axis. It was opined that AgNORs seem to reflect proliferation independent of cellular and nucleolar activity of tumor cells as well (Bankfalvi *et al*., 1998). It was supported by Bundgaard-Andersen *et al*. (2008) who found that the normal mammary gland group had mean values higher than both the hyperplastic and benign groups. The authors attributed this to high proliferative activity in normal mammary tissue of bitches in metestrus, which leads to an increased AgNOR value. Hence, as mentioned by Bukhari *et al*. (2007), the size and configuration of the dots are more important for interpretation of the results in malignant neoplasms. Therefore, AgNOR staining can be used as an adjunct to histopathology in differentiating benign and malignant tumors, especially in borderline cases.

Conclusion

The present paper discusses the AgNOR counts in different subtypes of benign and malignant canine mammary tumors. The AgNOR counts were high in malignant tumors when compared to benign tumors. This method of determining proliferative index of canine mammary tumors is easy to perform and economic and hence may be used in clinical routine.

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Conflict of Interests

There is no conflict of interest.

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