Neuropeptides bombesin and calcitonin induce resistance to etoposide induced apoptosis in prostate cancer cell lines

M. Salido, J. Vilches and A. López
Department of Cellular Biology, School of Medicine, University of Cadiz, Spain

Summary. Background: Neuroendocrine differentiation in prostatic carcinoma has been related to regulation of proliferation and metastatic potential and correlated with prognosis. More than 80% of prostate carcinomas initially respond to androgen ablation, but most relapse, due to the heterogeneous presence of androgen-dependent and independent clones. The pathways of cellular proliferation and apoptosis are inexorably linked to minimize the occurrence of neoplasia, and dysfunction of apoptosis is proposed as a pathogenic process in malignant tumors. Androgen-dependent prostatic cancer cells undergo apoptosis after androgen deprivation, but not androgen-independent ones due to a defect in the initiation step. Anyway, they retain the basic cellular machinery to undergo apoptosis. We suggest a possible role of neuroendocrine differentiation in the onset and regulation of apoptosis in prostatic neoplasia. Methods: LNCaP, PC-3 and DU 145 prostatic cancer cell lines were induced to undergo apoptosis after treatment with etoposide alone or plus androgen ablation. We tested the role of neuropeptides bombesin and calcitonin at modulating etoposide induced apoptosis. Results: Etoposide-induced apoptosis in all cancer cell lines was achieved. In LNCaP androgen ablation was also required. Apoptosis is prevented in all three lines when bombesin was added. Calcitonin addition prevents apoptosis in PC-3, LNCaP and in an etoposide dose-dependent way in DU 145. Conclusion: Neuropeptides bombesin and calcitonin can modulate the apoptotic response of prostate cancer cells by inducing resistance to etoposide-induced apoptosis, suggesting that neuropeptides can be used as a target of therapeutical approach in prostatic carcinoma.

Key words: Apoptosis, Modulation, Neuroendocrine peptides, Prostate carcinoma, Etoposide

Introduction

Over the last decade, prostate cancer has become the most commonly diagnosed cancer in men. Of all cancers, the incidence of prostate cancer increases most rapidly with age, with an average age at diagnosis of 70 years, with 80% of cases being diagnosed in men over 65. Due to detection at an earlier age, there has also been a rapid increase in men diagnosed under the age of 65. Mortality from prostate cancer has increased at a slower rate but overall has doubled in the last 50 years. Although typically diagnosed in men over the age of 65, the impact of the disease is still significant in that the average lifespan of a man who dies from prostate cancer is reduced by 9 to 10 years (Denmeade and Isaacs, 1996).

Growth of a cancer is determined by the relationship between the rate of cell proliferation and the rate of cell death. Only when the rate of cell proliferation is greater than cell death does the tumor growth continue. If the rate of cell proliferation is lower than the rate of cell death, then regression of the cancer occurs (Gerschenson and Rotello, 1992; Payne et al., 1995; Denmeade and Isaacs, 1996).

Metastatic prostate cancers, like the normal prostates from which they arise, are sensitive to androgenic stimulation of their growth, due to the presence of androgen-dependent prostatic cancer cells. These cells are androgen-dependent since androgens stimulate their daily rate of cell proliferation while inhibiting their rate of death. In contrast, following androgen ablation, androgen-dependent prostatic cancer cells stop proliferating and activate a cellular suicide pathway termed programmed cell death or apoptosis. This activation results in the elimination of these androgen-dependent prostatic cancer cells from the patient. Due to this elimination, 80-90% of all men with metastatic prostate cancer treated with androgen ablation therapy have an initial positive response. All of these patients relapse eventually to a state unresponsive to further antiandrogen therapy, no matter how completely given. This is due to the heterogeneous presence of androgen-
independent prostatic cancer cells within such metastatic patients with a rate of proliferation exceeding their rate of cell death even after complete androgen blockade is performed (Kerr et al., 1972, 1974; Nawaz et al., 1987; Kiprianou et al., 1990; Berges et al., 1993).

Attempts to use nonandrogen ablative chemotherapeutic agents to adjust the kinetic parameters of these androgen-independent prostatic cancer cells so that their rate of cell death exceeds their rate of proliferation have been remarkable in their lack of success. These agents have been targeted at inducing DNA damage directly or indirectly via inhibition of DNA metabolism or repair and are critically dependent on an adequate rate of proliferation to be cytotoxic. In vitro studies have demonstrated that when these cells are rapidly proliferating they are highly sensitive to the induction of programmed cell death via exposure to the same antiproliferative chemotherapeutic agent, which are of limited value in vivo, due to major differences in the rates of proliferation occurring in the two states. Likewise, for chemotherapeutic agents to be effective, not only must the cancer cells have a critical rate of proliferation but also a critical sensitivity to induction of cell death. Anyway, the proliferative rate for androgen-independent prostatic cancer cells is very low, explaining why antiproliferative chemotherapy is of limited value against metastatic prostatic cells. Based on this, what is needed is some type of cytotoxic therapy which induces the death of androgen-independent as well as androgen-dependent prostate cancer cells without requiring the cells to proliferate (Raghavan, 1988; Fairbairn et al., 1993; Borner et al., 1995; Wright et al., 1996).

An optimal approach is to activate the "programmed cell death" pathway within these cells leading to their suicide. Both androgen-dependent normal prostatic glandular cells and androgen-dependent prostatic cancer cells can be induced to undergo programmed cell death following androgen ablation and this process does not require the cells to be in the proliferative cell cycle (Kiprianou and Isaacs, 1988; English et al., 1989; Catchpole and Stewart, 1995; Earnshaw, 1995; Furuya et al., 1995).

The programmed cell death induced in the prostate by androgen ablation is cell type specific: only the prostatic glandular epithelial cells and not the basal epithelial cells or stromal cells are androgen-dependent and then undergo programmed cell death following castration (Bonkhoff et al., 1993; Oberhammer et al., 1993; Berchel et al., 1995; Bpkhoff, 1995; Tang et al., 1998; Bareton et al., 1999).

Additional studies have demonstrated that androgen ablation does not induce this programmed death process in androgen-independent prostatic cancer cells due to a defect in the initiation step (Kyriachou and Isaacs, 1989). Even with this defect, however, androgen-independent prostatic cancer cells retain the basie cellular machinery to undergo this programmed cell death. This was demonstrated using chemotherapeutic agents which arrest proliferating cells in various phases of the proliferative cell cycle and which subsequently induce their programmed (i.e. apoptotic) cell death (Berges et al., 1993; Oberhammer et al., 1993; Solary et al., 1993; Denneade and Isaacs, 1996; Salido, 1996; Furuya et al., 1997).

Neuroendocrine differentiation in prostatic adenocarcinomas has received increasing attention in recent years as a result of possible implications on prognosis and therapy. The incidence of neuroendocrine cells in tumors have been reported from 10% up to 100%, and serum markers of neuroendocrine differentiation correlating with histological grade and differentiation are being used in clinical research (di Sant'Agene, 1985, 1992, 1995; Abrahamson, 1989; Bonhoff and Wernet, 1991; Aprikian, 1993; Bonhoff et al., 1993; Cohen, 1993; Bataglia, 1994; Segal et al., 1994; Berchel et al., 1995; Bonhoff, 1995; Gkonos et al., 1995; Lee et al., 1995; Hoosein et al., 1996; Angelsen et al., 1997a,b; Kimura et al., 1997; Westin et al., 1997; Cussenot et al., 1998).

Among the best-described neuroendocrine peptide growth factors in the prostate are members of the bombesin family. Calcitonin is frequently found in neuroendocrine cells in the prostate but the physiologic effect of calcitonin in prostate cancer is unknown (Di Sant' Agene, 1986, 1995; Gkonos et al., 1995). In the present paper, a possible role of neuroendocrine cells and neuropeptides bombesin and calcitonin in disbalancing the ratio cell proliferation/cell death by inducing resistance to apoptosis is suggested. In this sense, we treated androgen-independent-PC-3 and DU 145- and androgen-dependent-LNCaP-prostatic cancer cells lines with the topoisomerase II inhibitor, etoposide, alone or in combination with bombesin, calcitonin or dihydrotestosterone to assess exogenous induction of apoptosis as a therapeutic option in this type of cancer and evaluate the influence of neuropeptides in the onset of this phenomenon, as attempts to use nonandrogen ablative agents to adjust the kinetic parameters of these androgen-independent prostatic cancer cells so that their rate of cell death exceeds their rate of proliferation have been remarkable in their lack of success (Raghavan, 1988; Berges et al., 1993; Wright et al., 1996).

Materials and methods

Cell lines

Three prostatic cancer cell lines were used: LNCaP, an androgen-dependent prostatic cell line derived from a lymph metastasis, suministrated by American Type Culture Collection (ATCC) and two androgen-independent cell lines: PC-3, a p53 deficient prostate cell line derived from a bone metastasis (Nuclear Iberica, Madrid, Spain) and Du 145 (American Type Culture Collection, Rockville, Maryland), derived from a brain metastasis of a prostatic carcinoma.

Androgen-independent cells were grown in Dulbecco Modified Essential Medium -DME ME- (ICN
Biomedicals, Aurora, Ohio) supplemented with 10% Foetal Bovine Serum -FBS- (Serva, Heidelberg, Germany), 4% penicillin-streptomycin (Biochrom, Berlin, Germany) and 0.4% gentamycin (Gibco, Paisley, Scotland) under standard conditions, in a water saturated atmosphere of 5% CO₂ until the experience was started. Androgen-dependent cells were grown in RPMI 1640 (ICN Flow, Costa Mesa, California) under the same conditions.

Treatment protocols

All experiments were started with unsynchronized exponentially growing cultures. Cells were seeded in microplates (Nunclon, Madrid, Spain) at a density of 100,000 cells/ml in each well and culture media supplemented with 5% FBS, 4% penicillin and 0.4% gentamycin were added and 48 hours later shifted (tg) to medium with etoposide (Sigma, St Louis, Missouri) added -from 2 mM stock solution in DMSO- at doses 80 µg/ml, 100 µg/ml, 150 µg/ml, alone or with neuro peptides bombesin (Sigma), dose 1 nm/ml, or calcitonin (Sigma) 500 pg/ml, to assess induction of apoptosis as well as its possible blockage by neuro peptides. Reference groups with neuro peptides and a control group with only culture media were added. The cells were examined at 0, 24 and 48 hours of treatment.

In the androgen-dependent cell line, a second protocol including hormonal deprivation was used. Androgen withdrawal was carried out by changing from RPMI containing 5% FBS after 2 days, to RPMI containing 5% Steroid Free Serum -SFS- (Biogenesis, Bournemouth, England) for 2 more days. The cultures were then treated as described previously adding a group treated with dihydrotestosterone -DHT- (Sigma)-1nM-.

Cell parameter analysis

Direct examination by phase contrast microscopy

With a Nikon Diaphot phase contrast microscopy adapted to a photographic system, we could observe morphological changes like cell surface alterations, blebbing, detachment and round up of treated cells.

Growth kinetics and cell viability

Determined by XTT viability assay and trypan blue exclusion, with trypan blue in culture media (0.5%). After incubation of cells with trypan blue v/v, non stained cells are regarded as viable cells, and blue cells are considered non viable, when observed in a hematocytometer. Percentages of viable cells: (Number of non stained cells/total cell number) x 100.

XTT assay (Boehringer Manheim, cat nº 1465015)

Briefly, cells were grown in a microtiter plate, 96 wells, flat bottom, in a final volume of 100 µl culture medium per well, in a humidified atmosphere (37 °C and 5% CO₂), during the assay. After 24 and 48 hours, 50 µl of the XTT labelling mixture was added to each well. Cells were incubated for 4 hours in humidified atmosphere and espectrophotometrical absorbance of cells was measured using an ELISA reader. Wavelength 450-500 nm.

Determination of apoptotic process

For microscopical quantification of apoptotic cells, we used citospin preparations obtained from in vitro cell cultures. Apoptotic cells round up and detach from the substrate. The sample is taken by collecting the supernatant, containing the floating apoptotic cells, followed by trypsinization of the rest of the monolayer, containing healthy cells. Both fractions were added together to reconstitute the total population and then centrifugated at 1000 rpm for 5 minutes to get the pellet. Cells were then washed twice in PBS and cytopsii by means of cytotubes, at 1500 rpm for 5 minutes. Samples were air dried and afterwards stained for their observation with light and fluorescence microscopy.

Haematoxylin-eosin stain

Air dried slides were fixed in 10% formaldehde and stained in haematoxylin and counterstained with eosin.

Apoptosis staining: (fluorescent DAPI)

Air dried slides were fixed in metacril (Panreac, Barcelona, Spain) at -20° for 20 minutes, air dried and stained with 4',6'-diamino-2-phenylindol -DAPI- (Serva) at room temperature and in darkness for 20 minutes, and mounted with antifading media O- phenylenediamine (Sigma) in glycerol (Merck, Darmstadt, Germany) and preserved in darkness at -20° C until examination. Fluorescence range 300-400nm.

Apoptosis staining (TUNEL)

Cells were treated as recommended in the kit protocol (Boehringer Manheim, cat nº 1684817). Briefly, formalin fixed cells were permeabilized after dehydrating and rehydrating the specimens and treatment with 0.5% pepsin, washed in distilled water and TBS, and endogenous peroxidase was blocked with the blocking solution suministered for 30 min at room temperature. For labelling reaction, 50 µl of labelling solution was added to each specimen, except negative controls, for 30 min at 37 °C. 50µl of converter POD was then added for 30 min at 37 °C. and then cells were washed in TBS before adding DAB solution, 50µl, for 15 min at room temperature. After washing, cells were counterstained with haematoxylin.

Percentages of apoptotic cells: After microscopical examination they were established as (number of apoptotic cells/total cell number) x 100.

Mean and SD of a representative of at least ten
In vitro study of apoptosis reversion

experiments and a two-tailed Student's t-test were performed for statistical validation of results.

Results

1.- Induction of cell death

Treatment with etoposide of androgen-independent prostatic cell lines resulted in a dose- and time-dependent cytotoxicity accompanied by induction of apoptosis and a significant decrease in the number of viable cells when compared to control groups. Maximal induction was achieved after 48 hours of treatment with 100 µg/ml dose in PC-3 cells and 80 µg/ml dose in Du 145 cells (Figs. 1, 2).

**Fig. 1.** Percentages of apoptosis in PC-3 cells at different doses and time intervals. b: bombesin 1 nm/ml; c: calcitonin 50 pg/ml. Doses in µg/ml for etoposide.

**Fig. 2.** Percentages of apoptosis in Du 145 cells at different doses and time intervals. b: bombesin 1 nm/ml; c: calcitonin 50 pg/ml. Doses in µg/ml for etoposide.

**Fig. 3.** Percentages of apoptosis in LNCaP cells at different doses and time intervals. aw: androgen withdrawal. b: bombesin 1 nm/ml; c: calcitonin 50 pg/ml; dht: dihydrotestosterone 1nM. Doses in µg/ml for etoposide.

**Fig. 4.** Cell viability assessed by XTT assay in all three cell lines after treatment. Androgen-dependent cell line LNCaP after androgen withdrawal. b: bombesin 1 nm/ml; c: calcitonin 50 pg/ml; dht: dihydrotestosterone 1nM. Doses in µg/ml for etoposide.
Since normal prostatic epithelium undergoes apoptosis after androgen withdrawal we evaluated the response of androgen-dependent cell line LNCaP to etoposide prior to and after an androgen withdrawal protocol. Maximal induction of apoptosis was achieved at 48 hours of treatment after androgen withdrawal at 100μg/ml concentration and no significant apoptosis was observed prior to androgen withdrawal protocol (Fig. 3).

2. Reversion of etoposide-induced apoptosis

Significative reversion of etoposide-induced apoptosis and an increase in cell viability were achieved when neurpeptides were added to etoposide treated groups. In Du 145 cell line maximal reversion was achieved at 24 hours treatment in the group receiving bombesin plus etoposide 80 μg/ml (p<0.05). Significative reversion (p<0.05) was also observed after 48 hours in the groups receiving etoposide 100 μg/ml plus bombesin. In those groups receiving etoposide plus calcitonin maximal reversion was reached after 48 hours treatment (p: 0.000) (Fig. 2).

In PC-3 androgen-independent cell line reversion of etoposide-induced apoptosis was significative after 48 hours of treatment with etoposide 100μg/ml plus bombesin (p<0.05) or calcitonin (p<0.0005) (Fig. 1).

In LNCaP androgen-dependent prostate cancer cell line after androgen withdrawal, significative reversion of induced apoptosis was seen after 48 hours of treatment in the 100μg/ml etoposide-treated group when neurpeptides bombesin (p<0.0005) and calcitonin (p: 0.0000) were added, being maximal after addition of bombesin. Reversion observed when DHT was added to etoposide was similar to that obtained with calcitonin plus etoposide and significatively lower than etoposide plus bombesin group. In the latter, percentages were similar to that obtained when DHT alone was added to LNCaP cells (Fig. 3).

Reference groups treated only with neurpeptides bombesin and calcitonin show an increased viability and a very low percentage of spontaneous cell death via apoptosis (Fig. 4).

The percentages of apoptosis were established after morphological examination of cultured cells, and classical morphological criteria were applied. Phase contrast exam revealed detachment and round up of cells from 12 hours of treatment, maximal at 48 hours, and membrane blebbing.

Living cell nuclei were round and consisted of blue fluorescent staining with one or two nucleolus, when examined by fluorescence microscopy, and predominated in control and neurpeptide treated groups significatively when compared to etoposide-treated groups and in different proportions in the combined treatment groups (Fig. 5).

In etoposide-treated groups, a significatively higher number of apoptotic cells in different stages were found. Typical apoptotic bodies were observed among cells recovered from the monolayers and stained for light or
fluorescence microscopy, though their number became substantial only after 24-48 hours. Smaller in size than monolayer cells, these bodies showed markedly shrunken cytoplasm and nucleus. Chromatin was heavily condensed up to frank pyknosis. Nuclei presented variable morphology, characteristically asynchronous.

Fig. 6. In the latter stages of apoptosis, apoptotic bodies may be phagocytosed by nearby cells. Du 145 cell line. Haematoxylin-eosin. x 100

Fig. 7. LNCaP cells treated with etoposide plus bombesin. Positive stained nuclei appear in apoptotic cells in contrast with negative stained control cell nuclei TUNEL, counterstained with haematoxylin. x 40
In vitro study of apoptosis reversion

representing first and second stages of the process in the majority, some with crescent shaped caps at the periphery, others with marked condensation and reduction in size or even multiple apoptotic bodies that may be phagocytosed by neighbouring cells or apoptotic bodies exhibiting pronounced signs of "secondary necrosis" or so called third phase of apoptosis. Membrane blebbing and cytoplasmic condensation were also observed (Figs. 6, 7).

Apoptotic cells were shrunken with respect to control cells, or neuropeptide or androgen-treated cells. Occasional mitoses were found in control groups, and in higher number in neuropeptide- treated groups, and a variable number of apoptotic phenomena were seen in those groups with combined treatments.

**Discussion**

Prostatic cancer is the most commonly diagnosed neoplasm and the second leading cause of male death. According to the kinetics of tumor growth, an increase in a neoplastic cell population is the result of imbalance between the two processes controlling tissue homeostasis: cell proliferation and cell death. Apoptosis, therefore, besides cell proliferation, comprises a critical intrinsic cellular defense mechanism against tumorigenic growth which, when supressed, may contribute to malignant development. We have shown that neuropeptides can mediate resistance to etoposide-induced apoptosis in androgen-independent and androgen-dependent prostatic cancer cell lines. Multiple genetic and epigenetic factors have been implicated in the oncogenesis and progression of prostate cancer, but the molecular mechanisms underlying the disease remain largely unknown. The primary importance of the apoptosis concept for oncology lies in its being a regulated phenomenon subject to estimulation and inhibition (Schwartzman and Cidlowski, 1993; Schwartz and Osborne, 1993; Stewart, 1994; Barbiero et al., 1995; Catchpole and Stewart, 1995; Bauer et al., 1996; Tu et al., 1996; Wang et al., 1996; Tang and Porter, 1997).

In this study, we examine the apoptotic activity of prostatic cancer cell lines, androgen-dependent and androgen-independent, in basal conditions and in response to the topoisomerase II inhibitor, etoposide in a range of doses. We also studied the influence on apoptosis and cell viability of androgens and neuropeptides bombesin and calcitonin.

We used etoposide because it is a well characterized inductor of apoptosis which selectively inhibits new DNA synthesis on the nuclear matrix and causes accumulation of cells in the G2/M phase. Human autopsy distribution of etoposide reveals high concentrations of etoposide on prostate tissue and several Phase I and II clinical trials with oral etoposide have been developed (Stewart et al., 1993; Berchem et al., 1995)

Androgen-dependent cell line LNCaP is a well differentiated human prostatic cancer cell line. Despite its malignant phenotype, it retains some of the characteristic features of its benign counterpart. An important property is the presence of a mutated, but functional androgen receptor which would allow the cells to respond to androgen stimulation (Gorzyczka et al., 1993; Berchem et al., 1995; Lee et al., 1995).

In this cell line, we described an in vitro model for prostate cancer treatment that suggests a potential benefit for combined androgen ablation and cytotoxic chemotherapy, developing an interaction of androgen ablation and etoposide. Our data suggest that cytotoxic agents that mediate apoptosis may be more effective in the milieu of androgen deprivation (Berges et al., 1993; Berchem et al., 1995; Salido et al., 1999).

LNCaP apoptosis has been described as initiated by a unique conflict between the growth suppressive activity of the retinoblastoma protein and growth promoting mitogenic signals. When cells receive mixed signals for growth they usually die. When the conflict is prevented by addition of specific growth factors to depleted medium, like bombesin or calcitonin, apoptosis is prevented. This reversion of apoptotic process occurs also with DHT in these cells, but to a lower degree (Berchem et al., 1995). An increased chemotaxis has also been described for LNCaP and PC-3 cells treated with calcitonin and also with calcitonin-gene related peptide, that may play an integral role in the regulation of prostate cell growth (Arends and Wylie, 1991; Geller et al., 1992; Gorzyczka et al., 1993; Ritchie et al., 1997; Zhao et al., 1997; Cussenot et al., 1998; Nagakawa et al., 1998; Carson et al., 1999; Markwalde and Reubi, 1999; Zhu and Wang, 1999).

Androgen ablation does not result in activation of programmed cell death of androgen-independent prostate cancer cells. In PC-3 cells continuous exposure to etoposide 100μg/ml for 48 hours was required to induce substantial apoptosis. This might be an expression for a low propensity of these cells to undergo apoptosis. It can be speculated that the absence of wild type p53 in PC-3 was a contributing factor (Gerschenson and Rotello, 1992; Berges et al., 1993; Clarke et al., 1993; Fairbairn et al., 1993; Wright et al., 1996; Ahn et al., 1997; Aprikian et al., 1997; Kim et al., 1997; Kiprianou et al., 1997; Wasilenko et al., 1997; Zhao et al., 1997; Baretton et al., 1999; Zhu and Wang, 1999).

When neuropeptides were added we observed reversion of etoposide induced apoptosis from 48 hours onwards with bombesin and calcitonin that were confirmed with our results on cell viability assays.

In DU 145 cell line, which does not express functional retinoblastoma protein, cells are resistant to apoptosis induced via PKC, but not p53. Thus, PKC activation by bombesin and calcitonin should inhibit or downregulate p21, activating p53 pathway and cell cycle progression and proliferation with reversion of etoposide-induced apoptosis. It should be stressed that the action of DNA topoisomerase inhibitors in triggering the endonucleolytic activity is modulated by tissue-
specific factors, in our case neuropeptides or androgens, and taking into consideration that with longer time of exposure the secondary effects may be predominant, as shown when cells are treated up to 72 hours (data not shown). Our results (Larrán et al., 1996) after administration of calcitonin alone show that the neuropeptide, as described by some authors, induces cell proliferation and increasing viability (del Bino, 1991; Prokocimer and Rotter, 1994; Barbiero et al., 1995; Berchem et al., 1995; Borner et al., 1995; Blut et al., 1997; Campbell et al., 1997; Ritchie et al., 1997; Zhao, 1997; Chapman et al., 1999).

Neuropeptide induced resistance to etoposide-induced apoptosis represents a novel mechanism-based approach which may help to identify novel drugs and/or develop new therapeutic regimens for the treatment of prostate cancers, using even these neuropeptides secreted by prostatic cells implied in prostatic carcinoma as a target in tumor therapy. Further studies will be conducted to investigate the point at which etoposide induced events could interact with bombesin or calcitonin signal transduction pathways. (Berchem et al., 1995; Sausville et al., 1996; Imam et al., 1997; Jungwirth et al., 1997; Tang and Porter, 1997; Wasielenko et al., 1997; Zhang and Degrot, 1997; Baretton et al., 1999; Chaudhary et al., 1999; Merkwald and Rueslin, 1999).

References


In vitro study of apoptosis reversion


Accepted March 7, 2000