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Cell-Free DNA Is Released From Tumor Cells Upon Cell Death: A Study of Tissue Cultures of Tumor Cell Lines

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To determine the source of cell-free DNA detected in the serum of cancer patients, we monitored the release of cell-free DNA and tumor markers from tumor cell lines using the cell culture technique. During the first 6 days of cell culture, we found very low concentrations of DNA and a steady increase of the CA 125 released from the SK-BR3 tumor cell lines during cell growth. A sudden increase of cell-free DNA detected in the cell medium coincided with cell death. As the cells died, the concentration of cell-free DNA declined but the concentration of CA 125 increased. The sudden release of cell-free DNA observed upon cell death was also found on the cell cultures of additional tumor cell lines, including A549, RD, and SK-N-SH cells. It appears that the majority of cell-free DNA detected in the serum of cancer patients comes from tumor cells following cell death and cell necrosis. The concentration of cell-free DNA released is related to the number of dead cells, whereas that of the tumor marker CA 125 is associated with the presence of the total number of cells (dead or alive). J. Clin. Lab. Anal. 17:103–107, 2003.

Key words: cell-free DNA; cell death; necrosis; tissue culture; tumor cell line

INTRODUCTION

There are two types of DNA in circulating blood: the DNA derived from blood lymphocytes, and the so-called cell-free circulating DNA. Cell-free DNA can be detected in both plasma and serum. A small amount of lymphocyte DNA may also be released into the serum during the coagulation of whole blood (1). Cell-free DNA is usually elevated in the serum and plasma of cancer patients (2–6). It has been reported that the increased cell-free DNA in the plasma of cancer patients has all of the characteristics of tumor DNA (4), such as decreased strand stability and the presence of specific oncogenes, tumor suppressor genes, and microsatellite alterations. Although previous studies have suggested that tumor DNA is released into the circulation and is enriched in the plasma and serum, there is no direct evidence indicating how much of the cell-free circulating DNA is derived from apoptosis or necrosis (1).

Fournie et al. (7,8) have suggested that dead tumor cells are the major source of circulating cell-free DNA. In the current investigation, we studied the release of DNA from tumor cells by cell tissue cultures. During the course of tumor cell growth, we measured the numbers of cells and the amount of DNA released into the cell medium. We also correlated the release of DNA with that of tumor markers, such as CA 125. We found that DNA was usually not detectable in the cell medium during the period of normal growth. Elevated DNA was released into the cell medium when the tumor cells started to die. The concentration of CA 125 was detectable early and rose proportionally to tumor cell growth, even after cell death.

MATERIAL AND METHODS

The trypsin (porcine pancreatic) used to remove attached cells was obtained from ICN Biomedicals (Costa Mesa, CA). Minimal Essential Medium (MEM) was purchased from Gibco BRL (Grand Island, NY), and the QIAmp 96 spin blood DNA extraction kit came from Qiagene (Santa Clara, CA). The PicoGreen double-strand DNA detection kit was purchased from Molecular Probe (Eugene, OR), and the metaphor gel

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was from FMC Bioproducts (Rockland, ME). The ethidium bromide, DNA tracking dye, and 1Kb DNA marker were all purchased from Sigma (St. Louis, MO). The Easy-Cast Minigel system and Seakem GTG agarose used for electrophoresis were obtained from ISC (Kaysville, UT).

Cell Lines

All tumor cell lines used in the current investigation were obtained from the American Tissue Culture Collection (ATCC, Rockville, MD), including SK-BR3 cells from breast adenocarcinoma, SK-N-AS cells from neuroblastomas, A549 cells from lung carcinomas, RD cells from rhabdomyosarcomas, and LNCaP cells from prostate carcinomas. Fresh-frozen MRC-5 cells from normal fibroblasts were obtained from Diagnostic Hybrids Inc. (Athens, OH).

Cell Tissue Culture

Cells were allowed to grow in 150 or 25 cm^2 tissue culture flasks (Falcon 3024). Each flask was seeded with approximately $2–20 \times 10^5$ cells in 25 or 5 mL of growth medium. At various stages of cell growth, cells were harvested on the first, third, fifth, seventh, ninth, and 11th days after seeding. The cell monolayer was detached by incubating the cells in 5 mL of 0.25% trypsin for 1–2 min. The cells were collected by rinsing each flask twice with 5 mL medium, and were concentrated by centrifugation counted by a Bright-Line hemacytometer (Hausser Scientific, Horsham, PA). The medium (supernatant) was subjected for determination of tumor marker. The cell-free DNA was extracted with a QIAamp 96 spin blood kit (Qiagen, Santa Clara, CA) and quantified with a PicoGreen double-strand DNA kit (Molecular Probe, Eugene, OR) (6).

Assay for CA 125 and Ectodomain of c-erbB-2

The concentrations of CA 125, CA 19-9, and CA 15-3 released into the cell medium were determined by the Abbot AsSym® system (Abbot Park, Chicago, IL) for c-erbB2, CA-125, CA 19-9, and CA 15-3. The concentration of the ectodomain of c-erbB-2 was determined by in-house ELISA, as described previously (9).

Electrophoresis

DNA extracted from the cell medium was concentrated to 200 μg/mL with a Savant DNA speed vac (Savant, Farmingdale, NY). Then 10 μL of concentrated DNA containing 2 μg DNA were loaded on the gel together with 2 mL of gel loading buffer (as little as 500 ng of DNA was used). Electrophoresis was performed with the Easy-Cast MiniGel system and was run at 90 volts for 2 hr in 0.09 mol/L Tris-boric-EDTA buffer, pH 8. At the end of electrophoresis, the gel was stained with 0.5 mg/mL ethidium bromide and photographed under UV light with Polaroid Gel Cam (Janesville, WI).

RESULTS

Release of Cell-Free DNA

In an attempt to determine the origin of cell-free DNA in the blood, we decided to grow SK-BR3 (a breast tumor cell line) in culture, and monitor the concentration of cell-free DNA and tumor markers released into the cell medium, and the proliferation of cells (cell number). During the first 6 days, only very low concentrations of DNA (approximately 100 ng/mL or less) were released. However, when the cells began to die, thousands of ng/mL of cell-free DNA were detected in the cell medium. After it reached a peak, the cell-free DNA began to decline, in proportion to the number of dead cells (Fig. 1A).

Release of CA 125 and c-erbB-2

While the SK-BR3 cells were growing, we also monitored various tumor markers released into the cell

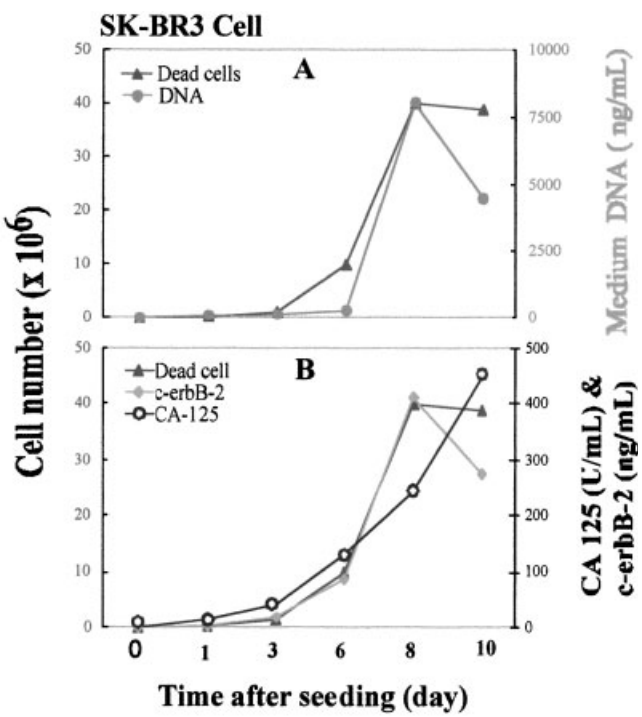

Fig. 1. Monitoring the release of DNA, CA 125, and the ectodomain of c-erbB-2 into the cell medium during cell culture of a SK-BR3 breast tumor cell. A significant amount of cell-free DNA was found in the cell medium when the cells started to die. Cell medium concentrations of CA 125 and ectodomain of c-erbB-2 increased proportionally to cell growth (cell number) and continued to increase upon cell death.

medium. To our surprise, we could not detect any significant concentration of CA 15-3 (a dominant tumor marker for breast cancer) in this breast tumor cell line, nor was there any detectable concentration of CA 19-9. However, both CA 125, a dominant tumor marker for serous ovarian cancer, was highly elevated, and the concentration increased proportionally with cell growth in the cell medium (Fig. 1B). The ectodomain of c-erbB-2 oncoprotein was also detectable at high concentrations, and the increased concentration of c-erbB-2 in the medium coincided with the increased number of SK-BR3 cells in the cell culture. Notably, the concentrations of both cell-free DNA and c-erbB-2 ectodomain decreased as the cells died, whereas CA 125 increased in concentration. It appears that the concentrations of CA 125 and c-erbB-2 ectodomain released into the cell medium are proportional to the total number of cells (dead or alive) present.

Release of Cell-Free DNA From Other Tumor Cell Lines

A sudden large increase in cell-free DNA was also observed in other human cell lines, including A549, a lung cancer cell; SK-N-SH, a metastatic neuroblastoma cell; MRC-5, a normal diploid lung cell; and rhabdomyosarcoma cells (Fig. 2). Cell necrosis is probably the major cause of large amounts of cell-free DNA being released into the cell medium and, eventually, into the blood.

Electrophoretic Pattern of Cell-Free DNA Release

The patterns of cell-free DNA release from the SK-BR3 tumor cell at different days of cell culture are shown in Fig. 3. Some cells began to die at day 6 of cell culture, and cell-free DNA began to be detectable in the cell medium at that time. As time progressed there were more dead cells, and more cell necrosis occurred. The DNA pattern had a smeared appearance and did not have the distinct ladder found in apoptotic cells. Most released DNA still remained as intact genomic DNA.

DISCUSSION

It appears that the elevated concentrations of cell-free DNA detected in cancer patients are largely caused by dead tumor cells that have undergone necrosis. Apoptosis apparently contributes much less to the cell-free DNA in the cell medium. Most of the cell-free DNA in the cell medium was not fragmented. During the early growth period of the tumor cells, cell-free DNA was detected in the cell medium, but the concentration was lower than that at cell death (Figs. 1 and 2). Cell-free DNA detected during the first 6 days of cell culture was approximately 100 ng/mL or less, whereas several

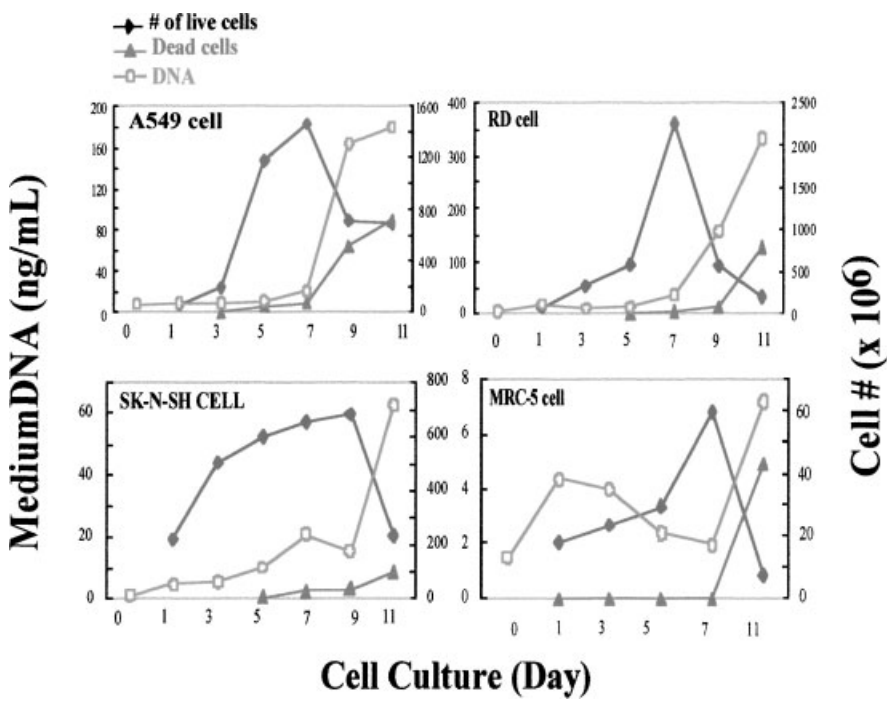

Fig. 2. Release of DNA upon cell death was also found during tissue culture of A549, RD, and SK-N-SH tumor cell lines. A similar pattern of cell-free DNA release was also found in MRC-5 cells (a normal cell line), but at a much lower concentration.

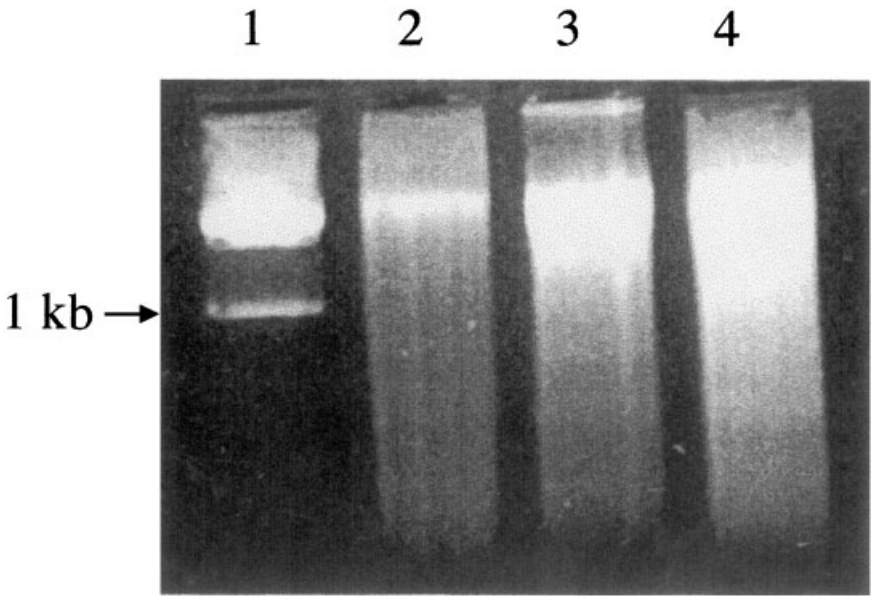

Fig. 3. Electrophoretic pattern of cell-free DNA released from the SK-BR3 tumor cell line during cell culture. 1) 1 kb DNA ladder; 2) cell medium cell-free DNA released at day 6 of cell culture; 3) at day 8; and 4) at day 10.

thousand ng/mL of DNA were detectable upon cell death. The release of cell-free DNA upon cell death was not limited to the SK-BR3 cells. Apparently, this is a common phenomenon among tumors: the same increase in cell-free DNA was observed in several other tumor cell lines when they started to die (Fig. 2).

Normal cell lines, such as MRC-5, exhibited a similar pattern but at a much slower rate of growth; furthermore, much less DNA was released upon cell death.

It was interesting to note that the breast tumor cell line did not secret CA 15-3. However, CA 125, a marker for serous ovarian cancer, was detected in the cell medium. We also did not detect any significant concentration of CA 19-9, which usually appears in all epithelial tumors. Furthermore, it is surprising that the level of CA 125 did not decline in response to cell death. A previous study (10) found that tumor markers monitored during the course of chemotherapy did not respond exactly according to the clinical status of the cancer patient, whereas both cell-free DNA and the ectodomain of c-erbB-2 did. Apparently, the tumor markers released during cell necrosis declined as cell death progressed, or as the number of live cells decreased. A similar result was noted in our earlier study of the release of free PSA from LNCaP (a prostate cancer cell line), CA 15-3 from MCF-7 (a breast cancer cell line), and chromogranin A from SK-N-AS (a neuroblastoma cell line) in cell cultures (11). In that earlier study, the concentration of released homocysteine, but not the tumor markers, decreased as the cells died. Apparently, the concentration of released cell-free DNA was proportional to the number of dead cells, and the concentration of released homocysteine correlated with the number of live cells. However, the concentration of tumor markers was related only to the total number of cells, regardless of whether they were dead or alive. The monitoring of homocysteine and cell-free DNA, in addition to currently used tumor markers, appears to be of benefit in the treatment of cancer patients.

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