Detection of argyrophil nucleolar organizer regions (AgNORs) and proliferating cell nuclear antigen (PCNA) in epithelial skin tumours from domestic animals

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SUMMARY

The objective of this study was to compare the chemical characterization of the AgNOR bodies and the immunohistochemical detection of the PCNA for differentiating benign and malignant epithelial tumours (n = 21) from various animal species (mainly dogs and cattle). Fibropapillomas / papillomas (n = 3) and fibropapillomatosis / papillomatosis (n = 3) in cattle, and perianal gland adenomas (n = 3), keracanthomas (n = 2) and epithelioma (n = 1) in dogs constituted the group of benign tumours whereas the identified malignant tumours were squamous cell carcinomas (n = 7) in cattle and in one sheep and carcinomas in dogs (n = 2). A complete AgNOR characterization with determination of the NAI (nuclear area index), the AgNAI (AgNOR area index), the AgNCI (AgNOR count index), the AgNAI/NAI (mean AgNOR area) and the AgNCI/NAI (AgNOR density) was performed and PCNA expression was explored in parallel by immunohistochemistry. Significant increases of AgNAI, AgNCI and AgNAI/NAI were evidenced in malignant tumours compared to benign ones whereas no significant difference of the PCNA (numbers of PCNA positive cells) was found. Significant and positive correlations found between NAI and AgNAI or mean AgNOR area and between AgNAI and the mean AgNOR area or the relative AgNOR area in both malignant and benign tumours suggest that the AgNOR size is an important parameter for cell proliferation. By contrast, the lack of correlation between AgNCI and the relative AgNOR area and between the AgNOR density and NAI or AgNCI or AgNAI/AgNCI in malignant tumours contrary to benign tumours would be due to the under-estimation of the enumeration of the AgNOR bodies in malignant tumours mainly regrouped as AgNOR clusters.

Keywords: Skin, epithelial tumour, malignancy, AgNOR, PCNA, domestic animal.

Introduction

Tumours are classified as benign and malignant based on biological behaviour. Both tumour types are differentiated with morphological criteria. They are generally diagnosed according to the cell morphology (typical and atypical cells, differentiation, and anaplasia), cell lining and organization, mitosis, giant cell formation, stroma structure, vascularity, growth rate and growth form, metastasis, recurrence, macroscopic structure and formation like criterion [10].

The assessment of cell proliferation usually provides useful information on the biological behaviour of tumours.
Recently, in addition to the well known mitotic index, new histochemical (AgNORs) and immunohistochemical (Ki67, Mib1, PCNA, etc...) techniques have become available [5]. The nucleolar organizer regions (NORs) are chromosomal loops of DNA involved in ribosomal synthesis. Associated with NORs some nucleolar proteins can be evidenced with silver stain methods (AgNOR proteins or AgNORs) [8] and are identified as black dots in the nuclei. Their size and number reflect nucleolar activity and cell proliferative capacity of tumours [7].

The histochemical detection of AgNORs and of the Proliferating Cell Nuclear Antigen (PCNA) has been used for evaluating the tumour cell proliferation and for differentiating benign and malignant tumours [5, 18, 21, 23, 26, 27]. There are numerous studies concerning AgNORs technique carried out for diagnostic and prognostic purposes [1, 19]. AgNORs counting have been used especially to determine the malignancy of many tumour types both in human and animals [1, 2, 4, 6, 14, 16, 19, 26, 29].

The aim of this study was to compare the discriminating value of AgNORs and PCNA histochemical detection between benign and malignant epithelial skin tumours in various animal species.

**Materials and Methods**

**MATERIALS**

Epithelial skin tumours used in this study (n = 21) were diagnosed between 1993 and 2006 at the Department of Pathology, Faculty of Veterinary, University of Selcuk, Turkey. The diagnosis of the tumour type was established after Haematoxylin and Eosin (H.E) staining on cellular criteria and they were classified as benign and malignant tumours according to the protocol by World Health Organization [12].

**HISTOCHEMICAL METHODS**

**AgNOR staining and quantification**

The tissues were cut 4 μm thick and stained for AgNOR [9]. After passing through xylene, alcohol and distilled water, the tissues were transferred in a mixture obtained by mixing 1 part of 2% gelatine in a 1% aqueous formic acid solution with 2 parts of 50% aqueous AgNO₃ solution and filtered through 0.2 μm filter in a dark room and incubated in the autoclave at 37°C for 20-25 minutes. Thereafter, tissues were transferred to bidistilled water and stored in 5% sodium thiosulfate for 5 minutes. Then tissues were again passed through alcohol, xylene, and bidistilled water and stained after mounted with synthetic glue.

The area without necrosis and inflammation were observed under the light microscope (Olympus, Model BX51TF, Olympus, Tokyo, Japan) using X100 oil immersion lens. Randomly selected areas were micro-photographed through digital imaging system (Olympus, DP12-BSW, version 01.03, Olympus, Tokyo, Japan) then the images were transferred to a computer for evaluating the nuclei area, areas of NOR dots within the nuclei and the NOR counts on 100 tumour cells using an image analyzing software (Digital Life Science Imaging, analySIS® LS Starter, 2.2, Build 1110, An Olympus Company, Münster, Germany). In each tumour case, the following indices were calculated: the Nuclear Area Index (NAI) (nuclear area / 100), the AgNOR Count Index (AgNCI) (total number of NOR dots / 100) and the AgNOR Area Index (AgNAI) (NOR area / 100), the AgNOR density (AgNCI / NAI) and the relative AgNOR area (AgNAI / NAI).

**PCNA staining and quantification**

The tissues cut 4 μm thick mounted on slides with lysine were incubated at 37°C overnight to be evaluated for PCNA immunohistochemistry. Hence at this stage, all staining procedures were made at room temperature. Following paraffin extraction and rehydration, the tissues were antigen retrieved for 30 minutes in a microwave in citrate buffer pH 6. After washing 2 times for 5 minutes with distilled water, 3% hydrogen peroxide was dropped to the tissues placed in Shandon’s Manual Staining Set (Shandon Sequenza, Thermos Shandon, Cheshire, England). After another washing step (2 times for 5 minutes in distilled water), the tissue sections were kept in protein block serum-free solution for 5 minutes (DakoCytomation X0909, USA) and were incubated 1 hour at room temperature with anti-rat PCNA monoclonal antibody diluted to 1:500 (monoclonal mouse anti-rat Proliferating Cell Nuclear Antigen, clone PC10, DakoCytomation N1529, USA). After washing twice with phosphate buffer saline (PBS) pH 7.4 for 10 minutes, sections were incubated with the biotin conjugated secondary antibody (DakoCytomation LSAB®/System HRP K0673) at room temperature for 10 minutes. Thereafter, tissue sections were again washed (twice in PBS for 10 minutes) then incubated with the streptavidine-peroxidase (DakoCytomation LSAB®/System HRP K0673) for 10 minutes at room temperature. After a last washing step, the enzyme substrate, DAB (3,3’-diaminobenzidine-tetrahydrochloride, DakoCytomation LSAB®/System HRP K0673) (1 to 2 drops) was added according to the manufacturer’s instructions for 5 minutes at room temperature. After counterstaining with haematoxylin, they were changed in alcohol and xylene twice and covered with lamellas. The negative controls were obtained by incubating the sections with PBS instead of the primary antibody.

Randomly selected areas were investigated under the light microscope (Olympus, Model BX51TF, Olympus, Tokyo, Japan), with X100 oil immersion lens, micro-photographed through digital imaging system then analysed for quantification with a digital image analyzer software: PCNA positive nuclei were counted in 1000 tumour cells in 10 different areas. The PCNA index (PCNAI) in percent was determined by dividing positive cell count to 1000.

**STATISTICAL ANALYSES**

Statistical analyses were made with the SPSS software (released 9.0 for windows, SAS Institute INC; Cary, NC,
USA) using the Student-t-test. The Pearson correlations and regression analyses were also calculated. The difference was considered as significant when p value was less than 0.05.

Results

Among the 21 epithelial tumour cases diagnosed (Table I), 12 were benign (6 were stemming from dogs and 6 from cattle) and 9 malignant (2 were stemming from dogs and 7 from cattle and sheep) according to the criteria from the World Health Organization [12]. In dogs, benign tumours were keratoacanthomas (n = 2), trichoepithelioma (n = 1) and perianal gland adenomas (n = 3, with epithelioma, one out of 3) whereas papillomatosis and fibropapillomatosis (n = 3) and papilomas and fibropapillomas (n = 3) were found in cattle. Only the epithelial part of the tumour was evaluated in cases of fibropapilloma and fibropapillomatosis for histochemical and immunohistochemical studies. The main type of malignant epithelial tumours encountered in this study was the squamous cell carcinoma (n = 7), particularly in cattle and in one sheep.

<table>
<thead>
<tr>
<th>Species</th>
<th>Epidemiological data</th>
<th>Tumour characterisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breed</td>
<td>Age (year)</td>
<td>Sex</td>
</tr>
<tr>
<td>Dog</td>
<td>NR</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>7</td>
</tr>
<tr>
<td>NR</td>
<td>NR</td>
<td>M</td>
</tr>
<tr>
<td>Terrier</td>
<td>12</td>
<td>NR</td>
</tr>
<tr>
<td>Kangal</td>
<td>10</td>
<td>M</td>
</tr>
<tr>
<td>Russian</td>
<td>13</td>
<td>M</td>
</tr>
<tr>
<td>Cattle</td>
<td>NR</td>
<td>1.5</td>
</tr>
<tr>
<td>Holstein</td>
<td>NR</td>
<td>F</td>
</tr>
<tr>
<td>Holstein</td>
<td>1.5</td>
<td>F</td>
</tr>
<tr>
<td>Holstein</td>
<td>1.0</td>
<td>F</td>
</tr>
<tr>
<td>Holstein</td>
<td>1.0</td>
<td>F</td>
</tr>
<tr>
<td>Charolais</td>
<td>2</td>
<td>M</td>
</tr>
<tr>
<td>Dog</td>
<td>German shepherd</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Japanese Spitz</td>
<td>10</td>
</tr>
<tr>
<td>Cattle</td>
<td>NR</td>
<td>4</td>
</tr>
<tr>
<td>Simmental</td>
<td>4</td>
<td>F</td>
</tr>
<tr>
<td>Holstein</td>
<td>5</td>
<td>F</td>
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<tr>
<td>Simmental</td>
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<td>F</td>
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<tr>
<td>Holstein</td>
<td>6</td>
<td>F</td>
</tr>
<tr>
<td>Holstein</td>
<td>7</td>
<td>F</td>
</tr>
<tr>
<td>Sheep</td>
<td>Akkaraman</td>
<td>9</td>
</tr>
</tbody>
</table>

NR: Not reported; M: Male; F: Female.

Table I: Types of benign and malignant epithelial tumours (n = 21) observed in different species (mainly dog and cattle).

AgNOR IDENTIFICATION AND QUANTIFICATION

The mean nuclear area index (NAI) was 42.9 ± 12.6 µm² in benign tumours (ranging from 26.89 to 62.75 µm²). On the other hand, in malignant tumours the mean NAI was 57.6 ± 19.0 µm² ranging between 31.70 and 87.40 µm². The NAI tended to be more elevated in malignant tumours than in benign tumours (Table II) but no significant difference (P > 0.05) was observed between the two types. By contrast, the indexes of AgNOR area (AgNAI), of AgNOR counts (AgNCI) and the relative AgNOR area (AgNAI/NAI) were significantly increased in malignant tumours (P < 0.05 for AgNAI and AgNAI/NAI and P < 0.001 for AgNCI) compared to benign ones whereas the mean AgNOR area (AgNAI/AgNCI) and the AgNOR density (AgNCI / NAI) were not significantly modified (Table II), although they tended to increase. While the AgNOR bodies were either large, circular or oval shaped in small quantities in benign tumours (figure 1A), they were rather small and in large quantities in malignant tumours (figure 1B).

THE PCNA INDEX

The mean PCNA index did not significantly differ between benign and malignant epithelial tumours (p > 0.05) (Table II). However, a weak immunostaining was noticed in benign tumours (figure 2A) whereas an intense labelling was obtained in malignant tumours (figure 2B).

PEARSON CORRELATIONS

Significant positive correlations were observed between the AgNAI (AgNOR area) and NAI (Nuclear area) (P < 0.01),
HATIPOGLU (F.) AND COLLABORATORS

**Table II :** AgNOR (Argyrophil nucleolar organizer regions) and PCNA quantification in benign (n = 12) and malignant epithelial tumours (n = 9) observed in different species (mainly dog and cattle).

<table>
<thead>
<tr>
<th></th>
<th>Benign tumours</th>
<th>Malignant tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 12)</td>
<td>(n = 9)</td>
</tr>
<tr>
<td>NAI (µm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>42.9 ± 12.6</td>
<td>57.6 ± 19.0</td>
</tr>
<tr>
<td>Min</td>
<td>26.89</td>
<td>31.70</td>
</tr>
<tr>
<td>Max</td>
<td>62.75</td>
<td>87.40</td>
</tr>
<tr>
<td>AgNAI (µm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>4.42 ± 1.94a</td>
<td>8.07 ± 4.01b</td>
</tr>
<tr>
<td>Min</td>
<td>2.54</td>
<td>3.79</td>
</tr>
<tr>
<td>Max</td>
<td>8.67</td>
<td>15.44</td>
</tr>
<tr>
<td>AgNOR (μm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>3.08 ± 0.73a</td>
<td>5.34 ± 1.43b</td>
</tr>
<tr>
<td>Min</td>
<td>1.65</td>
<td>3.12</td>
</tr>
<tr>
<td>Max</td>
<td>4.42</td>
<td>7.71</td>
</tr>
<tr>
<td>PCNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>59.0 ± 12.7</td>
<td>66.6 ± 6.3</td>
</tr>
<tr>
<td>Min</td>
<td>42.5</td>
<td>53.2</td>
</tr>
<tr>
<td>Max</td>
<td>88.8</td>
<td>77.4</td>
</tr>
</tbody>
</table>

**SD:** standard deviation; **Min:** minimal value; **Max:** maximal value; **NAI:** Nuclear area index; **AgNAI:** AgNOR Area Index; **AgNCI:** AgNOR Count Index; **AgNAI / NAI:** relative AgNOR area; **AgNAI / AgNCI:** mean AgNOR area; **AgNCI / NAI:** AgNOR density; **PCNA:** Proliferating Cell Nuclear Antigen.

The different superscripts a,b in the same row indicate significant difference (P < 0.05) between the 2 tumour types.

**Table III :** Correlations between AgNOR indices and PCNA index in benign (n = 12) and malignant epithelial tumours (n = 9) observed in different species (mainly dog and cattle).

<table>
<thead>
<tr>
<th></th>
<th>Benign</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 12)</td>
<td>(n = 9)</td>
</tr>
<tr>
<td>NAI</td>
<td>r = 0.758; P &lt; 0.01</td>
<td>r = 0.844; P &lt; 0.01</td>
</tr>
<tr>
<td>AgNAI</td>
<td>r = 0.530; NS</td>
<td>r = 0.657; NS</td>
</tr>
<tr>
<td>AgNCI</td>
<td>r = 0.663; P &lt; 0.05</td>
<td>r = 0.611; NS</td>
</tr>
<tr>
<td>AgNAI/NAI</td>
<td>r = 0.203; NS</td>
<td>r = 0.509; P &lt; 0.01</td>
</tr>
<tr>
<td>AgNAI/AgNCI</td>
<td>r = 0.716; P &lt; 0.01</td>
<td>r = 0.770; P &lt; 0.05</td>
</tr>
<tr>
<td>AgNCI/NAI</td>
<td>r = 0.220; NS</td>
<td>r = 0.872; P &lt; 0.01</td>
</tr>
<tr>
<td>PCNA</td>
<td>r = 88.8</td>
<td>r = 0.612; NS</td>
</tr>
<tr>
<td>PCNAI</td>
<td>r = -0.640; P &lt; 0.05</td>
<td>r = 0.623; NS</td>
</tr>
<tr>
<td></td>
<td>r = 0.332; NS</td>
<td>r = 0.356; NS</td>
</tr>
<tr>
<td></td>
<td>r = -0.644; NS</td>
<td>r = 0.163; NS</td>
</tr>
<tr>
<td></td>
<td>r = 0.187; NS</td>
<td>r = 0.014; NS</td>
</tr>
<tr>
<td></td>
<td>r = 0.144; NS</td>
<td>r = 0.230; NS</td>
</tr>
<tr>
<td></td>
<td>r = 0.207; NS</td>
<td>r = 0.118; NS</td>
</tr>
</tbody>
</table>

**NAI:** Nuclear area index; **AgNAI:** AgNOR Area Index; **AgNCI:** AgNOR Count Index; **AgNAI / NAI:** relative AgNOR area; **AgNAI / AgNCI:** mean AgNOR area; **AgNCI / NAI:** AgNOR density; **PCNA:** Proliferating Cell Nuclear Antigen. **NS:** Not significant.

**Discussion**

The skin is widely affected by tumours and other diseases. Sun light, ionized radiation, viruses, chemical substances, hormones, genetic and immunologic factors have an important role in the development of tumours. Skin and subcutaneous tumours occupy more than 30% of all tumours in dogs and more than 20% in cats [25].

The classification of skin tumours based on histological criteria alone is difficult and insufficient because different structural components according to the species can be observed for a same tumour type [25]. The tumour classification is generally based on the cellular origin: epithelial skin tumours mainly consist in basal and squamous cell carcinomas, papillomas, sweat gland tumours, hepatoid (perianal gland) tumours, trichoepitheliomas and keratoacanthomas (intracutaneous cornified epitheliomas); benign and malignant melanomas have a melanocytic origin and are considered as mesenchymal tumours whereas fibromas, fibrosarcomas, lipomas and haemangiomata originate from soft tissues of subcutaneous region [25, 28]. In the current study, the main epithelial tumour types encountered in dogs were peri-anal gland adenomas, keratoacanthomas and epithelioma whereas in cattle, papillomas and fibropapillomas were the more frequent benign tumours. The squamous cell carcinoma was the main type of the epithelial malignant tumours (n = 7), followed by a perianal gland carcinoma and an apocrine carcinoma.
Anisonucleosis characterized by differences in shape and size of the nucleus in atypical cells is an important criterion to differentiate malignant tumours from benign ones [10]. Although there was no statistically significant difference \((P > 0.05)\) for the nuclear area between benign and malignant tumours, nuclear area was larger in malignant cases. These measurements although not directly related to shape differences together with anisonucleosis could be used to differentiate between benign and malignant tumours. In addition, when the AgNOR number increases, the dimensions of NOR bodies decrease, and differences in shape, count and size also occur. The AgNOR increase in proliferative events, mainly evidenced by the diameter increase of AgNOR dots instead of the AgNOR counts, has been attributed to increases of rRNA and tDNA synthesis coupled to the nucleolar volume enhancement [20, 22] leading to amplification of protein synthesis in neoplastic tissues. This highlights the importance of the AgNOR area (AgNAI) measurements. In this way, a strong positive correlation between the nuclear area (NAI) and the AgNOR area (AgNAI) was found in benign and malignant epithelial tumours. A study carried out for comparing histological structure and tumour behaviour in dog seminomas revealed that the AgNOR nuclear area was more related to tumour malignancy than the AgNOR count [4]. In the present study, in agreement with that, the AgNOR areas were significantly higher \((P < 0.05)\) in malignant tumours than in benign cases but the AgNOR count (AgNCI) was also dramatically elevated in malignant cases compared to benign tumours \((P < 0.001)\). Furthermore, the relative AgNOR area (calculated by the ratio AgNAI / NAI) was also significantly enhanced in malignant tumours \((P < 0.05)\) and significantly and positively correlated with the AgNOR area (AgNAI) in both benign and malignant tumour types. This parameter was also positively and significantly correlated with the AgNCI but only in benign tumours, suggesting that in malignant tumours, the enumeration of the AgNOR bodies would be more difficult.
Indeed, there are some discrepancies about the AgNOR enumeration: some authors only count dots inside nucleoli [3] whereas others take in consideration all intra- and extranucleolar AgNOR dots [13] and each dot is more often counted as a single AgNOR [11]. In the present study, NOR dots in the nucleoli were not counted one by one, but NOR dots in nuclear area regrouped into clusters were counted, although it was difficult to count them one by one because of the absence of definite borders of NOR dots in the nucleoli due to staining. Moreover, many factors in AgNOR staining such as fixing solutions, temperature, time and pH affects the counting results [9, 17, 24, 26]. A prolonged staining incubation causes staining of whole part of the nucleoli, leading to the appearance of a giant size structure. Besides, many AgNOR bodies become like a single structured AgNOR dot when chromosomes carrying NOR bodies are joined in interphase. Therefore, the counting of AgNOR clusters instead of counting black dots one by one in nucleoli has been proposed for avoiding these artefacts. Consequently, the standardization of AgNOR staining techniques and counting methods is absolutely required for proposing the AgNOR measurements as relevant and objective markers of cell proliferation rate. Nevertheless, the AgNOR enumeration is already considered in human medicine as a prognostic marker in some tumours. A high AgNOR quantity is a marker of very malignant in human medicine as a prognostic marker in some tumours. Nevertheless, the AgNOR enumeration is already considered in human medicine as a prognostic marker in some tumours. A high AgNOR quantity is a marker of very malignant tumour phenotype, with poor prognosis, such as pharyngeal carcinoma, multiple myeloma, malignant thymoma, male breast cancer and prostate carcinoma [19].

It was reported that NOR dots were bigger but less abundant in benign tumours and on the contrary, smaller and more frequent in malignant prostate tumours in humans [1]. Although similar findings were found in the present study, the difference in AgNAI/AgNCI (mean AgNOR area) between benign and malignant epithelial tumours was insignificant \( (P > 0.05) \), probably because of the method for AgNOR count determination: the AgNCI was probably underestimated because AgNOR clusters which regrouped several AgNOR dots were counted as single AgNOR dots. However, strongly positive associations between AgNAI/AgNCI and NAI or AgNAI were found in the both tumour types. In the same way, the AgNOR density given by the formula AgNCI/NAI tended to be higher in malignant epithelial tumours but the differences with the benign cases was not significant. Besides, no significant correlation between the AgNOR density (AgNCI/NAI) and the other parameters characterizing the AgNOR was found in malignant tumours whereas this parameter positively correlated with the AgNOR numbers and negatively with NAI and the mean AgNOR area (AgNAI/AgNCI) in benign tumours. It was reported that two cell types in different density for uniform nuclear staining was observed. While the cells were heavily AgNOR stained in the S phase, they were weakly stained in other phases. These differences in staining density would complicate the enumeration of the AgNOR bodies and strengthen the necessity to take into consideration the cell cycle phases [5].

On the other hand, although the numbers of PCNA positive cells did not significantly differ between malignant and benign tumours, the PCNA staining intensity was globally higher in malignant cases. In agreement, it was reported that the PCNA index and the mitotic index were not significantly modified between cornified intra-cutaneous epithelioma and squamous cell carcinoma whereas the AgNOR count was significantly increased \( (P < 0.01) \) [15]. In the present study, similar findings (significant modifications of the AgNOR characteristics while the PCNA index was not modified) were also noted. In the present study, the PCNA index was determined regardless of staining density. Moreover, the absence of significant correlation between PCNAI and all parameters for AgNOR characterization suggest that PCNA was expressed more or less intensively whatever the cell cycle phases. Consequently, it is also necessary to standardize the method for PCNA quantification for taking into consideration simultaneously the number of positive cells and the staining intensity.

As a conclusion, contrary to the PCNA determination, a complete AgNOR characterization would be useful for differentiating malignant from benign epithelial tumours, the most relevant parameters being the AgNAI (AgNOR Area Index), the AgNAI/NAI (relative AgNOR area) and the AgNCI (count of AgNOR bodies). Because of difficulties of the enumeration of the AgNOR bodies, particularly when they were small and organized into clusters as in malignant tumours, the 2 other calculated parameters (mean AgNOR area - AgNAI / AgNCI and the AgNOR density - AgNCI / NAI) would be underestimated. Besides, the AgNOR characteristics would differ according to the tumour type (for example, between papillomatosis, keratoacanthomas, adenoma and epithelioma) and the heterogeneity in cell structure and cell behaviour inside the 2 groups of tumours (benign and malignant) would induce important value dispersion, and would compromise statistical analyses. Taking into consideration the animal species, the general structural tumour characteristics and the cell type origin would be more objective and would yield to more reliable outcomes. Whereas the AgNOR characterization is used in human medicine for differentiating benign and malignant tumours, for establishing a prognostic and for evaluating treatment efficiency, these applications remain uncertain in veterinary medicine: further investigations are needed for AgNOR characterization according to the animal species and to the tumour type.

Acknowledgements

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