

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.

For more information visit www.intechopen.com



Cytotoxic Endonucleases: New Targets for Prostate Cancer Chemotherapy

Xiaoying Wang, Marina V. Mikhailova and Alexei G. Basnakian
*University of Arkansas for Medical Sciences
and Central Arkansas Veterans Healthcare System
Little Rock, Arkansas
USA*

1. Introduction

Prostate cancer is one of the most common malignancies in Western countries and the world (Baade et al., 2009). It is the third most common cause of death from cancer in men of all ages and the most common cause of death from cancer in men over age 75. The current standard therapies for prostate cancer include radiation, surgery, hormonal therapy and chemotherapy (Debruyne, 2002; Freytag et al., 2007; Nelius et al., 2009; Rozkova et al., 2009). Chemotherapy is almost always a salvage therapy for advanced prostate cancer, and chemoresistance is emerging problem in prostate cancer therapy. Strategies to overcome the chemoresistance of prostate cancer cells have not been developed partially because mechanisms of it are unknown and likely to be numerous. Chemoresistance has a tendency to occur both to clinically established therapeutic agents and novel targeted therapeutics implicating both intrinsic and acquired mechanisms of drug resistance (Djeu & Wei, 2009). Most likely, these are the mechanisms which are universal for cytoprotection from cell death induced by various factors.

Cell death by apoptosis is one of the most universal mechanisms of cell response to injury. It plays the major role in carcinogenesis and prostate tumor progression. Suppression of apoptosis was proposed to cause inappropriate survival of genetically aberrant cells during carcinogenesis (Vineis, 2003). Cancer cells seem to be designed to propagate and survive in a new and hostile environment by suppressing their natural mechanisms of cell death. The neoplastic transformation of prostate epithelial cells is known to be associated with decreased apoptotic cell death (Inokuchi et al., 2009; Shilkaitis et al., 2000). The progression of prostate cancer, in particular, androgen-independent prostate cancer or prostate adenocarcinomas, was also shown to be associated with decreased apoptosis (Raffo et al., 1995; Singh & Lokeshwar, 2009). The latter is the predominant form of tumor cell demise caused by chemotherapeutic agents and it plays an important role in cancer chemosensitivity and radiosensitivity (Arnold & Isaacs, 2002; Debes & Tindall, 2004). Targeting various mechanisms of apoptosis to cure prostate cancer has been suggested in many studies, naming potential molecular targets and key apoptotic regulators such as upstream and downstream caspases, p53, Phosphatase and tensin homolog (PTEN), prostate apoptosis response gene-4 (Par-4), Bcl-2 (B-cell lymphoma 2) protein, transcription factor

NF-kappa B, serine/threonine protein kinase and others (Uzzo et al., 2008; Wang et al., 2004). Precisely, manipulating with sensitivity to cytotoxic agents to alter cancer progression has been suggested as a therapeutic approach for prostate cancer in some reports (McKenzie & Kyprianou, 2006; Watson & Fitzpatrick, 2005). However, for some reason, almost no attention was paid to apoptotic/cytotoxic endonucleases as potential targets.

2. Cytotoxic endonucleases in normal prostate and prostate cancer cells

Cytotoxic endonucleases, also called “apoptotic endonucleases,” are the initially recognized group of enzymes responsible for premortem and postmortem DNA fragmentation associated with cell death by apoptosis (Hengartner, 2001; Samejima & Earnshaw, 2005). Importantly, the same enzymes were later shown to provide DNA fragmentation that accompanies necrosis, autophagy, mitotic catastrophe and all other types of cell death. Therefore the term “apoptotic endonucleases” should be considered outdated.

Major representatives of cytotoxic endonucleases include: deoxyribonuclease I (DNase I) (Polzar et al., 1993), deoxyribonuclease II (DNase II) (Krieser & Eastman, 1998), Endonuclease G (EndoG) (Li et al., 2001), caspase-activated DNase (CAD) (Enari et al., 1998), and DNase gamma (Shiokawa et al., 1997). Some of these enzymes, for example DNases I and II had been known before 1960s. However the actual role of the cytotoxic endonucleases was clarified much later. Cytotoxic endonucleases were found in all studied cells and tissues, including the prostate (Koizumi, 1995; Napirei et al., 2004). The enzymes belong to the family of hydrolyses that cleave phosphodiether bonds in DNA. They differ in certain catalytic characteristics and DNA sequence specificity, and yet produce very similar type of DNA damage consisting of single-stranded or double-strand DNA breaks. Most harmful and hard to repair DNA breaks and double-stranded. They can be produced by so called “single hit” or “double hit” mechanism. In a “single hit” mode, both DNA strands are cut simultaneously at the same site. This mechanism is mainly characteristic of DNase II. The much more common mechanism is “double hit,” in which strands are cleaved independently to result in a double-strand DNA break if the single-stranded breaks coincide with a 2-base or less shift between them. This mechanism is characteristic of DNase I and all other endonucleases, except DNase II.

Independently from the mechanism, endonuclease-generated breaks have been shown to strongly interfere with DNA synthesis in both normal and cancer cells (Nagata, 2000). That is why, while sometimes considered downstream effectors of apoptotic cascades, the endonucleases can cause DNA fragmentation and imminent and irreversible cell death when acting alone after overexpression or introduction into the cell (Enari et al., 1998; Krieser & Eastman, 1998; Polzar et al., 1993). The endonucleases are commonly found active during cell death; however, the overall link between these enzymes and apoptosis is weak. Some of the endonucleases seem to be dispensable in normal apoptosis (Davidson & Harper, 2005; Irvine et al., 2005; Napirei et al., 2000). On the other hand, their participation in cell death in general after tissue injury seems crucial and evidence of this is overwhelming. Recent studies demonstrated that inactivation of the endonucleases causes protection of normal and cancer cells against a variety of injuries *in vitro* and *in vivo* (Basnakian et al., 2006; Basnakian et al., 2005; Napirei et al., 2006; Yin et al., 2007), suggesting that the endonucleases are essential for and mechanistically linked to injury-related cell death. In addition to causing cell death itself, the endonuclease are certainly

essential for clean up after cell death, removal of DNA from blood plasma, and destroying “foreign” DNA from bacteria and viruses consumed by cells (Buzder et al., 2009). These roles of cytotoxic endonucleases are less relevant to prostate cancer and thus will not be considered in this review.

Although all cells and tissues seem to express all endonucleases, the spectrum of them differs between the tissues. The reason for such redundancy of the cytotoxic endonucleases is not known, which allows speculation about the importance of DNA destruction from immediately prior to long after cell death.

The most expressed and active endonuclease in normal prostate is DNase I, previously also known as Ca/Mg-dependent endonuclease (Kyprianou et al., 1988; Kyprianou & Isaacs, 1988). Ca/Mg-dependent endonuclease-mediated DNA fragmentation is used as a marker of apoptosis in prostate cancer. The degradation of genomic DNA into nucleosome-sized fragments is an early event in castration-induced androgen withdrawal that involves death of the androgen-dependent epithelial cells following an increase of endonuclease activity (Banerjee et al., 2000; Brandstrom et al., 1994; Kyprianou et al., 1988).

DNase I is found in all studied species and tissues (Lacks, 1981). It is expressed mainly in tissues of the digestive system, though the specific activity of the enzyme varies between the organs (Gonzalez et al., 2001; Jacob et al., 2002; Lacks, 1981). In digestive tissues (intestine, pancreas, salivary glands), it is a secreted enzyme intended to hydrolyze DNA in the alimentary tract. In non-digestive tissues (including prostate), the role of DNase I is not known. Bovine or mouse DNase I bind specifically to G-actin and blocks its polymerization (Lacks, 1981). The enzyme from all sources endonucleolytically cleaves double- or single-stranded DNA to 3'OH/5'P-end oligonucleotides, requires divalent cations, particularly Ca²⁺ and Mg²⁺, is inhibited by Zn²⁺, and has a neutral pH optimum. Inside the cell, the enzyme is located in the cytoplasm (Peitsch et al., 1993). It has also been shown inside nuclei, but the mechanism of its introduction into nuclei has not been studied. Inhibition of DNase I by internalized nuclear anti-DNA antibodies was shown to provide protection of cells against apoptotic stimuli (Madaio et al., 1996). No known nuclear localization signal was identified in DNase I, and “leakage” through nuclear pores was suggested (Polzar et al., 1993). Among various organs and tissues, prostate, pancreas, salivary glands and kidney tubular epithelium have the highest levels of DNase I activity (Lacks, 1981; Polzar et al., 1993). Little is known about DNase I regulation *in vivo*. An alternative pre-mRNA splicing both in 5'UTR and in coding region was shown to be a mechanism of DNase I regulation (Basnakian et al., 1998; Basnakian et al., 2002). It is known that some DNase I isoforms can be generated by post-translational modification, namely mannose-type glycosylation of the protein (Lacks, 1981).

Studies of endonucleases associated with prostate cancer are very limited. Usually neoplastic transformation is associated with the decrease of endonuclease expression and activity in various cancers, thus making them “immortal” (Banfalvi et al., 2007; Basnakian et al., 2006; Basnakian et al., 1991; Wang et al., 2008). The most profound decrease of endonuclease activity was observed in malignant invasive prostate and breast cancer cells (Basnakian et al., 2006; Wang et al., 2008). The decrease of endonuclease activity had been also observed in other cancers and models of carcinogenesis (Basnak'ian et al., 1989; Basnakian et al., 1991). Immortalization of rat fibroblasts with the S1A segment of SA7 adenovirus also led to a significant decrease of endonuclease activity (Basnak'ian et al.,

1989). Another report indicated that an endonuclease activity is decreased in diethylnitrosamine (DEN)-induced hepatomas in rats compared to normal liver tissue (Basnakian et al., 1991). The decrease was proportional to the degree of dedifferentiation and the activity was the lowest in poorly differentiated tumors.

With the decrease of main prostate endonuclease, DNase I, the endonuclease activity in human prostate cancer cells is provided by EndoG. This endonuclease has a unique site-selectivity, initially attacking poly(dG).poly(dC) sequences in double-stranded DNA, as denoted by this enzyme's name. The enzyme also has RNase activity. EndoG predominantly resides in the intermembrane space of mitochondrion (Ohsato et al., 2002). Mammalian EndoG is synthesized as a 32 kDa propeptide in the cytoplasm and imported into mitochondria through a process mediated by its amino-terminal mitochondrion-targeting sequence (Cote & Ruiz-Carrillo, 1993; Ruiz-Carrillo & Renaud, 1987). The EndoG protein precursor is inactive (Ikeda & Kawasaki, 2001). The signal peptide is cleaved off after entering the mitochondria and the mature active 27 kDa EndoG is released from mitochondria during apoptosis, moves to the nuclei and cleaves nuclear DNA without sequence specificity (Li et al., 2001). EndoG expression varies in different tissues and in embryonic tissues the expression of EndoG is very low (Apostolov et al., 2007b). As opposed to DNase I, the enzyme has a greater activity on single-stranded nucleic acid substrates, single-stranded DNA and RNA. It preferentially cleaves non-canonical structures of DNA, damaged DNA, triplex DNA, and R-loops that appear non-specifically during transcription (Masse & Drolet, 1999). Cisplatin-treated DNA was shown to be preferentially cleaved by EndoG (Ikeda & Ozaki, 1997). EndoG requires either Mn^{2+} or Mg^{2+} ions, and is inhibited 15-fold at physiological ionic strengths (Widlak et al., 2001). Fe^{2+} and Zn^{2+} inhibit the enzyme activity. The EndoG gene in mice is a single copy gene, which consists of 3 exons (Prats et al., 1997). The loss of EndoG activity in *C.elegans* resulted in increased cell survival (Hengartner, 2001). However, EndoG knockout mouse is viable (Irvine et al., 2005). Reduction of EndoG in *C.elegans* using siRNA or genetic mutation affected normal DNA degradation, as revealed by staining with TUNEL assay, and resulted in the delayed appearance of cell corpses during development in *C.elegans* (Parrish et al., 2001). Thus in comparison to other endonucleases, EndoG is uniquely compartmentalized in mitochondria and it does not have known intracellular inhibitors (like DNase I or CAD). The EndoG location site may indicate that this enzyme is not an instrument of immediate response to cell injury.

EndoG seems to be particularly important in cancer cells because it regulates their sensitivity to chemotherapeutic agents (Basnakian et al., 2006). This report suggests the presence of EndoG in non-invasive breast cancer cells determines their sensitivity to apoptosis, which may be taken into consideration for developing the chemotherapeutic strategy for cancer treatment. In other cells, EndoG has been recognized as a key endonuclease in the caspase-independent apoptosis (Abbott et al., 2001; Bahi et al., 2006), mitotic catastrophe (Diener et al., ; Wang et al., 2008), and necrosis (Apostolov et al., 2007a; Jiang et al., 2006).

Because anticancer drugs induce apoptosis in cancer cells through endonuclease-mediated DNA fragmentation (Ploski & Aplan, 2001; Shrivastava et al., 2000), and the inhibition of endonucleases has a protective effect (Shrivastava et al., 2000), endonuclease should be considered as important mediators of cancer cell death and potential therapeutic targets for

the anticancer therapy. However, delivery of endonucleases or modulation of endonuclease activity are not currently used for cancer therapy, in particular, for prostate cancer therapy.

3. Modulation of EndoG by DNA methylation and histone deacetylation

Epigenetic changes are believed to be the most common alteration at the DNA level in prostate cancer (Schulz & Hatina, 2006; Walton et al., 2008). Two types of DNA epigenetic changes that are known to occur in prostate cancer include regional DNA hypermethylation and regional/global DNA hypomethylation. Hypermethylation of the promoter region that contains CpG island occurs in a large number of genes and is usually associated with gene silencing in the vast majority of prostate cancer cases (Li et al., 2005; Perry et al., 2006; Rennie & Nelson, 1998). Studies have shown that hypermethylation of this region may be eventually used as a tumor biomarker for early diagnosis and risk assessment of prostate cancer. Furthermore, the prevalence of epigenetic changes in prostate cancer and the potential reversibility of DNA methylation alterations by DNA methylation inhibitors suggest that these changes are a viable target for cancer chemotherapy and chemoprevention strategies (Egger et al., 2004; Kopelovich et al., 2003; Yoo & Jones, 2006).

Mammalian genome contains patterns of methylated cytosines for normal function, but until recently the structural organization of the methylation landscape of the human genome was unclear (Rollins et al., 2006). It has been reported that the human genome consists of short (<4 kb) unmethylated domains enriched in promoters, CpG islands, and first exons, embedded in a matrix of long methylated domains (Rollins et al., 2006). Analysis of promoter sequences of all known human cytotoxic endonucleases – described below – showed that EndoG is the only cytotoxic endonuclease that contains a CpG island, a segment of DNA with high G+C content and a site for methylation, in the promoter region (Wang et al., 2008).

A large number of studies have shown that methylation of promoter CpG islands plays an important role in gene silencing (Ruchusatsawat et al., 2006; Taghavi & van Lohuizen, 2006). The broadly accepted definition of a CpG island as a 200-bp fragment of DNA with G + C content greater than 50% and observed CpG/expected CpG ratio higher than 0.6 failed to exclude many sequences (such as *Alu* repeats and unknown sequences) that are not associated with regulatory regions of genes (Takai & Jones, 2002). Recent studies indicate that the usage of a modified algorithm to search for CpG islands using a more stringent definition (G + C content higher than 55% and a length greater than 500 bp with observed CpG/expected CpG ratio 0.65) resulted in the exclusion of the majority of *Alu* repetitive and unknown sequences associated with the 5' region of genes (Takai & Jones, 2002). In view of these considerations, we applied this algorithm to the analysis of endonuclease genes, which could be regulated by DNA methylation. All known human cell death endonucleases and their sequence variants were analyzed using the CpG Island Searcher program (available at <http://www.cpgislands.com> (Takai & Jones, 2003)): DNase 1, DNase 1L1 variants 1, 2, 3 and 4; DNase 1L2, DNase 1L3 (DNase gamma), DNase 2 α , DNase 2 β variants 1 and 2, L-DNase II (LEI), CAD and EndoG. Surprisingly, this analysis showed that EndoG is the only gene that satisfied the criteria of containing a long CpG island in the promoter and exon 1 of the gene.

The methylation status of the EndoG promoter/exon 1 in prostate cancer cells was then determined by using the methylation-sensitive McrBC-PCR method. McrBC is a bacterial endonuclease, that does not act on unmethylated DNA, but cleaves DNA containing 5-methylcytosine in one or both strands and thus nullifies PCR amplification (Nakayama et al., 2004). This experiment showed that in three studied prostate cancer cell lines, LNCaP, 22Rv1 and PC3, EndoG promoter methylation was the lowest in 22Rv1 cells and highest in PC3 cells. Further comparison of the three prostate cancer cell lines showed that EndoG is highly expressed in 22Rv1 and LNCaP cells. In PC3 cells, EndoG was not expressed and the EndoG gene CpG island was hypermethylated (Wang et al., 2008).

The expression of EndoG correlated positively with sensitivity to docetaxel, cisplatin and etoposide, and the silencing of EndoG by siRNA decreased the sensitivity of the cells to the chemotherapeutic agents in the two EndoG-expressing cell lines. To determine whether the level of EndoG expression affects the sensitivity of prostate cancer cells to chemotherapeutic drugs, we exposed the three cell lines to two anticancer agents, cisplatin (0-100 μM) and etoposide (0-300 μM), which are known to induce cell death *in vitro* (Fang et al., 2004; Lee et al., 2006). As expected, the two cell lines that expressed EndoG, 22Rv1 and LNCaP, were highly sensitive to both chemotherapeutic agents. EndoG-deficient PC3 cells, in contrast, were insensitive to these drugs in the range of concentrations used.

Further study determined that cisplatin-induced death of prostate cancer cells can be prevented by EndoG silencing. Although EndoG is known to participate in cell death, it was necessary to determine whether the role of EndoG was the same in prostate cancer cells subjected to injury by cytotoxic agents as has been described in other cells. To test a causal relationship, EndoG was silenced in 22Rv1 cells by applying siRNA. To show that siRNA was delivered to the cells, fluorescent DY547-labeled siRNA was used. After DY547-siRNA transfection, 22Rv1 cells were exposed to 80 μM cisplatin, a concentration that had induced significant cell death in the above experiments. Next, TUNEL assay was conducted to measure DNA fragmentation. The assay showed the DNA fragmentation was decreased, indicating that silencing of EndoG leads to significant decrease of EndoG expression and protects cells from DNA fragmentation. As expected, EndoG silencing resulted in the increased viability of cisplatin-treated 22Rv1 cells as measured using clonogenic assay. The results suggest EndoG is responsible for cisplatin-induced death in prostate cancer cells.

It is interesting that inhibition of DNA methylation induced EndoG and increased sensitivity of PC3 cells to cisplatin and etoposide. 5-aza-2'-deoxycytidine (decitabine), which is a DNA methylation inhibitor, caused hypomethylation of the EndoG promoter in PC3 cells, induced EndoG mRNA and protein expression, and made the cells sensitive to the chemotherapy agents. Using McrBC-PCR method, we determined that the treatment of PC3 cells with decitabine inhibited methylation of the CpG island in the EndoG gene. The same concentration of decitabine also increased EndoG expression as determined by real-time RT-PCR and Western blotting. These data clearly suggested EndoG expression is regulated by DNA methylation. Importantly, the induction of EndoG by demethylation caused a significant increase in sensitivity to cisplatin and etoposide.

Finally, the acetylation of histones by trichostatin A (TSA), a histone deacetylase inhibitor, induced EndoG expression in 22Rv1 cells, while it had no such effect in PC3 cells. These

data indicated EndoG may be regulated by methylation of its gene promoter, and partially by histone acetylation, and that EndoG is essential for prostate cancer cell death in the used models. Histone modification, in particular, histone acetylation, is another epigenetic mechanism that is important in regulation of genes in prostate cancer (Das et al., 2006; Egger et al., 2004; Wang et al., 2008; Yoo & Jones, 2006). To determine whether and how histone acetylation regulates EndoG expression, two prostate cancer cell lines were treated with TSA, and EndoG protein expression was studied using Western blotting. The exposure of the cells to TSA induced high levels of EndoG expression in EndoG-positive 22Rv1 cells, whereas in EndoG-deficient PC3 cells, EndoG was not induced. Again, EndoG induction by TSA caused increased sensitivity to cisplatin. These data demonstrated that chromatin acetylation is important for EndoG expression. Taken together with the above methylation experiments, these data indicate that DNA methylation plays a primary role in EndoG regulation as compared to histone acetylation. In other words, the CpG island of the EndoG gene has to be hypomethylated in order to allow regulation of EndoG expression by histone acetylation.

4. Endonuclease delivery to prostate cancer cells and tumors

Although these are attractive and potentially therapeutically useful approaches, modulations of endonuclease expression by DNA methylation or histone acetylation may not be a realistic approach because the specificity of epigenetic regulation is notoriously low. An alternative to these methods may be a gene delivery and overexpression in the target cancer cells. To determine whether overexpression of EndoG would make PC3 cells sensitive to the chemotherapy agents, the cells were transfected with human mature EndoG gene. To model chemotherapy *in vitro*, we used docetaxel, which is an FDA-approved the first line chemotherapeutic agent in castration-refractory prostate cancer (Oudard et al., 2007; Ryan et al., 2001). Despite survival benefits with docetaxel based chemotherapy, prognosis for castration-refractory prostate cancer patients usually is poor and patients typically show rapid progression (Oudard et al., 2005; Wang et al., 2008). Progressive prostate cancer is associated with the development, and subsequent expansion of tumor cells that are resistant to apoptotic triggers and dysregulation of apoptosis is often characterized by insufficient apoptosis (Kruslin, 2009; Mori et al., 1996). Therefore a delivery of EndoG gene was expected to increase sensitivity of prostate cancer cells to docetaxel.

As described below, this genetic manipulation resulted in significant increase of PC3 cells sensitivity to docetaxel and cisplatin *in vitro*. Similar results were obtained when PC3 cells were transfected with EndoG precursor gene suggesting that the drugs induce speedy processing of the protein to mature endonuclease.

PC3 cells were chosen because they have very low expression of EndoG (Wang et al., 2008). Human EndoG gene (NM 004435.2) was cloned in the mammalian expression vector pECFP.N1 to result in an expression of EndoG protein fused with the enhanced cyan fluorescence protein (CFP). The expression of the chimeric protein was confirmed by fluorescent microscopy. Cells were then treated with docetaxel and cell death was measured using lactate dehydrogenase (LDH) release assay. This experiment showed the sensitivity of the PC3 cells expressing EndoG-CFP to docetaxel was much higher than the cells expressing CFP alone (Figure 1). The same result was also observed in cisplatin-induced cells death:

EndoG overexpression resulted in an over 4-folds elevation of cisplatin-induced cell death (data not shown). We also have compared mature EndoG gene and precursor EndoG gene overexpression cytotoxicity and their effects on cisplatin-induced cell death, and found that both types of EndoG had familiar effect (data not shown).

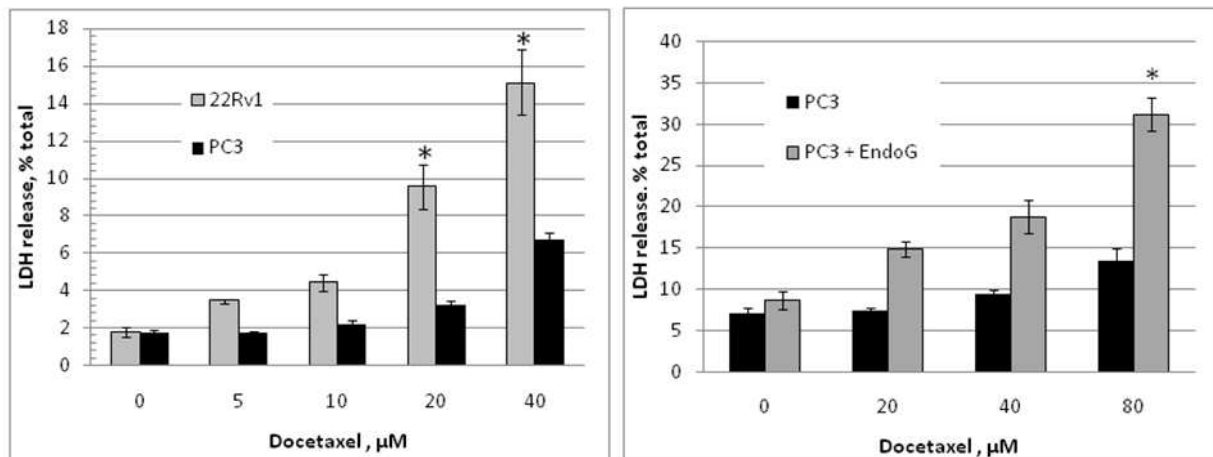


Fig. 1. EndoG expression enhances prostate cancer cells' sensitivity to docetaxel in vitro. Left panel: Cell death measured by LDH release assay in EndoG-expressing 22Rv1 and EndoG-negative PC3 cells, which were exposed to varying concentrations of docetaxel for 24h (n=4, *p<0.05). Right panel: Cell death measured by LDH release assay in PC3 cells with or without EndoG precursor overexpression 24 hrs after exposure to varying concentrations of docetaxel (n=4, *p<0.05).

Finally, parental PC3 cells and PC3 cells overexpressing human EndoG precursor were implanted in prostates of SCID mice to produce orthotopic tumors. The animals with xenografts were subjected to the docetaxel chemotherapy and the tumor size progression was monitored by high frequency ultrasound visualization. This experiment showed that EndoG-expressing tumors shrink in response to chemotherapy, while control tumors made of EndoG-negative parental PC3 cells were chemoresistant. To produce orthotopic xenografts, 8-weeks old male SCID mice were injected with human prostate cancer PC3 cells or EndoG gene-transfected PC3 cells by surgical orthotopic implantation. 2×10^5 cells were mixed with matrigel at 1:1 ratio (v/v) in a total volume of 20μl were injected in the left ventral prostate lobes after surgical opening of the lower abdomen skin and peritoneal membrane. Ultrasound image could identify prostate tumor as early for 6 days after implantation. Monitoring of the tumor growth showed that the prostate lobe eventually was occupied by the tumor and lost its original shape. At the 12th day, the mice received docetaxel (10mg/kg) via peritoneal cavity injection while the control mice received saline injection. Ultrasound images were taken at the 6, 12, and 18 days after orthotopic implantation. By day 18, PC3 xenograft tumors significantly grew up regardless of the docetaxel treatment; EndoG-PC3 xenografts without docetaxel treatment grew up less, while the docetaxel-treated EndoG-PC3 xenografts did not grow in size and instead shrunk (Figure 2). Histology analysis confirmed the EndoG overexpression in tumors, which coincided with positive TUNEL staining, thus confirming EndoG overexpression made xenografts sensitive to the docetaxel treatment.

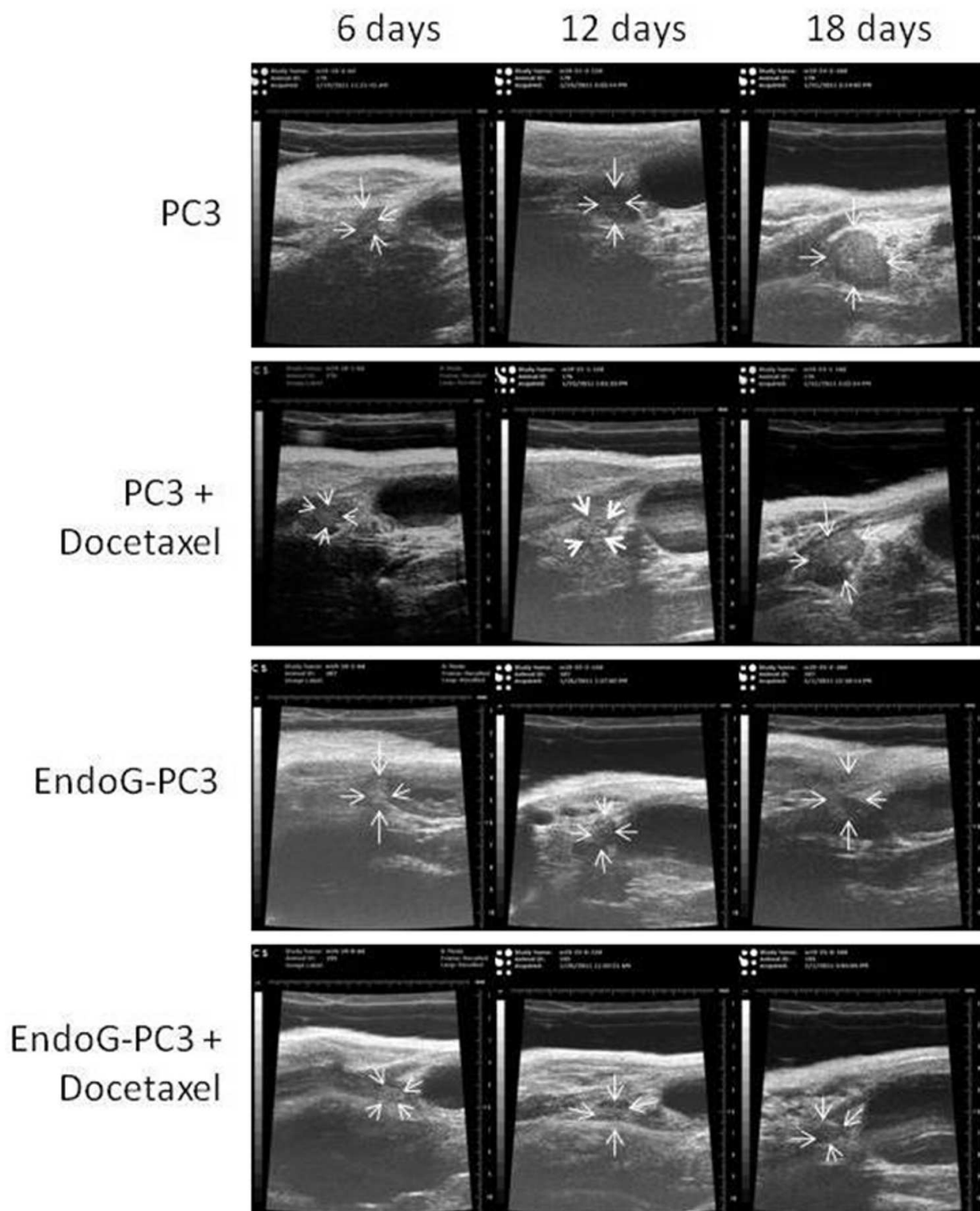


Fig. 2. EndoG expression facilitates docetaxel sensitivity of orthotopic PC3 xenograft tumors. Human prostate cancer PC3 cells were transfected with EndoG precursor gene. Parental EndoG-deficient cells or EndoG-expressing PC3 cells were implanted in ventral prostate. Docetaxel (10 mg/kg) was administered at the 12th day after implantation. Tumor sizes were monitored by intravital ultrasound sonography using VisualSonics Vevo 770 instrument. Arrows indicate tumor edges.

5. Conclusive remarks

Overall, our studies demonstrated that the expression and activity of the cytotoxic endonucleases are decreased in prostate cancer cells that are resistant to chemotherapy (Wang et al., 2008). This is consistent with previous studies of breast cancer, which also showed disappearance of DNase I in immortalized breast epithelial cells, and decrease of EndoG that coincided with dedifferentiation and invasiveness of breast cancer (Basnakian et al., 2006). EndoG is shown essential for prostate cancer cell death induced by chemotherapy. Expression of EndoG positively correlated with the sensitivity to chemotherapeutic agents cisplatin and etoposide, while the silencing of EndoG by siRNA in two cancer lines, 22Rv1 and LNCaP, decreased the sensitivity of the cells to the chemotherapeutic agents. In PC3 cell line, which does not express EndoG, the chemotherapeutic agent 5-aza-2'-deoxycytidine caused hypomethylation of the EndoG promoter, induced EndoG expression, and made the cells sensitive to both cisplatin and etoposide. In our latest studies described above, the overexpression of EndoG in PC3 cells made them also sensitive to docetaxel *in vitro* and *in vivo*. Therefore these studies demonstrate the first application of endonucleases as a helper drug for the chemotherapy of prostate cancer.

Because the mechanisms of chemo- and radiosensitivity of cells are very similar, these observations may be easily extrapolated to the radiotherapy of prostate cancer. Future studies may be necessary to determine the role of other epigenetic mechanisms in regulation of EndoG and their role in chemoresistance to prostate cancer and cancers of other organs. Chemotherapy is currently one of the frequently used therapeutic strategies for prostate cancer (Dyrstad et al., 2006; Kaku et al., 2006; Nakabayashi & Oh, 2006), and measurement of EndoG may be a potentially useful approach to evaluate chemosensitivity of cancer cells to determine optimal conditions for chemotherapy prior to the therapy.

If further *in vivo* studies confirm our observation that EndoG is a potential key mediator of prostate cancer cell death regulated by the methylation of *EndoG* gene promoter, future epigenetic therapeutics will need to be targeted to EndoG. A development of this approach may lead to similar therapeutic strategies for cancer of other organs.

Recent study determined that DNase I and EndoG, which represent most of DNase activity in prostate epithelial and many other cells and are linked in a single pathway, in which DNase I expression positively modulates EndoG expression (Yin et al., 2007). DNase I has the highest specific activity (per mg protein) among all known endonucleases, and it is the only endonuclease that can be directly incorporated into cells. The mechanisms of DNA destruction and the role in cell death are same between the two endonucleases. Therefore, it may not be necessary to deliver EndoG gene to prostate tumors and instead deliver DNase I protein packed in liposomes, which are attractive as vehicles because they have low toxicity. The only example of an endonuclease being applied for a therapy is human recombinant DNase I is used in complex therapy of cystic fibrosis. Future studies may lead to the first application of endonucleases as a helper drug for chemotherapy of prostate cancer.

6. References

- Abbott, D. W., Ivanova, V. S., Wang, X., Bonner, W. M., and Ausio, J., 2001, Characterization of the stability and folding of H2A.Z chromatin particles: implications for transcriptional activation: J Biol Chem, v. 276, p. 41945-9.

- Apostolov, E. O., Shah, S. V., Ok, E., and Basnakian, A. G., 2007a, Carbamylated Low-Density Lipoprotein Induces Monocyte Adhesion to Endothelial Cells Through Intercellular Adhesion Molecule-1 and Vascular Cell Adhesion Molecule-1: *Arterioscler Thromb Vasc Biol*.
- Apostolov, E. O., Wang, X., Shah, S. V., and Basnakian, A. G., 2007b, Role of EndoG in development and cell injury: *Cell Death Differ*, v. 14, p. 1971-4.
- Arnold, J. T., and Isaacs, J. T., 2002, Mechanisms involved in the progression of androgen-independent prostate cancers: it is not only the cancer cell's fault: *Endocr Relat Cancer*, v. 9, p. 61-73.
- Baade, P. D., Youlten, D. R., and Krnjacki, L. J., 2009, International epidemiology of prostate cancer: geographical distribution and secular trends: *Mol Nutr Food Res*, v. 53, p. 171-84.
- Bahi, N., Zhang, J., Llovera, M., Ballester, M., Comella, J. X., and Sanchis, D., 2006, Switch from caspase-dependent to caspase-independent death during heart development: essential role of endonuclease G in ischemia-induced DNA processing of differentiated cardiomyocytes: *J Biol Chem*, v. 281, p. 22943-52.
- Banerjee, S., Banerjee, P. P., and Brown, T. R., 2000, Castration-induced apoptotic cell death in the Brown Norway rat prostate decreases as a function of age: *Endocrinology*, v. 141, p. 821-32.
- Banfalvi, G., Trencsenyi, G., Ujvarosi, K., Nagy, G., Ombodi, T., Bedei, M., Somogyi, C., and Basnakian, A. G., 2007, Supranucleosomal organization of chromatin fibers in nuclei of Drosophila S2 cells: *DNA Cell Biol*, v. 26, p. 55-62.
- Basnakian, A. G., Topol, L. Z., Kirsanova, I. D., Votrin, II, and Kiselev, F. L., 1989, [Activity of topoisomerase I and endonucleases in cells transfected by a ras oncogene]: *Mol Biol (Mosk)*, v. 23, p. 750-7.
- Basnakian, A. G., Apostolov, E. O., Yin, X., Abiri, S. O., Stewart, A. G., Singh, A. B., and Shah, S. V., 2006, Endonuclease G promotes cell death of non-invasive human breast cancer cells: *Exp Cell Res*, v. 312, p. 4139-49.
- Basnakian, A. G., Apostolov, E. O., Yin, X., Napirei, M., Mannherz, H. G., and Shah, S. V., 2005, Cisplatin nephrotoxicity is mediated by deoxyribonuclease I: *J Am Soc Nephrol*, v. 16, p. 697-702.
- Basnakian, A. G., Boubnov, N. V., Kirsanova, I. D., and Votrin, II, 1991, Nuclear topoisomerase I and DNase activities in rat diethylnitrosamine-induced hepatoma, in regenerating and fetal liver: *Biochem Int*, v. 24, p. 429-37.
- Basnakian, A. G., Singh, A. B., and Shah, S. V., 1998, Rat kidney DNase I pre-mRNA is alternatively spliced both in 5'-untranslated region and in coding region. Proceedings of the ASN 31st Annual Meeting, Oct 25-28, 1998, Philadelphia, PA: *J Am Soc Nephrol*, v. 9, p. 573A.
- Basnakian, A. G., Singh, A. B., and Shah, S. V., 2002, Identification and expression of deoxyribonuclease (DNase) I alternative transcripts in the rat: *Gene*, v. 289, p. 87-96.
- Brandstrom, A., Westin, P., Bergh, A., Cajander, S., and Damber, J. E., 1994, Castration induces apoptosis in the ventral prostate but not in an androgen-sensitive prostatic adenocarcinoma in the rat: *Cancer Res*, v. 54, p. 3594-601.
- Buzder, T., Yin, X., Wang, X., Banfalvi, G., and Basnakian, A. G., 2009, Uptake of Foreign Nucleic Acids in Kidney Tubular Epithelial Cells Deficient in Proapoptotic Endonucleases: *DNA Cell Biol*.

- Cote, J., and Ruiz-Carrillo, A., 1993, Primers for mitochondrial DNA replication generated by endonuclease G: *Science*, v. 261, p. 765-9.
- Das, P. M., Ramachandran, K., Vanwert, J., Ferdinand, L., Gopisetty, G., Reis, I. M., and Singal, R., 2006, Methylation mediated silencing of TMS1/ASC gene in prostate cancer: *Mol Cancer*, v. 5, p. 28.
- Davidson, B. L., and Harper, S. Q., 2005, Viral delivery of recombinant short hairpin RNAs: *Methods Enzymol*, v. 392, p. 145-73.
- Debes, J. D., and Tindall, D. J., 2004, Mechanisms of androgen-refractory prostate cancer: *N Engl J Med*, v. 351, p. 1488-90.
- Debruyne, F., 2002, Hormonal therapy of prostate cancer: *Semin Urol Oncol*, v. 20, p. 4-9.
- Diener, T., Neuhaus, M., Koziel, R., Micutkova, L., and Jansen-Durr, P., Role of endonuclease G in senescence-associated cell death of human endothelial cells: *Exp Gerontol*.
- Djeu, J. Y., and Wei, S., 2009, Clusterin and chemoresistance: *Adv Cancer Res*, v. 105, p. 77-92.
- Dyrstad, S. W., Shah, P., and Rao, K., 2006, Chemotherapy for prostate cancer: *Curr Pharm Des*, v. 12, p. 819-37.
- Egger, G., Liang, G., Aparicio, A., and Jones, P. A., 2004, Epigenetics in human disease and prospects for epigenetic therapy: *Nature*, v. 429, p. 457-63.
- Enari, M., Sakahira, H., Yokoyama, H., Okawa, K., Iwamatsu, A., and Nagata, S., 1998, A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD: *Nature*, v. 391, p. 43-50.
- Fang, X., Zheng, C., Liu, Z., Ekman, P., and Xu, D., 2004, Enhanced sensitivity of prostate cancer DU145 cells to cisplatin by 5-aza-2'-deoxycytidine: *Oncol Rep*, v. 12, p. 523-6.
- Freytag, S. O., Stricker, H., Peabody, J., Pegg, J., Paielli, D., Movsas, B., Barton, K. N., Brown, S. L., Lu, M., and Kim, J. H., 2007, Five-year follow-up of trial of replication-competent adenovirus-mediated suicide gene therapy for treatment of prostate cancer: *Mol Ther*, v. 15, p. 636-42.
- Gonzalez, V. M., Fuertes, M. A., Alonso, C., and Perez, J. M., 2001, Is cisplatin-induced cell death always produced by apoptosis?: *Mol Pharmacol*, v. 59, p. 657-63.
- Hengartner, M. O., 2001, Apoptosis. DNA destroyers: *Nature*, v. 412, p. 27, 29.
- Ikeda, S., and Kawasaki, N., 2001, Isolation and characterization of the *Schizosaccharomyces pombe* cDNA encoding the mitochondrial endonuclease(1): *Biochim Biophys Acta*, v. 1519, p. 111-6.
- Ikeda, S., and Ozaki, K., 1997, Action of mitochondrial endonuclease G on DNA damaged by L-ascorbic acid, peplomycin, and cis-diamminedichloroplatinum (II): *Biochem Biophys Res Commun*, v. 235, p. 291-4.
- Inokuchi, J., Lau, A., Tyson, D. R., and Ornstein, D. K., 2009, Loss of annexin A1 disrupts normal prostate glandular structure by inducing autocrine IL-6 signaling: *Carcinogenesis*, v. 30, p. 1082-8.
- Irvine, R. A., Adachi, N., Shibata, D. K., Cassell, G. D., Yu, K., Karanjawala, Z. E., Hsieh, C. L., and Lieber, M. R., 2005, Generation and characterization of endonuclease G null mice: *Mol Cell Biol*, v. 25, p. 294-302.
- Jacob, M., Napirei, M., Ricken, A., Dixkens, C., and Mannherz, H. G., 2002, Histopathology of lupus-like nephritis in Dnase1-deficient mice in comparison to NZB/W F1 mice: *Lupus*, v. 11, p. 514-27.

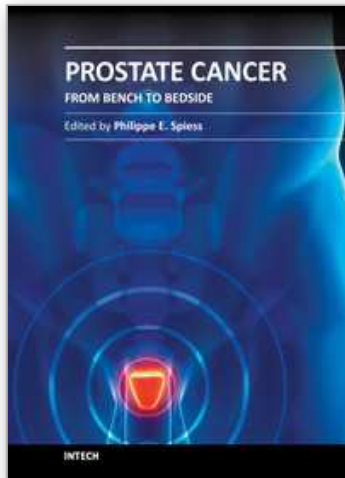
- Jiang, H., Sha, S. H., Forge, A., and Schacht, J., 2006, Caspase-independent pathways of hair cell death induced by kanamycin in vivo: *Cell Death Differ*, v. 13, p. 20-30.
- Kaku, H., Saika, T., Tsushima, T., Nagai, A., Yokoyama, T., Abarzua, F., Ebara, S., Manabe, D., Nasu, Y., and Kumon, H., 2006, Combination chemotherapy with estramustine phosphate, ifosfamide and cisplatin for hormone-refractory prostate cancer: *Acta Med Okayama*, v. 60, p. 43-9.
- Koizumi, T., 1995, Deoxyribonuclease II (DNase II) activity in mouse tissues and body fluids: *Exp Anim*, v. 44, p. 169-71.
- Kopelovich, L., Crowell, J. A., and Fay, J. R., 2003, The epigenome as a target for cancer chemoprevention: *J Natl Cancer Inst*, v. 95, p. 1747-57.
- Krieser, R. J., and Eastman, A., 1998, The cloning and expression of human deoxyribonuclease II. A possible role in apoptosis: *J Biol Chem*, v. 273, p. 30909-14.
- Kruslin, B., 2009, [Apoptosis in pathologic prostatic processes]: *Acta Med Croatica*, v. 63 Suppl 2, p. 49-52.
- Kyprianou, N., English, H. F., and Isaacs, J. T., 1988, Activation of a Ca²⁺-Mg²⁺-dependent endonuclease as an early event in castration-induced prostatic cell death: *Prostate*, v. 13, p. 103-17.
- Kyprianou, N., and Isaacs, J. T., 1988, Activation of programmed cell death in the rat ventral prostate after castration: *Endocrinology*, v. 122, p. 552-62.
- Lacks, S. A., 1981, Deoxyribonuclease I in mammalian tissues. Specificity of inhibition by actin: *J Biol Chem*, v. 256, p. 2644-8.
- Lee, M. G., Huh, J. S., Chung, S. K., Lee, J. H., Byun, D. S., Ryu, B. K., Kang, M. J., Chae, K. S., Lee, S. J., Lee, C. H., Kim, J. I., Chang, S. G., and Chi, S. G., 2006, Promoter CpG hypermethylation and downregulation of XAF1 expression in human urogenital malignancies: implication for attenuated p53 response to apoptotic stresses: *Oncogene*, v. 25, p. 5807-22.
- Li, L. C., Carroll, P. R., and Dahiya, R., 2005, Epigenetic changes in prostate cancer: implication for diagnosis and treatment: *J Natl Cancer Inst*, v. 97, p. 103-15.
- Li, L. Y., Luo, X., and Wang, X., 2001, Endonuclease G is an apoptotic DNase when released from mitochondria: *Nature*, v. 412, p. 95-9.
- Madaio, M. P., Fabbi, M., Tiso, M., Daga, A., and Puccetti, A., 1996, Spontaneously produced anti-DNA/DNase I autoantibodies modulate nuclear apoptosis in living cells: *Eur J Immunol*, v. 26, p. 3035-41.
- Masse, E., and Drolet, M., 1999, R-loop-dependent hypernegative supercoiling in *Escherichia coli* topA mutants preferentially occurs at low temperatures and correlates with growth inhibition: *J Mol Biol*, v. 294, p. 321-32.
- McKenzie, S., and Kyprianou, N., 2006, Apoptosis evasion: the role of survival pathways in prostate cancer progression and therapeutic resistance: *J Cell Biochem*, v. 97, p. 18-32.
- Mori, O., Hachisuka, H., Morita, M., Kiyokawa, C., and Sasai, Y., 1996, Apoptosis identified with DNA fragmentation in basal cell carcinomas: *Arch Dermatol Res*, v. 288, p. 258-61.
- Nagata, S., 2000, Apoptotic DNA fragmentation: *Exp Cell Res*, v. 256, p. 12-8.
- Nakabayashi, M., and Oh, W. K., 2006, Chemotherapy for high-risk localized prostate cancer: *BJU Int*, v. 97, p. 679-83.

- Nakayama, M., Gonzalvo, M. L., Yegnasubramanian, S., Lin, X., De Marzo, A. M., and Nelson, W. G., 2004, GSTP1 CpG island hypermethylation as a molecular biomarker for prostate cancer: *J Cell Biochem*, v. 91, p. 540-52.
- Napirei, M., Basnakian, A. G., Apostolov, E. O., and Mannherz, H. G., 2006, Deoxyribonuclease 1 aggravates acetaminophen-induced liver necrosis in male CD-1 mice: *Hepatology*, v. 43, p. 297-305.
- Napirei, M., Karsunky, H., Zevnik, B., Stephan, H., Mannherz, H. G., and Moroy, T., 2000, Features of systemic lupus erythematosus in Dnase1-deficient mice: *Nat Genet*, v. 25, p. 177-81.
- Napirei, M., Ricken, A., Eulitz, D., Knoop, H., and Mannherz, H. G., 2004, Expression pattern of the deoxyribonuclease 1 gene: lessons from the Dnase1 knockout mouse: *Biochem J*, v. 380, p. 929-37.
- Nelius, T., Klatte, T., de Riese, W., Haynes, A., and Filleur, S., 2009, Clinical outcome of patients with docetaxel-resistant hormone-refractory prostate cancer treated with second-line cyclophosphamide-based metronomic chemotherapy: *Med Oncol*.
- Ohsato, T., Ishihara, N., Muta, T., Umeda, S., Ikeda, S., Mihara, K., Hamasaki, N., and Kang, D., 2002, Mammalian mitochondrial endonuclease G. Digestion of R-loops and localization in intermembrane space: *Eur J Biochem*, v. 269, p. 5765-70.
- Oudard, S., Banu, E., Beuzeboc, P., Voog, E., Dourthe, L. M., Hardy-Bessard, A. C., Linassier, C., Scotte, F., Banu, A., Coscas, Y., Guinet, F., Poupon, M. F., and Andrieu, J. M., 2005, Multicenter randomized phase II study of two schedules of docetaxel, estramustine, and prednisone versus mitoxantrone plus prednisone in patients with metastatic hormone-refractory prostate cancer: *J Clin Oncol*, v. 23, p. 3343-51.
- Oudard, S., Banu, E., Scotte, F., Beuzeboc, P., Guyader, C., and Medioni, J., 2007, [New targeted therapies in hormone-refractory prostate cancer]: *Bull Cancer*, v. 94, p. F62-8.
- Parrish, J., Li, L., Klotz, K., Ledwich, D., Wang, X., and Xue, D., 2001, Mitochondrial endonuclease G is important for apoptosis in *C. elegans*: *Nature*, v. 412, p. 90-4.
- Peitsch, M. C., Polzar, B., Stephan, H., Crompton, T., MacDonald, H. R., Mannherz, H. G., and Tschopp, J., 1993, Characterization of the endogenous deoxyribonuclease involved in nuclear DNA degradation during apoptosis (programmed cell death): *Embo J*, v. 12, p. 371-7.
- Perry, A. S., Foley, R., Woodson, K., and Lawler, M., 2006, The emerging roles of DNA methylation in the clinical management of prostate cancer: *Endocr Relat Cancer*, v. 13, p. 357-77.
- Ploski, J. E., and Aplan, P. D., 2001, Characterization of DNA fragmentation events caused by genotoxic and non-genotoxic agents: *Mutat Res*, v. 473, p. 169-80.
- Polzar, B., Peitsch, M. C., Loos, R., Tschopp, J., and Mannherz, H. G., 1993, Overexpression of deoxyribonuclease I (DNase I) transfected into COS-cells: its distribution during apoptotic cell death: *Eur J Cell Biol*, v. 62, p. 397-405.
- Prats, E., Noel, M., Letourneau, J., Tiranti, V., Vaque, J., Debon, R., Zeviani, M., Cornudella, L., and Ruiz-Carrillo, A., 1997, Characterization and expression of the mouse endonuclease G gene: *DNA Cell Biol*, v. 16, p. 1111-22.
- Raffo, A. J., Perlman, H., Chen, M. W., Day, M. L., Streitman, J. S., and Buttyan, R., 1995, Overexpression of bcl-2 protects prostate cancer cells from apoptosis in vitro and confers resistance to androgen depletion in vivo: *Cancer Res*, v. 55, p. 4438-45.

- Rennie, P. S., and Nelson, C. C., 1998, Epigenetic mechanisms for progression of prostate cancer: *Cancer Metastasis Rev*, v. 17, p. 401-9.
- Rollins, R. A., Haghighi, F., Edwards, J. R., Das, R., Zhang, M. Q., Ju, J., and Bestor, T. H., 2006, Large-scale structure of genomic methylation patterns: *Genome Res*, v. 16, p. 157-63.
- Rozkova, D., Tiserova, H., Fucikova, J., Last'ovicka, J., Podrazil, M., Ulcova, H., Budinsky, V., Prausova, J., Linke, Z., Minarik, I., Sediva, A., Spisek, R., and Bartunkova, J., 2009, FOCUS on FOCIS: combined chemo-immunotherapy for the treatment of hormone-refractory metastatic prostate cancer: *Clin Immunol*, v. 131, p. 1-10.
- Ruchusatsawat, K., Wongpiyabovorn, J., Shuangshoti, S., Hirankarn, N., and Mutirangura, A., 2006, SHP-1 promoter 2 methylation in normal epithelial tissues and demethylation in psoriasis: *J Mol Med*, v. 84, p. 175-82.
- Ruiz-Carrillo, A., and Renaud, J., 1987, Endonuclease G: a (dG)_n X (dC)_n-specific DNase from higher eukaryotes: *Embo J*, v. 6, p. 401-7.
- Ryan, C. W., Stadler, W. M., and Vogelzang, N. J., 2001, Docetaxel and exisulind in hormone-refractory prostate cancer: *Semin Oncol*, v. 28, p. 56-61.
- Samejima, K., and Earnshaw, W. C., 2005, Trashing the genome: the role of nucleases during apoptosis: *Nat Rev Mol Cell Biol*.
- Schulz, W. A., and Hatina, J., 2006, Epigenetics of prostate cancer: beyond DNA methylation: *J Cell Mol Med*, v. 10, p. 100-25.
- Shilkaitis, A., Green, A., Steele, V., Lubet, R., Kelloff, G., and Christov, K., 2000, Neoplastic transformation of mammary epithelial cells in rats is associated with decreased apoptotic cell death: *Carcinogenesis*, v. 21, p. 227-33.
- Shiokawa, D., Ohyama, H., Yamada, T., and Tanuma, S., 1997, Purification and properties of DNase gamma from apoptotic rat thymocytes: *Biochem J*, v. 326 (Pt 3), p. 675-81.
- Shrivastava, P., Sodhi, A., and Ranjan, P., 2000, Anticancer drug-induced apoptosis in human monocytic leukemic cell line U937 requires activation of endonuclease(s): *Anticancer Drugs*, v. 11, p. 39-48.
- Singh, R. K., and Lokeshwar, B. L., 2009, Depletion of intrinsic expression of Interleukin-8 in prostate cancer cells causes cell cycle arrest, spontaneous apoptosis and increases the efficacy of chemotherapeutic drugs: *Mol Cancer*, v. 8, p. 57.
- Taghavi, P., and van Lohuizen, M., 2006, Developmental biology: two paths to silence merge: *Nature*, v. 439, p. 794-5.
- Takai, D., and Jones, P. A., 2002, Comprehensive analysis of CpG islands in human chromosomes 21 and 22: *Proc Natl Acad Sci U S A*, v. 99, p. 3740-5.
- Takai, D., and Jones, P. A., 2003, The CpG island searcher: a new WWW resource: *In Silico Biol*, v. 3, p. 235-40.
- Uzzo, R. G., Haas, N. B., Crispen, P. L., and Kolenko, V. M., 2008, Mechanisms of apoptosis resistance and treatment strategies to overcome them in hormone-refractory prostate cancer: *Cancer*, v. 112, p. 1660-71.
- Vineis, P., 2003, Cancer as an evolutionary process at the cell level: an epidemiological perspective: *Carcinogenesis*, v. 24, p. 1-6.
- Walton, T. J., Li, G., Seth, R., McArdle, S. E., Bishop, M. C., and Rees, R. C., 2008, DNA demethylation and histone deacetylation inhibition co-operate to re-express estrogen receptor beta and induce apoptosis in prostate cancer cell-lines: *Prostate*, v. 68, p. 210-22.

- Wang, G., Reed, E., and Li, Q. Q., 2004, Apoptosis in prostate cancer: progressive and therapeutic implications (Review): *Int J Mol Med*, v. 14, p. 23-34.
- Wang, Q. F., Tilly, K. I., Tilly, J. L., Preffer, F., Schneyer, A. L., Crowley, W. F., Jr., and Sluss, P. M., 1996, Activin inhibits basal and androgen-stimulated proliferation and induces apoptosis in the human prostatic cancer cell line, LNCaP: *Endocrinology*, v. 137, p. 5476-83.
- Wang, X., Tryndyak, V., Apostolov, E. O., Yin, X., Shah, S. V., Pogribny, I. P., and Basnakian, A. G., 2008, Sensitivity of human prostate cancer cells to chemotherapeutic drugs depends on EndoG expression regulated by promoter methylation: *Cancer Lett*, v. 270, p. 132-43.
- Watson, R. W., and Fitzpatrick, J. M., 2005, Targeting apoptosis in prostate cancer: focus on caspases and inhibitors of apoptosis proteins: *BJU Int*, v. 96 Suppl 2, p. 30-4.
- Widlak, P., Li, L. Y., Wang, X., and Garrard, W. T., 2001, Action of recombinant human apoptotic endonuclease G on naked DNA and chromatin substrates: cooperation with exonuclease and DNase I: *J Biol Chem*, v. 276, p. 48404-9.
- Yin, X., Apostolov, E. O., Shah, S. V., Wang, X., Bogdanov, K. V., Buzder, T., Stewart, A. G., and Basnakian, A. G., 2007, Induction of Renal Endonuclease G by Cisplatin Is Reduced in DNase I-Deficient Mice: *J Am Soc Nephrol*, v. 18, p. 2544-53.
- Yoo, C. B., and Jones, P. A., 2006, Epigenetic therapy of cancer: past, present and future: *Nat Rev Drug Discov*, v. 5, p. 37-50.

IntechOpen



Prostate Cancer - From Bench to Bedside

Edited by Dr. Philippe E. Spiess

ISBN 978-953-307-331-6

Hard cover, 528 pages

Publisher InTech

Published online 25, November, 2011

Published in print edition November, 2011

The present textbook highlights many of the exciting discoveries made in the diagnosis and treatment of prostate cancer over the past decade. International thought leaders have contributed to this effort providing a comprehensive and state-of-the art review of the signaling pathways and genetic alterations essential in prostate cancer. This work provides an essential resource for healthcare professionals and scientists dedicated to this field. This textbook is dedicated to the efforts and advances made by our scientific community, realizing we have much to learn in striving to some day in the not too distant future cure this disease particularly among those with an aggressive tumor biology.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Xiaoying Wang, Marina V. Mikhailova and Alexei G. Basnakian (2011). Cytotoxic Endonucleases: New Targets for Prostate Cancer Chemotherapy, Prostate Cancer - From Bench to Bedside, Dr. Philippe E. Spiess (Ed.), ISBN: 978-953-307-331-6, InTech, Available from: <http://www.intechopen.com/books/prostate-cancer-from-bench-to-bedside/cytotoxic-endonucleases-new-targets-for-prostate-cancer-chemotherapy>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen