

Levels of Apoptosis in Spontaneously Occurring Canine Tumors

Mincheol Choi¹, Susan M. LaRue*, and Edward L. Gillette*

College of Veterinary Medicine & Institute of Animal Medicine,
Gyeongsang National University, Chinju, Korea

*Department of Radiology and Radiation Biology,
Colorado State University, Fort Collins, Colorado, U.S.A.

자연발생된 개의 종양에서 Apoptosis의 수준

최민철¹ · 수존 엠 러루* · 에드워드 엘 질렛*

경상대학교 수의과대학 및 동물의학연구소,

*미국 콜로라도 주립대학 방사선 및 방사선 생물학 교실

요 약 : 65 마리 개의 자연 발생된 종양 샘플에서 apoptosis의 수준을 알아보고자 본 실험을 실행하였다. 65 종에는 골육종 23예, 임파종 11예, 다른 형의 육종 11예, 상피종 13예, 기타 종양 7예로 구성되었다. 대부분의 종양에서 다양한 범위의 apoptosis 백분율을 나타내었다. 80% 이상의 수준의 apoptosis가 전체의 55.4%(35 예)로 가장 높은 수준을 보였고, 20-39%의 수준을 보인 것은 전체의 7.7%(5 예)로 가장 낮은 수준을 보였다. 60-79%, 40-59% 및 20% 미만은 같은 수준인 12.3%(8 예)를 나타냈다. 특히 상피종에서 대부분의 종양이(69.2%) 80% 가 넘는 apoptosis를 나타내었다. 각 종류별 종양에서 평균 apoptosis의 백분율은 골육종, 임파종, 다른 형의 육종, 상피종 및 기타 종양에서 각각 72.7%, 61.4%, 71.1%, 74.8% 및 79.7% 이었다.

Key words : dog, tumors, apoptosis

Introduction

Normal development require the regulation of cell viability, growth, and differentiation to different lineages. Cell death occurs at specific times and sites in normal embryonic development. Apoptosis (or programmed cell death) is a mode of cell death, which is characterized by condensation of nuclear chromatin, compaction of cytoplasmic organelles, and changes at the cell surface. Biochemically, the most striking change in apoptosis is the digestion of nuclear DNA into oligonucleosomal length fragments. While necrosis is characterized by irregular

clumping of chromatin, marked swelling of organelles and focal disruption of membranes³, apoptosis occurs during normal embryonic development, in normal cells and spontaneously in malignant tumors. It is increased in tumors responding to irradiation, cytotoxic chemotherapy, heating and hormone ablation and can be regulated by genes^{4,6,12-14}.

The methods used to detect apoptotic cells include histological evaluation, gel electrophoresis and flow cytometry^{7,10}. More recently, the comet assay has been developed and found to be a sensitive method of detecting DNA strand breaks in individual cells. It is based on the ability of smaller negatively charged DNA fragments to migrate through an agarose gel in response to an electric current. Since apoptosis is characterized by extensive DNA cleavage, the comet assay should be useful in detecting not only the initial DNA damage which trig-

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¹Corresponding author

ers apoptosis, but also early stages of apoptosis when other methods may lack sensitivity⁹.

No studies have determined apoptosis in normal canine tumors, we examined levels of apoptosis in spontaneously occurring canine tumors using the comet assay.

Materials and Methods

Materials

65 client-owned dogs with tumors were evaluated at Colorado State University (CSU). Each dog had a clinical evaluation, regional and thoracic radiographs, serum biochemistry profile, and a complete blood count as the initial evaluation. Diagnosis of tumors was confirmed by histopathological findings. Before chemical and radiation therapy, each tumor sample was obtained from biopsies and surgical resection during operation.

The comet assay⁸

a standard protocol for comet preparation and analysis was adopted. With tumor samples, single cell suspension was made in 0.5-1 ml PBS(10^5 - 10^6 cells).

Agarose (1.5 ml) at 40°C was added to the 0.5 ml of cell suspension, and solution pipetted onto a poly-L-lysine treated slide. After lettering gel on cold surface 1 minute, slides were immersed in a lysing solution consisting of 0.03 M NaOH and 2 mM EDTA. Horizontal gel electrophoresis was performed in a fresh solution of 0.03 M NaOH, 2 mM EDTA at 0.5 V/cm for 25 min. After a 10 min rinse in distilled water, DNA was stained by immersing slides in 2.5 µg/ml propidium iodide (PI) for 10 min. Apoptotic cells were detected "by eye" on slides using a fluorescence microscope. Over 400 cells on a slides were analyzed for each sample.

Results

Fig 1 shows photomicrographs of comets from the same tumor cells. The extensive DNA fragmentation present in the apoptotic cell (Fig 1, left) allowed most of the DNA to migrate, whereas very

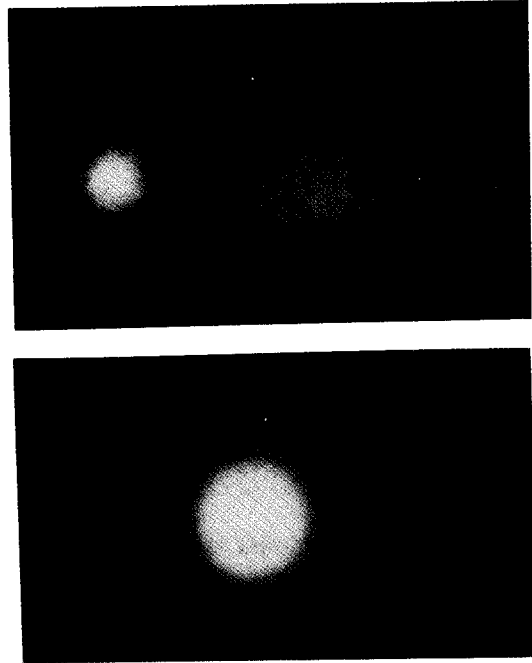


Fig 1. Apoptotic cell (left) and non-apoptotic cell (right) of the same tumor cells.

Table 1. Levels of Apoptosis on Spontaneously Occurring Canine Tumors

Types of tumors (No.)	Percent tumor apoptosis				
	<20%	20-39%	40-59%	60-79%	>80%
OSA (23)	2	4	0	4	13
LYM (11)	2	1	3	0	5
SCM (11)	2	0	1	3	5
CCM (13)	2	0	2	0	9
Others (7)	0	0	2	1	4
Total (65)	8	5	8	8	36

OSA=osteosarcomas, LYM=lymphomas, SCM=other types of sarcomas, CCM=carcinomas, Others=melanomas, mast cell tumors and hemangiopericytomas

little DNA was able to migrate from the cell which had not undergone apoptosis (Fig 1, right).

The incidence of apoptosis on spontaneously occurring canine tumors was presented in Table 1. In most types of tumors, level of over 80% apoptosis was the highest portion with an overall incidence of 55.4% (36 of 65), whereas level of 20-39% apoptosis was the lowest portion with an overall incidence of 7.7% (5 of 65). Especially in carcinomas,

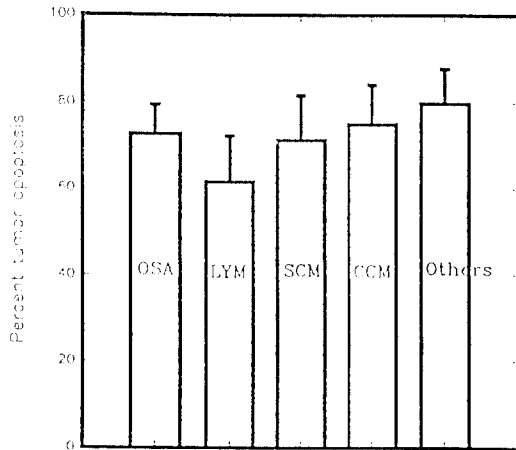


Fig 2. Mean and SEM of tumor apoptosis in dogs.

most tumor samples(69.2%) showed over 80% apoptosis (9 of 13).

In Fig. 2 the percent tumor apoptosis was presented with mean and SEM. The mean percent tumor apoptosis for each type of tumors (osteosarcomas, lymphomas, other type of sarcomas, carcinomas and others) was 72.7%, 61.4%, 71.1%, 74.8%, and 79.7%, respectively.

Except lymphomas, most types of tumors showed over 70% mean of apoptosis. There was no significant difference between the types of tumors.

Discussion

Apoptotic cell death characterized by extensive DNA fragmentation into oligonucleosomal subunits can be evaluated by electrophoretic, colorimetric, flow cytometric methods and the comet assay. Among them, electrophoretic and colorimetric methods are unable to determine the percentage of apoptotic nuclei or recognized the apoptotic cells in a heterogenous cell population⁷. The flow cytometry and comet assays for measuring apoptosis are fundamentally similar in that both depend on large strands of DNA remaining in nuclei while small strands are able to diffuse away. The comet assay detected apoptotic TK6 cells much earlier than a flow cytometry method and the only one currently available with sufficient sensitive to detect, in individual cells, initial DNA damage, repair of

that damage, and subsequent appearance of apoptotic cells⁹.

Determination of the percent tumor apoptosis in spontaneously occurring canine tumors is considered to be a valuable indicator of the response of the tumor to preoperative therapy. Although the mean value of 26.8 (range, 0% to 65.5) percent tumor necrosis for untreated osteosarcomas in dog has been reported¹¹, no studies have determined the percent tumor apoptosis in untreated canine tumors. In untreated human tumors, by using the flow cytometry, mean values of 19% and 37% of apoptosis-associated DNA strand breaks have been reported¹. In this study, twenty-two human solid tumors were examined. In diploid tumors (n=12) the percentage of cells with DNA strand breaks varied from 1% to 43% (mean=19%), and in aneuploid tumors this percentage varied from 15% to 51% (mean=37%). This study indicate that apoptosis is more frequent in populations of tumor cells than among normal cells of the same organs. The higher percent of cells with DNA strand breaks compared to the percent of cells with morphology typical of apoptosis suggest that the early phase of apoptosis, characterized by the appearance of DNA strand breaks, which precedes morphological changes (chromatin condensation and nuclear fragmentation), may be very long in individual cells of the tumors studied.

Compare to above human data, our data show higher apoptosis in canine tumors, which is maybe due to the fact that the comet assay is more sensitive in detecting early apoptotic change than flow cytometry method and the possibility of DNA damage in some cells during the procedure (mechanical separation of tumor cells, cell aspiration). As apoptosis is often known to be particularly prominent near foci of confluent necrosis³, sampling site is one of causing effects of enhanced apoptosis.

The percent tumor apoptosis along with percent tumor necrosis is predictive for tumor control in dogs.

Summary

Although the importance of apoptosis in cancer and cancer therapy become prominent, no studies

have determined levels of apoptosis on spontaneously occurring canine tumors.

So we examined the level of apoptosis in 65 canine tumor samples (23 osteosarcomas, 11 lymphomas, 11 other types of sarcomas, 13 carcinomas, 7 other types of tumors).

In most tumor samples, a wide range of percent apoptosis was shown. Level of over 80% apoptosis was the highest portion with an overall incidence of 55.4% (36 of 65), whereas level of 20-39% apoptosis was the lowest portion with an overall incidence of 7.7% (5 of 65). Levels of 60-79%, 40-59%, less than 20% showed the same 12.3% portion among the all samples (8 of 65). Especially in carcinomas, most tumor samples (69.2%) showed over 80% apoptosis (9 of 13). The mean percent tumor apoptosis for each type of tumors (osteosarcomas, lymphomas, other type of sarcomas, carcinomas and other types of tumors) was 72.7%, 61.4%, 71.1%, 74.8% and 79.7%, and 79.7%, respectively.

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