Flow Cytometric Analysis of DNA Ploidy in Canine Mammary Tumors

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ABSTRACT

DNA ploidy has been determined using flow cytometry in 23 nonmalignant and 34 malignant (primary and metastatic) mammary tumors from 46 dogs. This parameter was compared with clinical stage, histology, and estrogen and progesterone receptor analysis. Twenty-one of 34 cancers (61.8%) from 32 dogs were DNA aneuploid. Aneuploidy was also found in 4 of 23 nonmalignant tumors (17.4%) from 20 dogs. Regional lymph nodes were involved in 6 of 10 diploid and 3 of 9 aneuploid cancers of dogs with operable disease. The aneuploidy incidence was higher in dogs that had distant metastasis at initial diagnosis (8 of 11) than in those presented with local or locoregional disease (9 of 19), although this difference was not statistically significant. DNA aneuploidy incidence was not found to be related to histological tumor type, histological malignancy grade, nuclear grade, or steroid receptor presence. Heterogeneity in DNA content was found in 4 of 32 cancers (30 dogs) in samples from primary or locally recurrent lesions. In 3 of 16 cancers that were analyzed both at the primary and at secondary sites of growth, a significant variation in DNA content was observed. The degree of aneuploidy in the dog cancers was much lower than seen for human breast carcinomas with a relatively high frequency of hypoploid stemlines (7 of 34 cancers, 20.6%). The frequency distribution of DNA indices in dog mammary cancers indicates that aneuploidy evolution probably differs from that of human breast cancer.

INTRODUCTION

In women with breast cancer DNA aneuploidy, as defined by FCM analysis, has been associated with an unfavorable prognosis (1-4). A correlation of DNA aneuploidy with adverse prognostic factors such as a poor differentiation state (4-9) and steroid receptor negativity (4-8, 10) has been reported. The applicability of DNA ploidy analysis will probably be influenced by the variability of this feature among tumor cells of the same cancer. Heterogeneity of DNA content within primary breast cancers as shown by the appearance of multiple aneuploid stemlines has been observed in 0-19% of cases (1-5, 7, 8, 11). Still, analysis of DNA content in small series of various solid tumors (12, 13) and of breast cancer (11, 14, 15) revealed a close similarity of DNA ploidy in primary and secondary growths in the same patient in the great majority of cases.

Canine mammary cancer shares some important features with its human counterpart, such as the role of ovarian hormones in the pathogenesis (16), the frequent occurrence of metastasis (17), and possibly, the association between the presence of steroid-hormone receptors in primary tumors and prognosis (18). DNA index frequency distributions of canine and human solid tumors have been reported to display an interspecies similarity (19). The present study was undertaken in order to determine the DNA ploidy pattern in canine mammary tumors. Nuclear DNA content was analyzed by FCM in nonmalignant and malignant, both primary and metastatic, mammary tissue. In malignant tumors, results were compared with clinical stage, histological malignancy grade, and steroid receptor status.

MATERIALS AND METHODS

Animals. Tumorous and nonaffected mammary tissue was collected at surgery or autopsy from 48 female dogs for histopathological examination and determination of nuclear DNA content by FCM as well as steroid receptor analysis. Thirty-one dogs underwent surgery for tumors considered operable according to clinical criteria (including X-ray examination of the thorax) without further treatment, and 30 of these were followed up for at least 1 year or until death with subsequent autopsy. Seventeen other dogs were euthanatized at the owners' request because of advanced local or systemic malignant disease. Clinical staging of the dogs was done on the basis of the WHO TNM classification for tumors in domestic animals (20), where T stands for tumor size and fixation, N for the condition of the regional lymph nodes, and M for the absence/presence of distant metastasis. We did not follow the proposed grouping of stages, since we feel that histological examination does not carry enough weight in this classification system. Instead, a division into stage of disease was performed as follows: (a) local disease, any T1-T3, no evidence of regional node involvement, as verified by histology, or distant spread; (b) advanced local disease, T4 (inflammatory carcinoma), no evidence of regional node involvement or distant spread; (c) regional disease, any T, with axillary or superficial inguinal lymph node involvement verified by histology; (d) distant disease, distant metastasis present, including distant lymph node involvement.

Sample Preparation. Upon removal tissues were immediately placed in melting ice. Following euthanasia tissues were removed within 30 min. Macrosкопically tumorous tissues and in some cases nonaffected tissues well separated from tumorous areas were partly dissected, cleared of fat and necrotic parts, and cut in blocks of approximately 0.5 cm diameter. Several blocks and the remaining specimen were fixed in neutral phosphate-buffered 10% formalin for histopathological examination. The other blocks were quick-frozen in liquid nitrogen and stored at -70°C until analysis.

Histological Examination. The histological examination was done by one of the authors (W. M.) on hematoxylin-eosin stained sections according to the WHO classification for tumors in domestic animals (21). This examination included also sections adjacent to tissue blocks to determine receptor and DNA index in order to assess histological variability. Also, the relative amount of tumor cells (epithelial, myoepithelial, and/or mesenchymal) and of preexisting mammary epithelium was assessed as a percentage of tissue occupied by these components in microscopic sections. Twenty-three nonmalignant lesions from 20 dogs and 36 malignant lesions from 34 dogs were analyzed. This analysis included 7 animals where both types of lesion were studied. Distribution according to histological type is given in Table 2. Histological grading of carcinomas and sarcomas was performed as previously described (22).

Steroid-Receptor Analysis. ER and PR content was determined in all samples of the dogs in which nuclear DNA analysis was carried out. Tissue concentrations of ER and PR were determined as previously described (23). Absorbances at 414 nm were determined using a reference solution of 17β-estradiol at a concentration of 1 ng/ml and a standard curve to determine concentrations in the tissue homogenate.

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4 The abbreviations used are: FCM, flow cytometry; ER, estrogen receptor; PR, progesterone receptor; DI, DNA index.
negative (ER and PR negative) samples. At histological examination some samples of primary tumors were found to contain tumor as well as preexisting mammary epithelium. This latter structure, even if present in low quantity, may interfere with steroid receptor results. In nonaffected mammary glands with low (10% of surface area in microscopic sections) epithelium content ER and PR concentrations exceeding 100 fmol/mg protein have been observed by us (24). Steroid receptor results in primary tumor specimens with pre-existing mammary epithelium were therefore excluded.

Flow Cytometric Analysis of DNA Content. Frozen samples (n = 85) were thawed at 20°C and cut into small pieces with razor blades. Suspensions of single nuclei were prepared with the detergent-trypsin procedure of Vindolav et al. (25), and stained with propidium iodide (Sigma Chemical Co., St. Louis, MO).

From paraffin-embedded blocks of formalin-fixed specimens (n = 20) 35-μm sections were cut and deparaffinized in xylene. Cell suspensions from the deparaffinized tissue were prepared by treatment with 0.5% pepsin (Sigma No. P7000; Sigma) in saline for 30 min, according to the procedure of Hedley et al. (26).

The cells were stained with 4',6-diamidino-2-phenylindole dihydrochloride (Boehringer, Mannheim, Federal Republic of Germany). Stained samples were measured on an ICP 22 flow cytometer (Ortho, Westwood, MA). Filtered demineralized water was used as sheath fluid. For excitation of propidium iodide fluorescence, LP 515 and SP 560 filters were used in combination with a 560-nm chromatic beam splitter. Emission was measured using an LP 590 barrier filter. For excitation of 4',6-diamidino-2-phenylindole dihydrochloride fluorescence a UG 365 filter was used in combination with a 400-nm chromatic beam splitter. Emission was measured using a 435-nm barrier filter.

Nuclear suspensions of frozen material (n = 85) were analyzed with and without the addition of rainbow trout RBC or normal canine kidney cells as an internal ploidy standard and in some cases (n = 11) with both standards separately. The canine reference cells were prepared from frozen blocks of histologically normal kidney according to the method described for mammary tissue specimens. Rainbow trout RBC were found to have the same DNA content as diploid dog cells that were present in virtually all samples, and as canine kidney cells. The latter cells were found to have the same DI of 2.27 ± 0.018 (SE) (n = 18) compared to reference chicken RBC as the reported DI of 2.28 of rainbow trout RBC (27).

Some samples (n = 19) were also analyzed using chicken RBC as a standard. For samples prepared from paraffin-embedded tissue nonneoplastic cells, present in a sufficient quantity (>10%) as verified by histological examination, served as internal ploidy standard. Multiple samples of many breast tumors including all hypoploid tumors were reanalyzed to establish the accuracy of the measurement. In cases where a difference was noticed between 2 separate determinations, these were repeated 4 to 7 times in order to define the possible heterogeneity of a given specimen. Cell yields of all specimens permitted analysis of at least 10,000 cells in each assay, performed by either method.

Ploidy Assessment. The DI was defined as the ratio of the modal DNA values, i.e., the modal channel number of the G0-G1 peak in relation to the modal channel number of the G0-G1 fraction of diploid cells, which in frozen material was recognized by its position equal to the G0-G1 peak of standard rainbow trout RBC or normal canine kidney cells. For samples prepared from paraffin-embedded material this was not possible (26) but here nonneoplastic cells that were found in a sufficient quantity in all samples served as diploid reference. However, hypoploidy could not readily be discriminated in such samples from hyperploidy and was therefore included under the latter category.

Tumors with a distinct G0-G1 population with DI > 1.0 were defined as aneuploid. Tumors were considered tetraploid if a peak occurred with a DI of 1.90 to 2.10 consisting of more than 20% of the total number of analyzed cells and if a peak corresponding to G2-M cells of a tetraploid cell population was also present. When more than one aneuploid G0-G1 population was present the tumor was classified as multiploid.

Statistics. Differences in frequency distribution in data groups were assessed with the χ2 or Fisher's Exact Test. The level of significance was set at a P of 0.05.

RESULTS

The use of frozen as well as of paraffin-embedded material yielded satisfactory results of DNA FCM analyses in almost all tumors. The only exceptions were 2 dogs with regional lymph node positive breast cancer, where (the anaplastic) tumor specimens contained less than 10% of tumor cells both at primary and secondary sites. These cases, that at DNA content analysis did not have a detectable aneuploid G0-G1 peak, were excluded from further consideration (26). All other specimens examined had more than 10% tumor cells. The variation coefficient for the G0-G1 peak of diploid and of aneuploid cells was 2.27 ± 0.06 (n = 179) in frozen samples and 4.31 ± 0.31 (n = 29) in paraffin-embedded samples. DNA measurements in frozen and paraffin-embedded material from the same specimen (n = 7) yielded similar results. One embedded sample (a nonmalignant tumor) was classified as hyperploid that by definition could have been hypoploid. All other of the 13 samples (from 4 dogs) in which analysis was only possible to paraffin-embedded material, were found to be either diploid or tetraploid.

Nonaffected mammary gland tissue from tumor bearing dogs (n = 4) was found to be diploid.

Nonmalignant Tumors

Four of the 23 histologically nonmalignant tumors (from 20 dogs) had aneuploid G0-G1 peaks. One of 12 dogs that had initially and during follow-up nonmalignant disease only had an aneuploid tumor (DI, 1.10). The other 3 aneuploid nonmalignant tumors (DI, 1.09/1.76; 1.14; 1.34) were detected in 3 of the 8 dogs that also developed breast cancer (Table 1). The first of these 3 dogs had a multiploid nonmalignant tumor. The DI of one stemline (DI, 1.76) was similar to that of the subsequently observed metastasizing breast cancer (DI, 1.65). The second dog was at autopsy found to have one diploid and one hyperploid (Fig. 1B) nonmalignant tumor, together with a tetraploid metastasizing cancer. The third dog had a hyperploid benign lesion together with a diploid cancer. Independent review (Dr. A. L. Parodi, Ecole Vétérinaire, Alfort, France and Dr. D. E. Bostock, Faculty of Veterinary Medicine, Cambridge, United Kingdom) of the tumors of the 4 dogs with aneuploid nonmalignant lesions agreed with the original diagnosis in all tumors except one: this lesion, classified as nonmalignant by
Malignant Tumors

DNA Ploidy and Clinical Stage. Nineteen dogs were presented with local/locoregional cancers. In 13 dogs, including the 4 animals that had inflammatory carcinoma, distant spread was recognized at presentation at our clinic. Three of the 14 metastatic cancers that were found in these 13 dogs were not included in the comparison of clinical stage and ploidy status. In 2 dogs (Nos. 103 and 106) the primary cancer had been operated upon elsewhere and the third animal had bilateral cancers. In this latter dog (No. 96) we considered one (aneuploid) cancer metastatic. The clinical N-M stage of the other (aneuploid) tumor that had a DI and morphology different from that observed in the other tumor sites was considered inconclusive. The size of the primary tumor (T) and ploidy status in dogs of both groups were not correlated (data not shown).

In 9 of the 19 local/locoregional cancers an aneuploid DNA content was detected. Regional lymph node invasion was more common in diploid than in aneuploid tumors of this group, but the difference was not statistically significant. Eight of the 11 animals with distant spread at first diagnosis had aneuploid cancers (Table 1). Thus, aneuploidy was more frequent in dogs with clinically evident distant metastatic disease at first diagnosis than in dogs with local/locoregional cancers, although the difference was not significant (P = 0.17). Of the 17 animals that were followed up after surgery, 4 of the 9 diploid and 5 of the 8 aneuploid cancers gave rise to distant metastasis. One of these 17 dogs developed a heterolateral primary cancer after an interval of 2.5 years. Both tumors were confined to local growth only. The DNA content was found to be different (DI, 1.00 and 1.06, respectively).

In 21 (61.8%) of the total 34 cancers (from 32 dogs) aneuploid G0-G1 peaks were found. This proportion is significantly higher than that in nonmalignant tumors (4 of 23 = 17.4%; Table 1).

Histological Differentiation. In this series of cancers there was no correlation between histology and ploidy status (Table 2). Even in anaplastic tumors the proportion of aneuploid cases (5 of 9) was comparable to that in the other tumor types combined (15 of 25). Comparisons of histological malignancy grade and nuclear grade with ploidy status did not reveal significant correlations (Table 3).

Steroid Receptor Status. Conclusive receptor data became available from 25 cancers (24 dogs). From 3 cancers no material was available for receptor assay and from 6 cases the available tissue samples consisted of tumor mixed with preexisting epithelium, which prohibited the use of the data. Aneuploid tumors, irrespective of the site of tissue collection, were more frequently receptor negative than were diploid tumors. However, this difference was not significant (Table 4). Four cancers

Table 2 Aneuploidy in 57 canine mammary tumors in relation to type of histology

<table>
<thead>
<tr>
<th>Histology</th>
<th>No. of tumors</th>
<th>Diploid</th>
<th>Aneuploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lobular hyperplasia with epitheliosis</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Complex adenoma</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Fibroadenoma</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Benign mixed tumor</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Complex borderline tumor</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Solid carcinoma</td>
<td>15</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Anaplastic carcinoma*</td>
<td>9</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Tubular adenocarcinoma</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Complex papillary carcinoma</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Carcinosarcoma</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* Two anaplastic carcinomas have been excluded from analysis because of the low number of tumor cells observed in the histological sections.

Table 3 Relationship of histological malignancy or nuclear grades to DNA aneuploidy incidence in malignant tumors

<table>
<thead>
<tr>
<th>Histological malignancy grade</th>
<th>No. of cancers</th>
<th>Diploid</th>
<th>Aneuploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>15</td>
<td>5</td>
<td>10 (not significant*)</td>
</tr>
<tr>
<td>III</td>
<td>16</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nuclear grade</th>
<th>No. of cancers</th>
<th>Diploid</th>
<th>Aneuploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>19</td>
<td>8</td>
<td>11 (not significant*)</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

* The comparison of aneuploidy incidence in the histological malignancy or nuclear grades, considered separately ($\chi^2$ test) or in combination (first 2 grades together versus third grade, Fisher's Exact Test), did not reveal any significant relationship.

Fig. 1. DNA histograms of canine mammary tumors. A, benign mixed mammary tumor (frozen sample) showing a diploid profile; B, benign mixed mammary tumor (paraffin-embedded sample) excised at autopsy of a dog with a heterolateral metastasizing aneuploid (DI, 1.94) mammary osteosarcoma (not indicated). A small aneuploid G0-G1 peak (DI, 1.14) distinct from the prominent diploid peak was demonstrated. The proportion of tumor cells in microscopic sections (~60%) indicated heterogeneity of DNA content among tumor cell populations. No. refers to dog number; CRBC, chicken RBC.
had discordant receptor results at different tumor sites (Table 4). Yet, DNA content did not vary: 3 tumors were diploid and one aneuploid with constant DI, at all sites examined.

Heterogeneity in DNA Content. Four out of 32 cancers (30 dogs) had a heterogeneous DNA content in the primary/locally recurrent lesion: in 3 of these tumors a diploid G0-G1 peak or both a diploid as well as an aneuploid G0-G1 peak was identified in separate tissue blocks of the same specimen by multiple measurements. One dog (No. 78; see also Table 5) had a multiploid primary cancer.

In 18 metastasizing cancers DNA ploidy was determined at 2 or more sites (Table 5). In 2 aneuploid cases only metastatic lesions were examined, not showing an intersite variation in the DI. In 3 (aneuploid) cases of the other 16 cancers, differences between tumor cell populations with regard to the DI were demonstrated. In addition, in one dog (No. 108) the metastasizing tumor was defined as tetraploid at sites with a high density of tumor cells, whereas in samples with low tumor cellularity the classification was invariably diploid with the notation of a high fraction of G2-M cells. It is possible that the so-called G2-M cells were in fact tetraploid tumor cells but that their number in the nuclear preparations analyzed was below the 20% of all cells necessary for a tetraploid classification.

DNA Index Distribution. A presentation of stemlines of all cancers as characterized by the G0-G1 peaks in DNA distribution profiles is given in Fig. 4. Thirteen of 34 malignancies displayed only diploid G0-G1 populations. It is clear that the increase in DNA content in hyperploid cases is mostly moderate and that hypoploid stemlines are relatively common, with a frequency of 7 of 34 cancers (20.6%).

**DISCUSSION**

In this study DNA aneuploidy incidence was determined in clinically and histologically defined nonmalignant mammary lesions and mammary cancers in the dog. The finding of aneuploidy in 4 of the nonmalignant tumors is noteworthy; it may reflect the malignant potential suspected to exist in some cases of this condition (28). An independent review confirmed the original diagnosis in 3 of these 4 cases. One of the tumors was classified as borderline lesion by one of the reviewers. From each specimen multiple blocks were examined by both methods with similar results. Therefore, we think it unlikely that malignant cells were present in blocks used for FCM and not in blocks taken for histology. Perhaps early changes towards malignancy are detected by FCM that are not yet reflected by histomorphology of these lesions. The occurrence of aneuploidy in benign mammary tumors in the dog has recently been observed by others as well. Aneuploid tumor cells have also been

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**Table 4 Ploidy and receptor status in canine mammary cancer**

<table>
<thead>
<tr>
<th>Tumor site</th>
<th>No. of cancers</th>
<th>R+*</th>
<th>R−</th>
<th>R+/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>I, local</td>
<td>9</td>
<td>3/4</td>
<td>4/5</td>
<td></td>
</tr>
<tr>
<td>IIA, local + metastatic</td>
<td>6</td>
<td>1/1</td>
<td>2/3</td>
<td>0/2</td>
</tr>
<tr>
<td>IIB, metastatic</td>
<td>10</td>
<td>0/2</td>
<td>6/6</td>
<td>1/2</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>4/7</td>
<td>12/14</td>
<td>1/4</td>
</tr>
</tbody>
</table>

* R+, positive receptor status, ER and/or PR levels ≥ 5 fmol/mg cytosolic protein; R−, negative receptor status; R+/−, discordance of receptor status between different tumor sites.
* Local, primary, and/or locally recurrent cancer specimens.
* Conclusive receptor results obtained in regional and/or distant metastatic lesions only.
* In the combined groups I, IIA, and B (discordant cases excluded) no significant relationship between ploidy and receptor status was found (P = 0.18).

**Table 5 DI in different tumor sites of canine mammary cancers**

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Local</th>
<th>Tumor site</th>
<th>Relative node</th>
<th>Distant</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00 (1)*</td>
<td></td>
</tr>
<tr>
<td>73</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00 (1)</td>
<td></td>
</tr>
<tr>
<td>74</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00 (1)</td>
<td></td>
</tr>
<tr>
<td>88</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00 (2)</td>
<td></td>
</tr>
<tr>
<td>95</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00 (3)</td>
<td></td>
</tr>
<tr>
<td>98</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00 (4)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1.06</td>
<td>1.06</td>
<td>1.04</td>
<td>1.08 (1)</td>
</tr>
<tr>
<td>8</td>
<td>1.04</td>
<td>1.04</td>
<td>1.00 (1)</td>
<td>1.06 (1)</td>
</tr>
<tr>
<td>54, local</td>
<td>1.65</td>
<td>1.58 (1)</td>
<td>1.70 (1)</td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>1.40</td>
<td>1.30 (1)</td>
<td>1.30 (1)</td>
<td></td>
</tr>
<tr>
<td>68</td>
<td>1.29</td>
<td>1.30 (2)</td>
<td>0.79 (1)</td>
<td></td>
</tr>
<tr>
<td>89</td>
<td>0.79</td>
<td>0.88 (1)</td>
<td>0.92 (2)</td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>0.89</td>
<td>0.89 (1)</td>
<td>0.92 (2)</td>
<td></td>
</tr>
<tr>
<td>103</td>
<td>1.08 (2)</td>
<td></td>
<td>1.08 (1)</td>
<td></td>
</tr>
<tr>
<td>106</td>
<td>0.79</td>
<td>0.77/1.56 (1)</td>
<td>1.57 (1)</td>
<td></td>
</tr>
<tr>
<td>78</td>
<td>0.79/1.62</td>
<td>1.56 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>108</td>
<td>1.00/1.94</td>
<td>2.03 (1)</td>
<td>1.00 (2)</td>
<td></td>
</tr>
<tr>
<td>108</td>
<td>0.89</td>
<td>0.77/1.56 (1)</td>
<td>2.00 (1)</td>
<td></td>
</tr>
<tr>
<td>110</td>
<td>2.00</td>
<td>1.75/1.98 (1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number of tumors.
studies of nuclear DNA content have not (8) or rarely (31) identified adenomas of the salivary glands (30). On the other hand, FCM of human fibrocystic breast disease (29) and in pleomorphic canine mammary cancers. In cases where a diploid G0-Gi peak was accompanied by an aneuploid peak, only the latter is included in this chart.

Our present data did not demonstrate a significant correlation between histological type, malignancy grade, or nuclear grade and ploidy status. In particular the lack of a correlation between ploidy status and nuclear grade seems puzzling. However, hypoploid tumors were relatively common and the increase in DNA content in hyperploid cancers was rather small in this series. Thus, an increase in nuclear volume, interpreted as sign of anaplasia, may be expected to be absent or moderate in most tumors. In another study of solid canine tumors of varying histology, cytological differentiation and ploidy were found to be related (19). Although opinions differ on the relationship of histological tumor type and ploidy status of human breast cancer (4, 8, 33), many authors have indicated the existence of a relationship between cytological (4, 5, 11, 33) or histological differentiation (6–9, 33) and ploidy status. In this study receptor positive tumors (irrespective of site of tissue collection) more often were DNA diploid than receptor negative tumors, although the difference was not significant. In existing mammary epithelium in primary cancers that may be responsible for false-positive results (24). In the evaluable cases of this study receptor positive tumors (irrespective of site of tissue collection) more often were DNA diploid than receptor negative tumors, although the difference was not significant. In canine cancers with intersite discordance of receptor status there was no concomitant change in ploidy status.

In at least one of the benign as well as in 4 of the primary/recurrent cancer specimens the occurrence of 2 or more cell populations with different DNA content was demonstrated. Both multiploidy established in one assay as well as variation in ploidy status among different tissue blocks of the same specimen were found. The latter factor has been reported to be rare in human breast cancer as studied by image cytometry (38) in contrast to, e.g., renal cancer, where heterogeneity in ploidy status in different samples of the same tumor has been seen frequently (39). Multiploidy defined as the occurrence of 2 or more aneuploid peaks within primary breast cancers has been observed in 0 to 19% of cases in the human (1–5, 7, 8, 11) and in 2 of 15 mammary cancers in the dogs studied earlier (19).

![DNA histograms of primary cancer (A) and metastases (B) removed at autopsy 18 months after operation from the same dog (frozen samples). Apart from the diploid peak present in all specimens, a hypoploid peak can be discerned in the primary lesion (DI, 0.79) and the pleural metastasis (DI, 0.77) but not in the lung metastasis. Both distant tumor sites contained a hyperploid peak (DI, 1.56 and 1.57, respectively) possibly originating from a polyploidization of the primary hypoploid G0-G1 tumor stem line. No. refers to dog number; CRBC, chicken RBC.](image)

![DNA indices of stemlines present in 34 primary and/or metastatic canine mammary cancers. In cases where a diploid G0-G1 peak was accompanied by an aneuploid peak, only the latter is included in this chart.](image)
Three of 18 cancers in this study had significant intersite variation with regard to DNA content. In 2 of these dogs the difference possibly resulted from a polyploidization of the primary hypoploid G0-G1 tumor stemline. The hypoploid fraction was not detectable in (some of) the metastases. Perhaps the generated hypoploid cells do have a growth advantage over parent hypoploid cells. In an early study on human cancers using image cytometry of Feulgen-stained sections, the incidence of aneuploid cells was reported to be increased in metastases as compared to the primary site (40). Later studies using FCM or image cytometric analysis of DNA content of human solid tumors, including breast cancers, have indicated a close similarity of DNA ploidy in primary and secondary growth in the great majority of patients (10-15, 41). Marked intersite differences in DNA ploidy, however, have been observed at higher frequency in some human neoplasms such as renal (42) and lung carcinoma (43).

In the present series of canine cancers the degree of aneuploidy of hypoploid tumors was relatively low compared to that found in human breast cancer (4, 5, 7, 8, 11). A similar DNA index distribution pattern can be discerned from the data of another FCM study of various solid tumors in the dog (19).

Further, hypoploid cancers in our study population seemed to be more frequent than in the human (4, 32). More studies are necessary to confirm these findings. Yet, in another canine malignancy, namely osteosarcoma, karyologically confirmed hypoploid cases have been found frequently (44). Other investigators recently reported on the loss of chromosomes in 3 of 6 hypoploid cases have been found frequently (44). Other investigators recently reported on the loss of chromosomes in 3 of 6 canine mammary cancer cell lines isolated in culture (45). Thus, the occurrence of a chromosomal loss in solid neoplasms of the dog may be more common than in the human. Perhaps the dog karyotype permits loss of chromosomes during the process of transformation and progression without a loss of viability of cells more often than does the karyotype in man. All autosomes in the canine karyotype (consisting of 78 chromosomes) exhibit a telocentric or acrocentric configuration, in contrast to the predominantly metacentric configuration of human autosomes (46). The distribution of genes coding for vital cellular functions over a larger number of chromosomes might enable canine cells to overcome chromosomal loss to a greater extent. Chromosomal loss may even be a major factor in the generation of a malignant phenotype of cells, since it can imply the loss of suppressor or antioncogenes (47, 48).

In conclusion, FCM studies of DNA ploidy in canine mammary tumors have demonstrated the occurrence of aneuploid cells within some benign lesions and in the majority of malignant lesions. No significant correlation with clinical stage and histological malignancy grade could be established in mammary cancers, although the proportion of aneuploid cases was greater among tumors that had developed distant metastasis at initial presentation than among tumors without detectable metastasis. Heterogeneity of DNA content was observed within some benign and locally removed malignant lesions as well as between different sites of some of the metastasizing cancers.

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DNA PLOIDY IN CANINE MAMMARY TUMORS


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