Invited Review

Cell proliferation and apoptosis in prostate cancer: significance in disease progression and therapy

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Summary. Recent biochemical and genetic studies have substantially increased our understanding of death signal transduction pathways, making it clear however, that apoptosis is not a single-lane, one-way street. Rather, multiple parallel pathways have been identified. For instance, analysis of bcl-2, bax, p53, and caspase knockout mice while establishing distinct roles for each of these apoptotic players, they also provided valuable information for the design of specific inhibitors of apoptosis. Thus blocking one pathway, as in caspase knockout mice, what we observe is not a complete suppression of apoptosis but rather a delay in apoptosis induction (Hakem et al., 1998; Kuida et al., 1998). In view of nature’s means of ensuring activation of a compensatory apoptotic response, when one pathway fails in developing prostate cancer therapeutic interventions, the challenge remains to further dissect individual apoptotic pathways. Advances in our understanding of the integrated functions governing prostate cell proliferation and cell death, clearly suggest that effective prostate cancer therapies are not only molecularly targeted, but that are also customized to take into account the delicate balance of opposing growth influences in the ageing gland. In this review we discuss the evidence on the significance of molecular deregulation of the key players of this growth equilibrium, apoptosis and cell proliferation in prostate cancer progression, and the clinical implications of changes in the apoptotic response in disease detection and therapy.

Key words: Prostate cancer, Apoptosis, Cell cycle, Caspases, Bcl-2, Therapy

Introduction

Prostate cancer threatens to become an American epidemic accounting for an estimated 37,000 fatalities in 1999 (Landis et al., 1999). It is the leading male malignancy in the United States, superceding the once prevalent lung cancer status (Landis et al., 1999). Prostate cancer mortality results from metastasis to the bone and lymph nodes and progression from androgen-dependent to androgen-independent prostatic growth (Isaacs et al., 1994). Radical prostatectomy, androgen-ablation therapy and radiotherapy are considered curative for localized disease, however, no treatment for metastatic prostate cancer is available that effectively increases patient survival (Raghavan, 1988; Crawford, 1989; Lezefsky et al., 1994).

Induction of apoptosis represents a powerful weapon against advanced prostate cancer. The therapeutic significance of apoptosis in the treatment of prostate cancer stems from evidence demonstrating that like normal prostate epithelial cells, prostate cancer cells in androgen-dependent tumors undergo apoptosis in response to androgen deprivation (Kyprianou and Isaacs, 1988; Kyprianou et al., 1990). A similar apoptotic pathway of cell destruction can be activated by chemotherapeutic drugs or ionizing irradiation in androgen-independent prostate cancer cells (Sklar et al., 1993; Furuya et al., 1994; Kyprianou et al., 1994; Palayoor et al., 1997). Compelling evidence from several investigators suggests that the tumorigenic growth of the prostate depends on the evasion of the normal homeostatic control mechanisms, where cell proliferation exceeds cell death (Berges et al., 1995; Tu et al., 1996). A general understanding of how cell proliferation and cell death are regulated by extracellular signals requires the identification of mechanisms underlying these processes. Additionally, pharmacological manipulation or genetic targeting of the major apoptosis regulators, such as the bcl-2 and caspase family of proteins, represent clinically attractive avenues for exploring effective therapeutic strategies for prostate cancer. Since cell proliferation follows an orderly
progression through the cell cycle, it is also of paramount importance to have an in-depth knowledge of the mechanisms that control cell division. This review will encompass the molecular alterations occurring in prostate cancer that affect the regulation of cell cycle progression and apoptosis induction, as well as the potential development of therapeutic interventions aimed at enhancing the apoptotic responses to treatment.

**Deregulation of apoptotic and proliferative pathways in prostate tumorigenesis**

**Regulation of cell proliferation and cell cycle progression**

Factors that govern cell proliferation must coordinate regulation two distinct processes: the cellular biosynthesis that drives accumulation of cell number and progression throughout the cell cycle. During the past several years, advances in our understanding of the cell cycle regulatory machinery have indicated that disruption of the normal cell cycle is a critical step in cancer development (Hartwell and Kastan, 1994; Sherr, 1996; Del Sal et al., 1996). Abnormalities of various components of the cell cycle have been identified in several types of human cancer (Arber et al., 1996; Hall and Peters, 1996; Porter et al., 1997; Thomas et al., 1998). As the major regulatory events leading to cell proliferation and differentiation occur within the G1 phase of the cell cycle, attention has been focused on altered expression of the G1 cyclins and cyclin-dependent kinases (Cdk) as key events in tumorigenesis (Del Sal et al., 1996).

The G1 cyclins, including three D-type cyclins and cyclin E, regulate the progression of cells through the G1 phase of the cell cycle through interactions with specific Cdks. Each of these cyclin/Cdk complexes is activated at a specific point during G1 and has a specific set of substrates. Cyclin E is a late G1 cyclin, which, along with its catalytic subunit Cdk2, is involved in phosphorylation of the Rb protein. The activation of the cyclin E/Cdk2 complex is the rate-limiting event for cell transition into the S phase of the cell cycle, and overexpression of cyclin E accelerates the G1 to S phase transition (Ohshubo et al., 1995; Porter et al., 1997; Oyama et al., 1998). The activity of the cyclinE/Cdk2 complex is primarily regulated by the Cip/Kip family of Cdk inhibitors (CKI), which include the p21WAF1, p27Kip1, and p57Kip2 proteins (Polyak et al., 1994; Sgambato et al., 1997).

Overexpression of p27Kip1 in mammalian cells induces a G1 block in the cell cycle. Conversely, defective regulation at the p27Kip1 checkpoint in the cycle can result in uncontrolled cellular proliferation. The key role of p27Kip1 in regulating cell proliferation is manifested in the p27-knockout mice, which exhibit gigantism, increased tumorigenesis and organ hyperplasia (Nakayama et al., 1996). Several lines of evidence from numerous studies demonstrated that loss or decreased p27Kip1 expression is a prognostic marker in several human malignancies including breast (Porter et al., 1997), colorectal (Ciaparrone et al., 1998), esophageal (Jiang et al., 1993; Singh et al., 1998), gastric (Mori et al., 1997), pulmonary carcinomas (Esposito et al., 1997), prostate (Guo et al., 1997; Cote et al., 1998; De Marzo et al., 1998; Thomas et al., 1998) and bladder (Del Pizzo et al., 1999). Interestingly enough, tumor-specific mutations of the p27Kip1 gene have not been identified in human cancers. Instead, the low levels of p27Kip1 in certain tumors have been attributed to tumor-specific enhanced proteosome-mediated degradation of the protein (Loda et al., 1997). Guo and co-workers were the first to demonstrate a correlation between loss of p27Kip1 protein expression and increasing tumor grade and proliferative status in prostate cancer (Guo et al., 1997). Other investigators subsequently described a significant association between loss of p27Kip1 immunoreactivity in prostate carcinoma and increased probability of recurrence and decreased survival (Cote et al., 1998). It was also postulated that down-regulation of p27Kip1 in secretory epithelial cells of high grade prostatic intraepithelial neoplasia (PIN), is responsible for the dramatically increased proliferation rate of these pre-malignant cells (De Marzo et al., 1998); this evidence implicates that absence of p27Kip1 removes a critical block in cell cycle progression in prostate tissue leading to the development of the malignant phenotype of prostate epithelial cells. Taken together these studies strongly support a role for p27Kip1 as a candidate prognostic marker for predicting disease progression and metastatic potential in prostate cancer patients.

Growth arrest also correlates with a dephosphorylation and activation of the retinoblastoma tumor suppressor gene (Rb), a transcriptional repressor that is targeted to cell cycle genes through interaction with the E2F family of transcription factors (Weinberg, 1995). Rb controls the transition of cells from G1 to S phase and its activity is modulated by G1-specific cyclin-dependent kinases that phosphorylate and inactivate Rb in the late G1 phase of the cell cycle. The Rb protein remains highly phosphorylated in G2 phase. Several lines of evidence indicating Rb mutations and altered patterns of pRb expression in a wide of human malignancies, have confirmed the concept the Rb gene is the prototype tumor suppressor gene (Cordon-Cardo, 1995). Such changes in the Rb gene have been associated with aggressive behavior and poor clinical outcome in specific tumors such as in bladder and lung cancer, and in prostate cancer Rb mutations have been characterized as primary events in the early phases of tumor progression (reviewed by Cordon-Cardo, 1995). In recent years, significant data linked Rb activity with apoptosis induction in diverse cellular settings (Morana et al., 1996; Wang et al., 1996). In the case of prostate, studies by Day and co-workers documented a functional role for Rb in signaling apoptosis in prostate cancer cells, by anchorage disruption and inducible overexpression and activation of protein kinase C (Day et al., 1997, 1999). Consequently the concept has been
promoted that inactivation of the Rb apoptotic pathway may be a critical regulatory control that is lost during the metastatic progression of prostate cancer.

**The p53 tumor suppressor gene**

The p53 tumor suppressor gene is as a transcription factor that functions in regulating cell cycle progression, DNA repair and apoptosis (Kastan et al., 1991; Soussi and May, 1996). In response to DNA damage, p53 functions to monitor genomic integrity and to reduce the occurrence of mutations, either by inhibiting the cell from entering the cell cycle enabling the cell to repair the DNA, or by triggering apoptosis (Levine et al., 1991; Montenarh, 1992; Vogelstein and Kintzler, 1992). The identification of p53-responsive genes that mediate these outcomes is a matter of widespread interest. In response to genotoxic damage, the p53-dependent transcriptional regulation of p21WAF-1/CIP1 mediates G1 cell-cycle arrest (Gartel et al., 1996). p21WAF-1/CIP1 inactivates cyclin-cdk complexes that target Rb phosphorylation (Weinberg, 1995). In this context, p21WAF-1/CIP1 serves as an effector of cell cycle arrest in response to p53 checkpoint activation pathway. It is believed that the growth deregulation produced by Rb inhibition is counteracted by apoptosis induction orchestrated by normal p53 function. These studies imply a potential link between p53 and Rb in cell cycle regulation, apoptosis and tumor progression. Additionally, wild-type p53 can transcriptionally upregulate bax and downregulate bcl-2 (Miyashita et al., 1994; Soussi and May, 1996). Therefore, p53 may regulate apoptosis by modulating the ratio of bcl-2 to bax, which has been established as an important determinant of apoptosis (Korsmeyer, 1995; Kroemer, 1997).

Diverse types of cancer possess somatic mutations in the p53 gene (Holstein et al., 1991). In the case of prostate cancer, immunohistochemical evidence suggests that the frequency of p53 mutations in tumors from radical prostatectomy specimens varies between 47-80% (Van Veldhuizen et al., 1993; Henke et al., 1994; Bauer et al., 1995; Strickler et al., 1996; Johnson et al., 1998). Additionally, clustered p53 immunoreactivity in localized prostate cancer correlates with a higher incidence of tumor recurrence compared to lesions which lacking p53 mutations (Yang et al., 1996). Significantly enough, recent evidence indicates that p53 mutations found in the primary tumor are clonally expanded in metastases and that these sites within the primary tumor define regions with high metastatic potential (Stapleton et al., 1997; Navone et al., 1999). Furthermore, loss of p53 can occur by loss heterozygosity on chromosome 17 which occurs in approximately 20% of prostate cancers (Nigro et al., 1989; Carter et al., 1990; Johnson and Hamdy, 1998). Moreover, p53 protein expression in localized primary prostate carcinomas has been correlated with higher Gleason score, pathological stage, and proliferation (Harris and Holstein, 1993; Heidenberg et al., 1996; Yang et al., 1996). In advanced prostate cancer, increases in p53 mutations occur with the highest incidence in androgen-independent tumors (Harris and Holstein, 1993; Thomas et al., 1993; Heidenberg et al., 1996; Yang et al., 1996). The overall evidence implicates p53 protein expression as an adverse prognostic indicator in prostate cancer.

At the mechanistic level, the contribution of p53 inactivation to the resistance of prostatic epithelial cells to the effects of androgen-deprivation remains highly controversial. It has been shown that the level of p53 mRNA and protein increases in the regressing rat ventral prostate following castration (Montenarh, 1992). As a result of the increased p53, the prostate epithelial cells re-enter into a defective cell cycle and apoptosis ensues (Colombel et al., 1992; Furuya et al., 1995). On the other hand, the increase in p53 has been attributed to DNA repair and not cell cycle events (Amundson et al., 1998). The role of p53 in apoptosis induction following castration in unclear since studies using p53-/- mice demonstrate that the increased p53 is not essential (Berges et al., 1993). Therefore, the p53 tumor suppressor gene may play key roles in the apoptotic response in a context dependent fashion where normal and malignant prostate cells can undergo apoptosis via both p53-dependent and -independent pathways (Berges et al., 1993; Borner et al., 1995).

**Transforming growth factor-β1: modulating prostate apoptosis and proliferation**

Induction of prostate apoptosis in the presence of physiological levels of androgens, depends on other signals being received by the cell that in addition to apoptosis, can also influence cell proliferation. Transforming growth factor-β1 (TGF-β1) is a multifunctional growth factor involved in the regulation of proliferation, extracellular matrix production and degradation, cell differentiation, and apoptosis induction (Wrana et al., 1994). TGF-β signaling results from the interaction of TGF-β with its cell surface receptors, type I (TβRI) and type II (TβRII) (Lin et al., 1992; Wieser et al., 1992). TGF-β selectively binds to TβRII which allows for recognition by TβRI. Once recognized, a stable ternary complex forms and the TGF-β signaling pathways are initiated by the mutual phosphorylation of TβRI and TβRII, both of which are serine/threonine kinases (Wrana et al., 1994). Loss of function or expression of TβRI and/or TβRII may contribute to the absence of TGF-β-mediated growth inhibition and apoptosis.

TGF-β1 has long been established as a physiological regulator of prostate growth via its ability to inhibit cell proliferation and induce apoptosis (Kyprianou and Isaacs, 1989; Martikainen et al., 1990). TGF-β1 may serve as an autocrine growth inhibitory factor in the normal and malignant prostate (Wilding et al., 1989). TGF-β1 can simultaneously inhibit normal prostatic epithelial cell proliferation and directly activate prostatic...
apoptosis in the presence of physiological levels of androgens (Kyprianou and Isaacs, 1988, Kyprianou et al., 1990; Sutkowski et al., 1992). Deregulation of TGF-β1 expression or loss of sensitivity to it effects, would abrogate both the anti-proliferative and apoptotic programs, leading to an enlarged population of prostate epithelial cells (Guo et al., 1997). While serving a growth-inhibitory/apoptotic role in the normal prostate, overproduction of TGF-β1 in prostate cancer may contribute to tumor progression. In accord with this concept, is data indicating an increase in epithelial TGF-β1 expression in benign and malignant prostate compared to the normal gland (Tu et al., 1996).

By virtue of the central regulatory role played by TGF-β1 in coordinating the normal processes of cell growth and differentiation, an imbalance in either production of and/or response to TGF-β1, results in serious perturbations of normal growth regulatory mechanisms that are involved in tumor development and progression (Glick et al., 1994; Markowitz et al., 1994). Escape from the negative growth control imposed by TGF-β1, could be due to either loss of specific receptors for TGF-β1, or alterations in the post-receptor signaling pathways of TGF-β1 (Geiser et al., 1992; Massague et al., 1994). The cellular circuitry that mediates the apoptotic signals of TGF-β1 may also involve oncogene and tumor suppressor gene products. TGF-β1 can suppress transcription of the c-myc protooncogene or it can retain the Rb protein in its unphosphorylated, growth suppressive state, in cells whose growth is inhibited by TGF-β1 (Laiho et al., 1990; Geiser et al., 1992). In addition, mutant p53 decreases the responsiveness of human epithelial cells to TGF-β1 probably by functioning as a checkpoint control of the cell cycle (Gerwin et al., 1992; Reiss et al., 1993). Growth suppression by TGF-β1 has been linked with a concomitant induction of prostate apoptosis, in normal and neoplastic prostate epithelial cells, evidence implicating TGF-β1 as a physiological apoptotic intermediate in the prostate (Martikainen et al., 1990; Hsing et al., 1996).

The intracellular events leading to TGF-β1 negative growth effect appear to include induction of inhibitors of cyclin-cyclin dependent kinase complexes, such as p27Kip1, p21WAF1/CIP1, and p15 INK4 (Hannon and Beach, 1994; Polvak et al., 1994). Mechanistically, p27Kip1 protein links TGF-β1 to cell cycle arrest, by being released from cyclin D1-Cdk4 complexes upon TGF-β1 treatment of TGF-β1 sensitive cells (Serrano et al., 1993; Polvak et al., 1994). This allows p27Kip1 to be associated with cyclin E-cdk2 complexes thus blocking its kinase activity (Polvak et al., 1994). Growing evidence points to the potential involvement of a dysfunctional TGF-β1 signaling pathway in prostate tumorigenesis. Recent studies from this laboratory, as well as by other investigators, have demonstrated a marked decrease in the expression of TGF-β1 receptors RI and RII in prostatic tumors, a loss that correlates with tumor grade (Williams et al., 1996; Guo et al., 1997). Enforced expression of TGF-β RII receptor in the LNCaP prostatic carcinoma cell line restores sensitivity to TGF-β1, which results in growth arrest and apoptosis induction (Guo and Kyprianou, 1998). More significantly, in vivo, these LNCaP TRII cells exhibit suppressed tumorigenicity through downregulation of bcl-2 expression and induction of caspase-1 expression (Guo and Kyprianou, 1999). Prostatic tumors with functional TGF-β1 receptors may harbor downstream defects that impair the intracellular transduction of TGF-β1 signal. Since our recent findings documented the involvement of cyclin-dependent kinase inhibitors, p21WAF1 and p27Kip-1 as post-receptor effectors transducing the TGF-β1 apoptotic signal in malignant prostate cells, these molecules represent critical intracellular targets for TGF-β1’s negative growth effects during prostate tumorigenesis (Guo and Kyprianou, 1998).

**Clinical significance of apoptosis in prostate cancer**

Bcl-2 was discovered as a proto-oncogene via its association with the t(14;18) chromosomal translocation characteristic of follicular lymphoma and its oncogenic potential confirmed in a transgenic mouse model of t(14;18) (Tsujimoto et al., 1984; Cleary and Sklar, 1985; McDonnell et al., 1989, 1991). Enforced bcl-2 expression resulted in suppression of apoptotic cell death, rather than from enhancement of cell proliferation (Vaux et al., 1988; Hockenbery et al., 1990). It is now known that the bcl-2 gene is a member of a multigene family where the members function either to inhibit or to promote apoptosis (Bruckheimer et al., 1998).

Members of the bcl-2 gene family all possess at least one bcl-2 homology domain and numerous bcl-2 family members have been identified based on these homologous regions (Oltvai et al., 1993; Tanaka et al., 1993; Yin et al., 1994, 1995; Hanada et al., 1995; Adams and Cory, 1998; Reed, 1998) (Fig. 1). Functionally the homology domains allow hetero- and homodimerization between the bcl-2 family members which regulates the apoptotic response (Korsmeyer et al., 1993; Yin et al., 1994; Yang et al., 1995b, Hanada et al., 1995; Sedlak et al., 1995; Adams and Cory, 1998; Reed, 1998). Additionally, the BH4 domain allows bcl-2 to interact with non-bcl-2 protein which may also regulate bcl-2 function Bcl-2 (Reed, 1998). Initially, it was considered that interactions between the bcl-2 family members with respect to homodimerization versus heterodimerization, determined the commitment to undergo cell death (Korsmeyer et al., 1993; Yin et al., 1994; Hanada et al., 1995; Sedlak et al., 1995; Yang et al., 1995b). However, individual bcl-2 family members have been shown to independently regulate apoptosis questioning the requirement for heterodimerization (Knudson and Korsmeyer, 1997). Lastly, caspase cleavage of bcl-2 results in a bax-like pro-apoptotic molecule (Cheng et al., 1997). Clearly bcl-2 and its fellow family members
Clinical significance of apoptosis in prostate cancer

play multiple roles in the regulation of apoptosis which involve complex interacting partners and signaling pathways. The protagonists of a common apoptotic scenario, bel-2 and bel-xL, play roles in blocking the loss of mitochondrial membrane potential, the release of cytochrome c, caspase activation, and Apaf-1 activation (Adams and Cory, 1998; Dragovich et al., 1998; Green and Reed, 1998; Minn et al., 1998; Nunez et al., 1998; Reed, 1998). Bel-xL binds to Apaf-1 thereby sequestering Apaf-1 from interaction with caspase 9 and cytochrome c which is inhibited by bak and bik, two pro-apoptotic family members (Nunez et al., 1998).

In normal prostate tissue, bcl-2 expression is restricted to the basal cells of the glandular epithelium and these cells are resistant to the effects of androgen deprivation (McDonnell et al., 1992; Colombel et al., 1993; Shabaik et al., 1995). Alternatively, the secretory epithelial cells, which lack detectable bcl-2 expression, undergo apoptosis following androgen deprivation (McDonnell et al., 1992; Colombel et al., 1993; Shabaik et al., 1995; Tu et al., 1996). Several investigators demonstrated that bcl-2 expression could contribute to prostate cancer progression following androgen ablation therapy (McDonnell et al., 1992; Colombel et al., 1993; Shabaik et al., 1995; Tu et al., 1996). Several in vivo studies demonstrated that bcl-2 overexpression could contribute to prostate cancer progression following androgen ablation therapy (McDonnell et al., 1992; Colombel et al., 1993; Shabaik et al., 1995; Apakama et al., 1996). Additional in vivo studies demonstrated that bcl-2 overexpressing prostate carcinoma cell lines were more resistant to apoptosis induction following hormone ablation when grown as tumor xenografts (Raffo et al., 1995; Westin et al., 1997; Beham et al., 1998). In patients with locally advanced, or metastatic prostate cancer receiving hormonal therapy, bcl-2 overexpression is an adverse prognostic indicator (Colombel et al., 1993; Shabaik et al., 1995; Apakama et al., 1996). Furthermore, bcl-2 expression in prostate carcinoma cell lines has been associated with decreased apoptotic response following treatment with various chemotherapeutic agents and ionizing radiation (Hermann et al., 1996; Kyprianou et al., 1997).

The bel-2 family members bel-xL, bax, and Mcl-1, are expressed in all tumors, however, the intensity of immunoreactivity was stronger in higher grade lesions (Krajewska et al., 1996).

The caspase family of proteases: signaling and executing apoptosis

The mechanism of apoptosis is remarkably conserved across species and executed by a cascade of sequential activation of initiator and effector caspases. The caspases are a family of cysteine proteases that are expressed as inactive pro-enzymes in normal, healthy cells; however, upon activation, the pro-enzyme is processed and the active heterotetramer is formed (Cohen, 1997; Wolf and Green, 1999). Active caspases selectively cleave target protein substrates at the carboxyl terminus of specific aspartate residues (Cohen, 1997; Wolf and Green, 1999). Within the caspase family, caspases-1, -2, -8, and -10 have been implicated in the

Molecular Alterations and Potential Therapeutic Interventions During Prostate Cancer Progression

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<th>Therapeutic Interventions:</th>
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<tr>
<td>WT p53</td>
<td>Hormone Ablation</td>
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**Fig. 1.** Summary of the molecular alterations and the potential therapeutic interventions during prostate cancer progression from androgen-dependent to androgen-independent disease.
initiation of apoptosis, whereas caspases-3, -6, -7, and -9 have been shown to be involved in the execution of apoptosis (Cohen, 1997; Wolf et al., 1999). The importance of caspase pro-domains in the regulation of caspase activity has recently become evident by the recognition of a family of adapter inhibitory apoptotic proteins (IAP), capable of binding the caspases and sequestering their proteolytic activity (Roy et al., 1997). Caspase activation involves a distinct, temporal cascade demonstrating a hierarchy within the caspase family (Slee et al., 1999). The activator caspase-8 is an integral component of the apoptosis death-inducing mechanism upstream and in receptor-mediated apoptosis activation of caspase-8 represents a cell death commitment point (Sun et al., 1999). Activation of caspase-1 is also believed to be an important step in apoptosis initiation (Troy et al., 1996). At a later stage in the apoptotic process, other effector caspases are activated that are responsible for the characteristic morphological features of the apoptotic phenotype.

Caspases cleave both structural proteins involved in cell architecture and functional proteins in cell cycle regulation and DNA repair. The inactivation of the DNA repair enzyme PARP by caspase-mediated cleavage, is a crucial event in the cellular commitment to undergo apoptosis (Lazebnik et al., 1994). Caspase-3 mediated cleavage of p21WAF1 re-directs the cells from undergoing growth arrest to apoptosis, leading to the acceleration of chemotherapy-induced apoptotic process in cancer cells (Zhang et al., 1999). An expanding body of recent evidence suggests that the caspase cascade is involved in the execution of apoptosis in prostate cancer cells in response to diverse stimuli, including lovastatin and Fas-mediated signaling, TGF-β1, and okadaic acid (Marcelli et al., 1998, 1999; Bowen et al., 1999; Guo and Kyprianou, 1999). In addition, blockade of caspase activity by CrmA has been shown to suppress androgen-ablation induced apoptosis in LNCaP prostate cancer cells in vitro and in vivo (Srikanth and Kraft, 1998). Several caspase inhibitors can suppress TGF-β mediated apoptosis and have been utilized to separate the anti-proliferative and apoptotic responses elicited by TGF-β (Chen and Chang, 1997; Brown et al., 1998). Certain proteins of the bcl-2 family functionally interact with caspases to block or amplify the apoptotic signal. Strong evidence exists to suggest that bcl-2 and bcl-xL prevent cytochrome c release from the mitochondria, which in turn prevents the activation of caspase-9 (Chen et al., 1997; Brown et al., 1998). Caspase-8 cleavage of the anti-apoptotic protein Bid into an active carboxyl fragment has been shown to induce cytochrome c release from the mitochondria in the fas-mediated apoptotic pathway (Li et al., 1998).

The mitochondrial connection

Mitochondria play a key part in the regulation and signaling of apoptosis (Wolf and Green, 1999). Indeed a variety of critical events in apoptotic signaling focus on the functional integrity of mitochondria, including, the release of cytochrome c, calcium efflux, disruption of electron transport and energy metabolism, the generation of reactive oxygen species and alterations in intracellular redox potential (Kroemer et al., 1997). Cytochrome c, which is usually present in the mitochondrial intermembrane space, is released into the cytosol following induction of apoptosis by different stimuli including chemotherapeutic and DNA damaging agents. The release of cytochrome c from the mitochondria and its subsequent binding to caspase-9 (resulting in the transactivation of procaspase 9 by Apaf-1) can trigger the sequential activation of caspase-3, an apoptosis executioner (Zou et al., 1999).

Targeting apoptosis for therapeutic interventions in prostate cancer

Androgen ablation therapy and radiotherapy: the standard therapeutic approaches

When prostate cancer metastasizes, patients typically undergo hormone-ablation therapy. It is important to understand what leads to this eventual failure in order to individualize therapy and develop new effective treatments for advanced androgen-independent prostate cancer (Bernier et al., 1993). The fact that the majority of prostate cancer cells maintain their sensitivity to androgens has important palliative implications. Castration-induced androgen ablation leads to tumor regression and apoptosis induction of androgen-dependent cell populations (Kyprianou et al., 1990; Kyprianou, 1997). This provides the molecular basis of why certain metastatic forms of prostate cancer can be controlled for months, even years, following androgen ablation. Failure of cells to undergo apoptosis in response to castration-induced androgen withdrawal, is the direct result of a biochemical defect in their initiation step in activating the apoptotic process. Androgen-independent cells however, after having escaped the killing effects of androgen ablation, maintain their capacity to undergo apoptosis in response to non- androgen ablative means (Kyprianou, 1994). Indeed, a rapidly growing body of evidence has established the use of chemotherapeutic agents capable of arresting androgen insensitive prostate cells in various proliferative phases of the cell cycle and subsequently inducing their apoptotic death (Furuya et al., 1994; Kyprianou, 1994; Eisman et al., 1998). Bcl-2 overexpression may allow prostate cancer cells to survive in the absence of androgens and suggests that hormone ablation therapy actually may select bcl-2 expressing, apoptosis resistant cells within tumor cell populations (McDonnell et al., 1992; Colombel et al., 1993). The ability of androgen-independent prostate cancer cells to undergo apoptosis has had profound therapeutic implications by targeting the pharmacological modulations of apoptosis for prostate
Clinical significance of apoptosis in prostate cancer

Radiation therapy, either as external beam irradiation or brachytherapy, has been proven to be relatively effective treatment for localized prostate cancer, with local tumor control and improvement of patient survival (Schellhammer, 1994). The cellular response to lethal doses of ionizing irradiation, by undergoing apoptosis, has been recognized as the molecular mechanisms of radiation-induced cell killing in various human tumor cells, including prostate cancer cells (Sklar et al., 1993; Palyoor et al., 1997; Rupnow et al., 1998). Loss of the apoptotic response to ionizing irradiation has therefore been linked to a radiation-resistant phenotype. Bcl-2 overexpression in human prostate cancer cells increases radiation resistance by delaying radiation-induced apoptosis (Kyprianou et al., 1997). At the clinical setting, we have recently demonstrated that prostate cancer patients with an elevated bcl-2/bax ratio have an increased risk of clinical failure to radiotherapy (Mackey et al., 1998). Thus, assessment of the expression profiles of these apoptotic players may have significant implications in the selection of treatment strategies and radioresistant tumors with high bcl-2/bax ratios will be candidates for more aggressive strategies. The involvement of the apoptotic status in predicting clinical radioresistance gains support from a more recent study indicating that bcl-2 and p53 expression in localized prostate cancer was associated with treatment failure to external beam radiation therapy (Scherr et al., 1999).

Pro-apoptotic gene directed therapy

New non-hormonal therapies have been traditionally used in hormone refractory disease, but none have been proven effective in increasing survival. Since bcl-2 expression is observed in the highly resistant hormone-independent prostate cancers, therapeutic strategies directed at suppressing bcl-2 function have been devised. One group has focused on decreasing bcl-2 expression by generating an adenovirally expressed hammerhead ribozyme targeting bcl-2 (Dorai et al., 1997, 1999). A different approach using anti-sense oligonucleotides (ODN) against bcl-2 has demonstrated a downregulation of bcl-2 expression, which occurred in a dose- and sequence-specific manner (Gleave et al., 1999; Miyake et al., 1999). In in vivo models with the androgen-dependent Shionogi breast cancer and LNCaP prostate cancer cell lines, the emergence of hormone-independent tumors was dramatically decreased by systemic administration of the anti-sense bcl-2 ODN (Gleave et al., 1999; Miyake et al., 1999). Therefore, bcl-2 provides a suitable target to suppress tumor growth by directly promoting apoptosis within the context of bcl-2 overexpressing tumors (Fig. 1).

Gene therapy using p53 adenoviral and retroviral constructs has demonstrated that overexpression of wild-type p53 will result in apoptosis in both in vivo and in vitro models and regression of tumors in lung cancer patients (Fujjwara et al., 1993; Roth et al., 1996). In prostate cancer, several studies documented that adenoviral p53 decreased cell viability and can inhibit tumor growth in nude mice (Eastham et al., 1995; Srivastava et al., 1995; Yang et al., 1995a; Blagosklonny et al., 1996; Ko et al., 1996; Bruckheimer et al., 1999). Currently, phase one trials for prostate cancer using adenoviral p53 are underway.

Caspase activation: apoptosis execution in a therapeutic setting

The role the caspases play at both the commitment and execution phases of apoptosis has recently led to the exploration and design of new therapeutic modalities for advanced prostate cancer. In theory, caspases can be targeted at two levels: gene expression (gene therapy), and post-translationally (zymogen cleavage and caspase activation). Gene therapy directed at common downstream effectors caspase-3 and caspase-7, should bypass intracellular checkpoint genes that limit apoptosis making the cell unable to escape its death. The downstream effector caspase, caspase-7, has been shown to be activated following apoptosis induction by several agents including lovastatin and okadaic acid in prostate cancer cells (Bowen et al., 1999; Marcelli et al., 1999). Of major significance is the observation that overexpression/direct activation of caspase-7 induces apoptosis in bcl-2 overexpressing LNCaP prostate cancer cells (Marcelli et al., 1999). Considering the significant role of bcl-2 expression in prostate cancer progression to androgen-independent disease (McDonnell et al., 1992) and since caspase activation occurs downstream from the bcl-2 checkpoint (Kroemer, 1997), these findings have important clinical implications, as they suggest that caspase-7 activation can induce apoptosis in bcl-2 overexpressing prostate tumors. One could thus promote caspase-7 as a potential therapeutic gene candidate for prostate cancer therapy.

Chemical-induced dimerization (CID) of transfected caspase pro-forms may also prove an effective therapeutic strategy for androgen-independent prostatic tumors. The "proximity" model of transproteolysis for caspase activation supports that notion that the weak proteolytic activity of two procaspases being brought into close contact is sufficient to cleave one another, form active enzymes, and thereby begin the proteolytic cascade (Stellar, 1998). This model of chemical induced dimerization using modified pro-caspase molecules, termed artificial death switches, is being considered as a potential anti-cancer treatment (MacCorkle et al., 1998). Moreover, direct activation of caspases, through CID within the human prostate smooth muscle cells has been implicated as a potential effective treatment for benign prostatic hyperplasia (Slawin et al., 1998). This directly provides the rationale for designing conditional alleles based on CID and implementing chemical induced apoptosis as a novel and effective therapeutic strategy for prostate cancer.

Recent immunohistochemical studies from this
laboratory have demonstrated that a significant decrease in caspase-1 protein expression correlates with prostate tumor progression in prostate cancer patients (Newman et al., 1999). If prostate cancer cells require de novo synthesis of caspasas, timing the coordination of administration of chemotherapeutic agents that inhibit transcription or translation with those that induce apoptosis, could prove critical in determining the effectiveness of therapy. The ability of certain agents to increase expression of caspasas and subsequently prime cells for apoptosis may prove effective as adjuvant-based therapy (Fig. 1). Studies in our laboratory demonstrated the potential of the prostate growth modulator TGF-81 to induce prostate cancer cell apoptosis via upregulation of caspase-1 expression (Guo and Kyprianou, 1999). Furthermore, it has recently been reported that induction of apoptosis in androgen-independent prostate cancer cells in response to FTY720, a fungal-derived metabolite requires caspase-3 activation (Wang et al., 1999). Finally, recent evidence points to the mitochondrial respiratory chain as a functionally powerful therapeutic target for androgen-independent prostate cancer (Joshi et al., 1999). Considering that there is a disruption in the pro- and antioxidant balance during prostate cancer progression, these findings gain high clinical significance in targeting the electron transport chain of the mitochondria for the treatment of advanced prostate cancer.

Fas antigen/CD95 is a unique cell surface receptor protein that can initiate certain intracellular signaling pathways leading to apoptosis when engaged by its natural ligand (Fas), or when non-specifically activated by divalent antibodies against its internal domain (Nagata, 1996). The interaction of Fas with Fas ligand, FasL, leads to the aggregation of Fas cytoplasmic domains (DD) and increases the affinity of the Fas DD for the Fas intracellular domains, the adapter molecule FADD (Fas-associated with death domain) (MORT1). The end result of this Fas activation process is an unmasking of the proteolytic activity of caspase-8, an effector component of the apoptotic machinery. Probably by transproteolysis, the aggregation of caspase-8 contributes to the initiation of a protease cascade that includes caspase-1 and caspase-3, ultimately leading to the irreversible cleavage of multiple proapoptotic targets. Mitochondrial derived factors, primarily cytochrome c, seem to be essential for the activation of the most downstream members of this protease-death cascade, including caspases-3, -7 and -9.

The significance of the Fas antigen signaling cascade in prostate apoptosis has been demonstrated in the normal rat prostate following castration-induced apoptosis (de la Taille et al., 1999). Furthermore, evidence emerging from several studies implicates activation of the Fas-Fas ligand pathway in sensitizing androgen-independent human prostate cancer cells to undergo apoptosis in response to various chemotherapeutic agents (Roklin et al., 1998; Costa and Cotter, 1999), as well as lymphocyte-mediated cell killing (Frost et al., 1997). Thus if prostate epithelial cells harbor an intact Fas signaling pathway, sensitization of androgen-independent tumors to anti-Fas-induced apoptosis becomes an appealing therapeutic target with potential clinical application in the effective treatment of advanced prostate cancer.

References


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Clinical significance of apoptosis in prostate cancer


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