Comparison of DNA Aneuploidy of Primary and Metastatic Spontaneous Canine Osteosarcomas

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ABSTRACT

Spontaneous canine osteosarcomas were analyzed for DNA aneuploidy and percentage of S phase cells using flow cytometry. Forty-eight dogs were studied in which both a primary tumor and subsequent metastases were available. The DNA index distributions for the primary tumors and the metastases were quite similar. However, when individual primary tumors and metastases derived from them were compared, many of the cases had different ploidy values. The tumor cells were also analyzed for percentage of S phase. The diploid metastases had less than 17% S phase cells, whereas the aneuploid metastases had up to 40% S phase cells. There was a direct correlation between the DNA index and the percentage of S phase in the metastases.

INTRODUCTION

The nature of metastatic disease and its relationship to the primary tumor from which it originates has been discussed and studied at length (1–3). However, the precise nature of the selective processes leading to metastasis is not clearly defined. Experimental animal model systems, such as the mouse B16 melanoma, have been used to analyze the clonal characteristics of primary and metastatic tumors using clonal DNA markers (4, 5). These systems are highly specific and may not have general validity in spontaneous tumors growing in animals or humans.

The advent of flow cytometry allowed for a rapid and accurate measurement of the DNA content of cells derived from tumors (6). A large fraction of tumors have altered DNA content compared to normal cells. This DNA aneuploidy usually has a precise value for a given tumor, represented by a distinct peak on a DNA histogram. Variations in DNA aneuploidy between a primary tumor and subsequent metastases could be used as a marker for changes occurring during the development of metastatic disease. Of course, it would only measure rather large changes that occurred. Nevertheless, significant changes in the DNA aneuploidy of metastases versus the primary tumor would indicate that the cell populations in the metastases had an altered genome compared to the primary tumor.

This study analyzes the DNA ploidy characteristics of spontaneously occurring osteosarcomas in 48 different dogs where both primary and subsequent metastases were available. Significant differences were obtained between specific primary tumors and their metastases. Additionally, the percentage of S phase cells in diploid metastatic tumors was smaller than for the aneuploid metastases.

MATERIALS AND METHODS

Tumor samples were obtained from a surgical specimen in most cases, although a needle or trephine biopsy was obtained in a few cases. In about one third of the cases, from two to five separate biopsies were obtained from the primary tumor to minimize sampling error. Most samples of metastatic lesions were obtained immediately following euthanasia and included metastases to lung, liver, bone, skin, lymph nodes, and muscle. The majority were lung metastases. Each sample was divided into two parts, with half undergoing histological analysis for confirmation that tumor was present. The remainder was processed for flow cytometry as described below.

Individual cells were obtained by mincing the tumor specimen into small pieces with a scalpel and then mashing it in phosphate-buffered saline in a Stomacher (VWR) for 30 s to 1 min. This treatment released individual cells with a high yield and resulted in better coefficients of variation than mashing the tissue through a wire mesh. Blood was drawn from the dog with the tumor or from donor dogs at the time of the biopsy. Lymphocytes were obtained by centrifuging the blood through Histopaque (Sigma) with a density of 1.077. In all cases lymphocytes were mixed in a parallel sample as an internal standard. Cells were fixed in 50% ethanol in phosphate-buffered saline and then refrigerated prior to staining with chromomycin A3. The flow cytometer (7) was operated with an argon ion laser power of 100 mW at 458 nm. DNA histograms were analyzed by a computer model utilizing a second-order polynomial for S phase (8) or by the program MULTICYCLE (Phoenix Flow Systems) to analyze overlapping populations for S phase percentage. Not all samples were amenable to meaningful analysis for S phase by either model. In cases where overlapping populations were not clearly resolved or there was significant overlap between the S phases of the two populations, the S phase results were not used. Also, if background was large, the histograms were not fitted. Thus, the S phase results reported here are dependable.

RESULTS

A total of 64 dogs with osteosarcoma metastases were analyzed for DNA aneuploidy. Only 5 of these metastases had more than one aneuploid population present. In 48 cases, biopsies were obtained from both the primary tumor and subsequent metastases. A distribution of DNA index for both the primary tumor and its corresponding metastases is given in Fig. 1. Although there are some minor differences in the distribution of these tumors, the percentage of primary tumors that were aneuploid (56%) is quite similar to the percentage of metastatic tumors that were aneuploid (63%). Also, the overall shapes of the distributions are quite similar.

In 36 dogs, metastases were obtained from more than one location, including metastases from lung (the most common location), liver, bone, lymph nodes, and soft tissue. The DI3 of different metastases from the same animal frequently varied with respect to each other, as well as to the primary tumor. Fig. 2 shows the range of different DI values for both the primary tumor and the subsequent metastases for 48 dogs. Each different DI value is shown for each dog. In 9 cases of primary tumors and 5 cases of metastases, more than one DI value was associated with a single sample. The other cases with more than one DI value represent differences between different samples from the same animal. There is considerable heterogeneity in DI

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1. The abbreviations used are: DI, DNA index; SI, S index (S phase percentage).

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Fig. 1. Histogram of the DNA index of primary (■) and metastatic (□) spontaneous canine osteosarcomas.

Fig. 2. Comparison of the DNA index of primary and metastatic canine osteosarcomas. The individual values of DI for the primary tumor (■) or the metastases (▲) are given for each dog analyzed. As described in the text, the variation in ploidy derives both from multiple samples and polyclonal populations within a single sample.

Fig. 3. Distribution of percentage of S phase cells of osteosarcoma metastases.

between the primary tumor and the metastases. The metastases were also analyzed for percentage of S phase. The distribution of SI is shown in Fig. 3. This distribution is skewed to the right, but most of the metastases ranged between 5% and 20% S phase, with a mean value of 14%. When SI is plotted against DI (Fig. 4), there is a clear difference in SI between aneuploid and diploid metastases. The diploid metastases had an SI of less than 17% with one exception, whereas the aneuploid metastases showed a slight linear correlation (r = 0.37) between DI and SI, with the majority of them having an SI of >15% and a range of up to 40%.

DISCUSSION

Few studies have compared the ploidy characteristics of primary tumors with those of metastases derived from them. Frankfurt et al. (9, 10) evaluated the ploidy and percentage of S phase of human primary and metastatic solid tumors. They obtained similar DI and SI distributions for both primary and metastatic tumors. However, they did not compare the metastatic tumors with the primary tumors from which they were derived. Data from this study also show similar overall DI distributions for primary and metastatic canine osteosarcomas. However, when each individual metastasis was directly compared with the primary tumor from which it originated, there was considerable variation in ploidy. Thus, different metastases within the same animal may derive from different initial metastatic cells. This is consistent with the hypothesis that tumor populations evolve and cells with metastatic potential may be different from cells in the primary tumor (11).

In the present study, 5 of 48 (10%) metastases and 9 of 48 (19%) primary tumors had more than a single stemline present. This indicates that metastatic clones are usually derived from a single cell, but in some cases they may be derived from more than one initial cell. Alternatively, genetic evolution may take place in the metastasis also. It may be that the lower percentage of metastases that are multiclonal is due to a shorter period of time for genetic evolution to take place than in the primary tumor. Korczak et al. (11) used genetically tagged tumor cells to show that in some cases a metastasis was derived from two different progenitor cells.

At least one of the metastases had a different DNA index, compared to its parent primary tumor, in 67% of the dogs analyzed in this study. Also, different metastases in the same animal frequently differed in DI. However, it is possible that metastatic cells of the same ploidy were present in the primary tumor but were not present in the biopsy or were in such a small proportion that they were not observed in the DNA histogram.

The overall distribution of the percentage of cells in S phase in metastases is quite broad and skewed to large S phase values. However, the diploid metastases had a narrower range of S...
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phase than the aneuploid metastases. This is at variance with a larger sample of 130 primary canine osteosarcomas, where the diploid and aneuploid tumors had the same range in SI (data not shown). This may mean that the growth kinetics of the aneuploid metastases was significantly more rapid than for the diploid metastases. This can only be determined by additional studies utilizing bromodeoxyuridine uptake, however. This is very similar to results reported by Frankfurt et al. (9). The metastatic tumors also show a correlation between SI and DI for aneuploid tumors.

These results confirm that metastases frequently derive from unique subpopulations of the primary tumor that have undergone genetic changes. While the present study focuses on changes in ploidy, it is clear that other phenotypic changes would be associated with these genetic changes. The value of this study is that the results are from spontaneous tumors in dogs rather than experimental systems. These results are for canine osteosarcomas, but canine osteosarcoma is considered to be a good model for human osteosarcoma since there are many biological and histological similarities between osteosarcomas in both species (12). Therefore, the results are likely to be relevant for human osteosarcoma also. Only very limited work has been done on ploidy of human osteosarcomas, and none of the studies compared ploidy of metastases to that of the primary tumor. Osteosarcomas in humans and dogs may be somewhat unique in the frequency of polyclonicity. These results demonstrate the value of using spontaneous canine tumors as an experimental system to provide further information on the natural progression of cancer in vivo.

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