Apoptotic Conversion: Evidence for Exchange of Genetic Information between Prostate Cancer Cells Mediated by Apoptosis

Alexandre de la Taille, Min-Wei Chen, Martin Burchardt, Dominique K. Chopin, and Ralph Buttyan

Abstract

Changes in the outer membrane of apoptotic cells can induce neighboring cells to become phagocytic. Using genetically marked prostate cancer cell lines, we explored the possibility that genetic information might be transferred from an apoptotic cell to a phagocytic neighbor. Neomycin-resistant LNCaP cells that overexpress bcl-2 (LNCaPbcl-2/neo-r) were cocultured with hygromycin-resistant LNCaP cells (LNCaPPhygr-r). The cocultures were then transiently exposed to serum starvation to induce apoptosis of LNCaPPhygr-r cells. Surviving cells were then coculted in medium containing both antibiotics. Likewise, this change was required; cocultures not exposed to serum starvation yielded no dual-selectable clones. Analysis of DNA extracted from a dual-resistant clone demonstrated that the restriction endonuclease pattern of the neo-r gene was unaltered when compared with the parental LNCaPbcl-2/neo-r. However, the hygr-r gene demonstrated an altered restriction endonuclease pattern in the dual-resistant derivative compared with the parental LNCaPPhygr-r cell line. This is evidence that genetic information can be transferred from one prostate cancer cell to another through the process of apoptosis, and we term this form of genetic transfer “apoptotic conversion.”

Introduction

Viable cells normally maintain an asymmetric distribution of PS3 within the outer membrane; most of the PS residues are oriented toward the inner surface of the plasma membrane. However, during the later stages of apoptosis, this asymmetry is lost, and PS residues appear on the outer surface of the plasma membrane as well (1–5). This biochemical alteration in the membranes of apoptotic cells provides the basis for the use of annexin V binding to identify cells undergoing apoptosis (6, 7). Likewise, this change is known to be a potent inducer of phagocytosis by neighboring cells in a likely attempt to rapidly eliminate the apoptotic cell from the tissue before it can further degrade and activate inflammatory processes (8–10). Indeed, apoptotic bodies are often found in tissues as apoptotic cell debris. However, during the later stages of apoptosis, the restriction endonuclease pattern of the neo-r gene was unaltered when compared with the parental LNCaPbcl-2/neo-r. However, the hygr-r gene demonstrated an altered restriction endonuclease pattern in the dual-resistant derivative compared with the parental LNCaPPhygr-r cell line. This is evidence that genetic information can be transferred from one prostate cancer cell to another through the process of apoptosis, and we term this form of genetic transfer “apoptotic conversion.”

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Materials and Methods

Cell Lines. Parental LNCaP cells were obtained from the American Type Culture Collection (Manassas, VA). The derivation of LNCaPbcl-2/neo-r was described previously (15). These cells have been characterized previously, and they are known to be highly resistant to in vitro apoptotic stimuli such as serum starvation or phorbol ester treatment. LNCaPPhygr-r cells were derived from the parental LNCaP cells after transfection with an expression plasmid containing the hygromycin resistance gene (SelectaVecta–hyg, Novagen, Madison, WI). The clonal variant selected for further study was tested for its ability to undergo apoptosis in response to serum starvation and was found to be as sensitive as parental LNCaP cells (15). All cell lines described were cloned after selection in antibiotic-containing medium using a cloning ring procedure as described previously (15).

Cell Culture Protocol. Cocultures were established by seeding LNCaPbcl-2/neo-r and LNCaPPhygr-r cells at a 1:1 concentration in normal medium, and monolayers were allowed to form for 24 h (cell count at 24 h after seeding was 1.4 million cells/flask). The cocultures were then exposed to a minimum volume (1 ml in a 25-cm² flask) of serum-free medium (RPMI 1640) for 48 h, a condition that preferentially induces the apoptosis of LNCaPPhygr-r cells (15). The surviving cells were then washed and incubated with normal medium (containing serum) for an additional 48 h. After this period, the cells were trypsinized and split into three flasks. Selection medium containing both neomycin (Geneticin at a concentration of 400 µg/ml medium; Sigma, St. Louis, MO) and hygromycin B (at a concentration of 150 µg/ml medium; Sigma, St. Louis, MO) was then added, and incubation was continued for 3 weeks (with fresh medium added at 3-day intervals). Colonies were isolated and expanded in selective medium. As controls, each of the cell lines used in this study (i.e., parental LNCaP, LNCaPbcl-2/neo-r, and LNCaPPhygr-r) were cultured separately and then treated with the same protocol as the cocultures. Finally, we studied cocultures that were subject to a similar protocol, with the exception that they were never exposed to a period of serum starvation and therefore lacked exposure to an apoptotic stimulus. Colony counts from the cocultures exposed to serum starvation were statistically compared with the control results using the Student’s t test.

RT-PCR and PCR. RNA was extracted from clones and parental cell lines using the RNAzole B reagent of Tel-Test, Inc. (Friendswood, TX). DNA was

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extracted from these cells using a high molecular weight DNA extraction kit (DNA isolation kit; Boehringer Mannheim, Indianapolis, IN).

For cDNA synthesis, an aliquot containing 1 μg of total RNA was added to 0.5 μg of oligodeoxynucleotidyl acid primer (Life Technologies, Inc.) and brought to a final volume of 20 μl. The samples were placed at 65°C for 5 min and then chilled on ice. The primer-annealed RNA was added to 30 μl of master reaction mixture, so that the final concentrations of the following components were achieved: (a) 1 mM of each deoxynucleotide triphosphate; (b) 50 mM Tris-HCl (pH 8.3); (c) 75 mM KCl; (d) 3 mM MgCl₂; (e) 10 mM DTT; and (f) 10 units/reaction of Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.). The reaction was incubated at 42°C for 15 min, and the enzyme was then heat-inactivated at 95°C for 15 s.

Specific PCR primers were designed from each of the bacterial antibiotic resistance genes (neo-r and hygr-r), based on their available sequences in GenBank using the computer program Oligo (version 4.0; National Biosciences, Plymouth, MN). The primers for these genes were as follows: neo-r gene, GGCTATTCGGCTATGACTGG (5' primer) and TGGTCGAATGGCAGGTGA (3' primer); and (b) hygr-r gene, TTCCGGACCCGAAAGGA (5' primer) and AGATGTTGGCAGGCTCTGT (3' primer).

Oligonucleotide primers for human glyceraldehyde-3-phosphate dehydrogenase were obtained from Clonetech, Inc. (Palo Alto, CA). The PCR reaction was done in a total volume of 50 μl containing one-fifth of the reverse transcription reaction, with a final concentration of 20 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM of each deoxynucleotide triphosphate, 2.5 units of Taq polymerase, and 10 pm of each primer. A total number of 30 cycles were completed with the following specifications: (a) cycle 1, 95°C (4 min); (b) cycles 2–31, 95°C (1 min), 60°C for neo-r primers and 58°C for hygr-r primers (1 min), and 72°C (1 min); and (c) cycle 32, 72°C (15 min). Aliquots of the reaction were electrophoresed on 2% agarose gels in 40 mM Tris acetate (pH 8.0)/1 mM EDTA buffer. The gels were stained with ethidium bromide, viewed under UV light, and photographed. For PCR, 0.1 μg of DNA was used, and the PCR procedure was the same as that described for the RT-PCR.

**Southern Blot Analysis of DNA.** DNAs extracted from the parental cell lines and from the dual-antibiotic-resistant clones were digested to completion with BamHI or NcoI restriction endonucleases (Boehringer Mannheim). The digested DNAs were electrophoresed on a 0.7% agarose gel, and then the DNA was transferred to a nylon membrane (Boehringer Mannheim) by capillary blotting. The Southern blots were probed with 32P-labeled cDNA probes for human PSA, neo-r, or hygr-r that were obtained by PCR amplification of plasmids containing the cDNAs. The probes were labeled with 32P as described previously using the nick translation kit (Boehringer Mannheim). After overnight hybridization to the denatured probes, the blots were washed in a successive series of solutions containing decreasing amounts of SSC, and the filters were exposed to Kodak XAR-5 film to produce an autoradiograph.

**Results**

The two LNCaP-derivative cell lines (LNCaP<sup>neo-r</sup>, LNCaP<sup>hygr-r</sup>) were cosedseed into a cell culture plate at a 1:1 ratio. The coculture was exposed for a brief period to serum starvation to selectively induce apoptosis of the LNCaP<sup>hygr-r</sup> cells. The surviving cells were then exposed to a medium containing both neomycin and hygromycin. After 3 weeks of growth in this dual-selective medium, several colonies were observed in the dishes derived from cocultured treated cells, whereas no colonies were observed in flask treated similarly that had only monocultures of parental LNCaP cells, LNCaP<sup>neor</sup>, LNCaP<sup>hygr</sup>, or LNCaP<sup>neorhygr</sup> cells treated in this manner; nor were any colonies present in cocultures that were not pre-exposed to serum starvation. The mean colony number/flask resulting from the cocultures exposed to serum starvation was 7 ± 5. This was found to be statistically different from any of the control cultures (P = 0.02).

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selected after exposure to both antibiotics. These results suggest that the hygr-r gene marker was disrupted and transferred to a new chromosomal site during the apoptotic process from the LNCaP<sup>neo-r</sup> cells to the LNCaP<sup>hygr-r</sup> cells, as we would expect, given our prediction that the LNCaP<sup>hygr-r</sup> cells would be the apoptotic “donor” cells under these conditions.

**Discussion**

We have demonstrated here that cocultures of unique, genetically tagged prostate cancer cells can give rise to progeny cells containing both genetic markers when they are exposed to an apoptotic stimuli that preferentially kills one of the cell types in the coculture. We believe this is direct evidence that genetic information can be transferred from one (apoptotic) cell to another by the process of phagocytosis, and we have termed such an event apoptotic conversion. Whereas it is intuitive that such events are likely to occur, given the manner in which the DNA in apoptotic cells is degraded as well as the tendency of apoptotic bodies to be phagocytosed by neighboring cells, we believe that such events are not likely to influence normal cells of the body. Cells in normal adult tissues experiencing apoptosis are not undergoing proliferation, a condition that is generally necessary for the appropriate incorporation and subsequent transmission of genetic information (following standard transfection). However, apoptotic conversion likely has a high potential to lead to genetic transfer of abnormal information among cancer cells in tumors with high rates of apoptosis, such as prostate cancer (16, 17). Given the extensive genetic heterogeneity that has been found among the cancer cells in primary prostate tumors (18), it is possible that this process contributes to the acquisition of multiple genetic defects within some of the tumor cells, especially if the cancer patient is treated with a regimen that preferentially induces the apoptosis of only one of the genetic variants present in the tumor. Such an action might also explain the preferential development of more aggressive cancer cell types after therapy that occurs in some cancer patients. Moreover, it is possible that a similar event is responsible for the development of mouse cell-derived tumors in immune-deficient mice that are xenografted with human cancer specimens (19).

Additional experiments involving the use of mixed cell types in a tumor xenograft might help show the relevance of this phenomenon to human cancers. However, based on our results, we now propose that apoptotic conversion describes a new means of passing genetic information directly from a dying eukaryotic cell to a living eukaryotic cell, and that this process has the potential to mediate the passage of genetic defects between cancer cells in a tumor.

**Fig. 2.** Southern blot analysis demonstrates the presence of two genetic markers (neomycin and hygromycin resistance) in dual-selected clones of LNCaP cells. DNA was extracted from parental LNCaP (Lane 1), LNCaP<sup>neo-r</sup> (Lane 2), LNCaP<sup>hygr-r</sup> (Lane 3), or LNCaP<sup>hygr-neo-r</sup> cells (Lane 4). These DNAs were digested with NcoI (left panel) or BamHI (middle and right panels), electrophoresed, and blotted onto a nylon filter. The Southern blots were hybridized with a 32P-labeled cDNA probe for neomycin resistance (neo-r probe, left panel), hygromycin resistance (hygr-r probe, middle panel), or human PSA (PSA probe, right panel) and exposed to X-ray film for autoradiography.
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