Histol Histopathol (2000) 15: 1211-1223

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Cellular and Molecular Biology

Invited Review

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

N. Kyprianou, E.M. Bruckheimer and Y. Guo

Division of Urology, and Department of Molecular Biology and Bicohemistry, University of Maryland School of Medicine, Baltimore, Maryland, USA

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 equlibrium, 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

Offprint requests to: Dr. Natasha Kyprianou, University of Maryland School of Medicine, Division of Urology, Department of Surgery, 22, South Greene Street, Baltimore, MD 21201. USA. Fax: 410-706-0311. e-mail: nkyprianou@smail.umaryland.edu

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 coordinately regulate 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 G_1 phase of the cell cycle, attention has been focused on altered expression of the G_1 cyclins and cyclin-dependent kinases (Cdk) as key events in tumorigenesis (Del Sal et al., 1996).

The G_1 cyclins, including three D-type cyclins and cyclin E, regulate the progression of cells through the G_1 phase of the cell cycle through interactions with specific Cdks. Each of these cyclin/Cdks complexes is activated at a specific point during G_1 and has a specific set of substrates. Cyclin E is a late G_1 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 G_1 to S phase transition (Ohtsubo 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 $p21^{WAF-1}$, $p27^{Kip1}$, and $p57^{Kip2}$ proteins (Polyak et al., 1994; Sgambato et al., 1997).

Overexpression of $p27^{Kip1}$ in mammalian cells induces a G_1 block in the cell cycle. Conversely, defective regulation at the $p27^{Kip1}$ checkpoint in the cycle can result in uncontrolled cellular proliferation. The key role of $p27^{Kip1}$ 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 $p27^{Kip1}$ 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 $p27^{Kip1}$ gene have not been identified in human cancers. Instead, the low levels of $p27^{Kip1}$ 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 $p27^{Kip1}$ 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 $p27^{Kip1}$ immunoreactivity in prostate carcinoma and increased probability of recurrence and decreased survival (Cote et al., 1998). It was also postulated that down-regulation of $p27^{Kip1}$ 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 $p27^{Kip1}$ 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 $p27^{Kip1}$ as a candidate prognostic marker for predicting disease progression and metastatic potential in prostate cancer patients.

Growth arrest also correlates with a de-phosphorylation 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 G_1 to S phase and its activity is modulated by G_1-specific cyclin dependent kinases that phosphorylate and inactivate Rb in the late G_1 phase of the cell cycle. The Rb protein remains highly phosphorylated in G_2 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 supprerssor 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 or inducible over-expression 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-reponsive genes that mediate these outcomes is a matter of widespread interest. In response to genotoxic damage, the p53-dependent transcriptional regulation of $p21^{WAF-1/CIP1}$ mediates G_1 cell-cycle arrest (Gartel et al., 1996). $p21^{WAF-1/CIP1}$ inactivates cyclin-cdk complexes that target Rb phosphorylation (Weinberg, 1995). In this context, $p21^{WAF-1/CIP1}$ 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 (Hollstein 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 Hollstein, 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 Hollstein, 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-ß1: 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-ß1 (TGF-ß1) 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-ß signaling results from the interaction of TGF-ß with its cell surface receptors, type I (TßRI) and type II (TßRII) (Lin et al., 1992; Wieser et al., 1993). TGF-ß selectively binds to TßRII which allows for recognition by TßRI. Once recognized, a stable ternary complex forms and the TGF-ß signaling pathways are initiated by the mutual phosphorylation of TßRI and TßRII, both of which are serine/threonine kinases (Wrana et al., 1994). Loss of function or expression of TßRI and/or TßRII may contribute to the absence of TGF-ß-mediated growth inhibition and apoptosis.

TGF-ß1 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-ß1 may serve as an autocrine growth inhibitory factor in the normal and malignant prostate (Wilding et al., 1989). TGF-ß1 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-ß1 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-ß1 in prostate cancer may contribute to tumor progression. In accord with this concept, is data indicating an increase in epithelial TGF-ß1 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-ß1 in coordinating the normal processes of cell growth and differentiation, an imbalance in either production of and/or response to TGF-ß1, 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-ß1, could be due to either loss of specific receptors for TGF-ß1, or alterations in the post-receptor signaling pathways of TGF-ß1 (Geiser et al., 1992; Massague et al., 1994). The cellular circuitry that mediates the apoptotic signals of TGF-ß1 may also involve oncogene and tumor suppressor gene products. TGF-ß1 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-ß1 (Laiho et al., 1990; Geiser et al., 1992). In addition, mutant p53 decreases the responsiveness of human epithelial cells to TGF-ß1 probably by functioning as a checkpoint control of the cell cycle (Gerwin et al., 1992; Reiss et al., 1993). Growth suppression by TGF-ß1 has been linked with a concomitant induction of prostate apoptosis, in normal and neoplastic prostate epithelial cells, evidence implicating TGF-ß1 as a physiological apoptotic intermediate in the prostate (Martikainen et al., 1990; Hsing et al., 1996).

The intracellular events leading to TGF-ß1 negative growth effect appear to include induction of inhibitors of cyclin-cyclin dependent kinase complexes, such as $p27^{Kip1}$, $p21^{WAF-1/CIP1}$, and p15 INK4 (Hannon and Beach, 1994; Polyak et al., 1994). Mechanistically, $p27^{Kip1}$ protein links TGF-ß1 to cell cycle arrest, by being released from cyclin D1-Cdk4 complexes upon TGF-ß1 treatment of TGF-ß1 sensitive cells (Serrano et al., 1993; Polyak et al., 1994). This allows $p27^{Kip1}$ to be associated with cyclin E-cdk2 complexes thus blocking its kinase activity (Polyak et al., 1994). Growing evidence points to the potential involvement of a dysfunctional TGF-ß1signaling 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-ß1 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-ß RII receptor in the LNCaP prostatic carcinoma cell line restores sensitivity to TGF-ß1, which results in growth arrest and apoptosis induction (Guo and Kyprianou, 1998). More significantly, in vivo, these LNCaP TßRII 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-ß1 receptors may harbor downstream defects that impair the intracellular transduction of TGF-ß1 signal. Since our recent findings documented the involvement of cyclin-dependent kinase inhibitors, $p21^{WAF-1}$ and p27Kip-1 as post-receptor effectors transducing the TGF-ß1 apoptotic signal in malignant prostate cells, these molecules represent critical intracellular targets for TGF-ß1's negative growth effects during prostate tumorigenesis (Guo and Kyprianou, 1998).

The Bcl-2 familty of proteins: multifunctional regulators of apoptotic pathways

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

play multiple roles in the regulation of apoptosis which involve complex interacting partners and signaling pathways. The protagonists of a common apoptotic scenario, bcl-2 and bcl-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). Bcl-xL binds to Apaf-1 thereby sequestering Apaf-1 from interaction with caspase 9 and cytochrome 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; Apakama et al., 1996). Additional in vivo studies demonstrated that bcl-2 overexpressing prostate carcinoma cell 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 bcl-2 family members bcl-x_L, 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

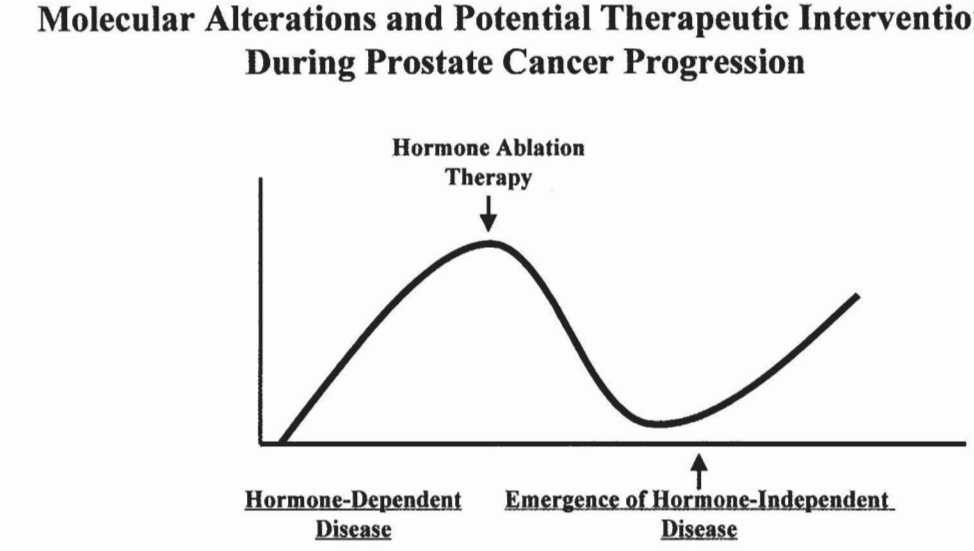

Fig. 1. Summary of the molecular alterations and the potential therapeutic interventions during prostate cancer progression from androgen-dependent to androgen-independent disease.

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), cabable 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 $p21^{WAF-1}$ 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-ß1, 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-ß mediated apoptosis and have been utilized to separate the anti-proliferative and apoptotic responses elicited by TGF-ß (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-x_L prevent the activation of the caspase cascade by blocking 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 (Berner 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; Eiseman 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

tumors in the androgen-independent state (Fig. 1).

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 (Fujiwara 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 excecution 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 caspases, 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 caspases 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-ß1 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

Adams J.M. and Cory S. (1998) The bcl-2 protein family: arbiters of cell survival. Science 281, 1322-1326.

Apakama I., Robinson M.C., Walter N.M., Charlton R.G., Royds J.A., Fuller C.E., Neal D.E. and Hamdy F.C. (1996). Bcl-2 overexpression combined with p53 protein accumulation correlates with hormone-refractory prostate cancer. Br. J. Cancer, 74, 1258-1262.

Amundson S.A., Myers T.G. and Fornace A.J. (1998). Roles for p53 in growth arrest and apoptosis: putting on the brakes after genotoxic stress. Oncogene 17, 3287-3299.

Arber N., Hibshoosh H., Moss S.F., Sutter T., Zhang Y., Begg M., Wang S., Weinstein I.B. and Holt P.R. (1996). Increased expression of cyclin D1 is an early event in multistage colorectal carcinogenesis. Gastroenterology 110, 669-674.

Bauer J.J., Sesterhenn I.A., Mostofi F.K., McLeod D.G., Srivastava S. and Moul J.W. (1995). p53 nuclear protein expression is an independent prognostic marker in clinically localized prostate cancer patients undergoing radical prostatectomy. Clin. Cancer Res. 1, 1295-1300.

Beham A.W., Sarkiss M., Brisbay S., Tu S.-M., von Eschenbach A.C. and McDonnell T.J. (1998). Molecular correlates of bcl-2-enhanced growth following androgen-ablation in prostate carcinoma cells in vivo. Int. J. Mol. Med. 1, 953-959.

Berges R.R., Furuya Y., Remington L., English H.F., Jacks T. and Isaacs J.T. (1993). Cell proliferation, DNA repair and p53 function are not required for programmed cell death of prostatic glandular cells induced by androgen ablation. Proc. Natl. Acad. Sci. USA 90, 8910-8914.

Berges R.R., Vukanovic J., Epstein J.I., CarMichel M., Cisek L., Johnson D.E., Veltri R.W., Walsh P.C. and Isaacs J.T. (1995). Implication of cell kinetic changes during the progression of human prostatic cancer. Clin. Cancer Res. 1, 473-480.

Berner A., Nesland J.M., Waehre H., Silde J. and Fossa S.D. (1993). Hormone resistant prostatic adenocarcinoma: an evaluation of prognostic factors in pre-and post-treatment specimens. Br. J. Cancer 68, 380-384.

Blagosklonny M.V., Schulte T., Nguyen P., Trepel J. and Neckers L.M. (1996). Taxol-induced apoptosis and phosphorylation of Bcl-2 protein involves c-Raf-1 and represents a novel c-Raf-1 signal transduction pathway. Cancer Res. 56, 1851-1854.

Borner M.M., Myers C.E., Sartor O., Sei Y., Toko T., Tropel J.B. and Schneider E. (1995). Drug-induced apoptosis is not necessarily dependent on macromolecular synthesis of proliferation in the p53-negative human prostate cell line PC3. Cancer Res. 55, 2122-2128.

Bowen C., Voeller H.J., Kikly K. and Gelmann E.P. (1999). Synthesis of pro-caspase-3 and -7 during apoptosis in prostate cancer cells. Cell. Death Differ. 6, 394-401.

Brown T.L., Patil S., Basnett R.K. and Howe P.H. (1998). Caspase inhibitor BD-fmk distinguishes transforming growth factor ß-induced apoptosis from growth inhibition. Cell. Growth Dev. 9, 869-875.

Bruckheimer E.M., Cho S.H., Sarkiss M., Herrmann J. and McDonnell

T.J. (1998). The bcl-2 gene family and apoptosis. Adv. Biochem. Eng. Biotechnol. 62, 75-105.

Bruckheimer E.M., Schumacher G., Beham A., Honda T., Brisbay S.M., Roth J.A., Logothetis C. and McDonnell T.J. (1999) Molecular determinants of cell death induction following adenoviral-mediated gene transfer of wild-type p53 in prostate cancer cells. (Submitted for publication).

Carter B.S., Ewing C.M., Ward W.S., Treiger B.F., Alders T.W., Schalken J.A., Epstein J.I. and Isaacs W.B. (1990). Allelic loss of chromosomes 16q and 10q in human prostate cancer. Proc. Natl. Acad. Sci. USA 87, 8751-8755.

Chen R.-H. and Chang T.-Y. (1997). Involvement of caspase family proteases in transforming growth factor-ß-induced apoptosis. Cell Growth Diff. 8, 821-827.

Cheng E.H., Kirsch D.G., Clem R.J., Ravi R., Kastan M.B., Bedi A., Ueno K. and Hardwick J.M. (1997). Conversion of Bcl-2 to a Bax-like death effector by caspases. Science 278, 1966-1968.

Ciaparrone M., Yamamoto H., Yao Y., Sgambato A., Cattoretti G., Tomita N., Monden T., Rotterdam H. and Weinstein I.B. (1998). Localization and expression of $p27^{Kip1}$ in multistage colorectal carcinogenesis. Cancer Res, 58, 114-122.

Cleary M.L. and Sklar J.C.M. (1985). Nucleotide sequence of a t(14;18) chromosomal breakpoint in follicular lymphoma and demonstration of a breakpoint-cluster region near a transcriptionally active locus on chromosome 18. Proc. Natl. Acad. Sci. USA 82, 7439-7443.

Cohen G.M. (1997). Caspases: the executioners of apoptosis. Biochem. J. 326, 1-16.

Colombel M., Olsson C.A., Ng P.Y. and Buttyan R. (1992). Hormone-regulated apoptosis results from reentry of differentiated prostate cells onto a defective cell cycle. Cancer Res. 52, 4313-4319.

Colombel M., Symmans F., Gil S., O'Toole K.M., Chopin D., Benson M., Olsson, C.A., Korsmeyer S. and Buttyan R. (1993). Detection of the apoptosis-suppressing oncoprotein bcl-2 in hormone-refractory human prostate cancers. Am. J. Pathol. 143, 390-400.

Cordon-Cardo C. (1995). Mutations of cell cycle regulators. Biological and clinical implications for human neoplasia. Am. J. Pathol. 147, 545-560.

Costa P.A. and Cotter T.G. (1999). Camptothecin sensitizes androgen-independent prostate cancer cells to anti-fas induced apoptosis. Br. J. Cancer 80, 371-377.

Cote R.J., Shi Y., Groshen S., Feng A.C., Cordon-Cardo C., Skinner D. and Lieskovosky G. (1998). Association of $p27^{Kip1}$ levels with recurrence and survival in patients with stage C prostate carcinoma. J. Natl. Cancer Inst. 90, 916-920.

Crawford E.D. (1989). Combined androgen blockade. Urology 34, 22-26.

Day M.L., Foster R.G., Day K.C., Zhao X., Humphrey P., Swanson P., Postigo A.A., Zhang S.H. and Dean D.C. (1997). Cell anchorage regulates apoptosis through the retinoblastoma tumor suppressor/ E2F pathway. J. Biol. Chem. 272, 8125-8128.

Day M.L., Zhao X., Vallorosi C.J., Putzi M., Powell C.T., Lin C. and Day K.C. (1999). E-cadherin mediates aggregation-dependent survival of prostate and mammary epithelial cells through the retinoblastoma cell cycle control pathway. J. Biol. Chem. 274, 9656-9664.

de la Taille A., Chen M.W., Shabsigh A., Bagiella E., Kiss A. and Buttyan R. (1999). Fas antigen/CD-95 upregulation and activation during castration-induced regression of the rat ventral prostate gland. Prostate 40, 89-96.

De Marzo A.M., Meeker A.K., Epstein J.I., Coffey D.S. (1998).Prostate stem cell compartments: expression of the cell cycle inhibitor $p27^{Kip1}$ in normal, hyperplastic, and neoplastic cells. Am. J. Pathol. 153, 911-919.

Del Pizzo J.J., Borkowski A., Jacobs S.C. and Kyprianou N. (1999). Loss of cell cycle regulators $p27^{Kip1}$ and cyclin E in transitional cell carcinoma of the bladder correlates with tumor grade and patient survival. Am. J. Pathol. 155, 1129-1136.

Del Sal G., Loda M. and Pagano M. (1996). Cell cycle and cancer: critical events at the G_1 restriction point. Crit. Rev. Oncol. 7, 127-142.

Dorai T., Olsson C.A., Katz A.E. and Buttyan R. (1997). Development of a hammerhead ribozyme against bcl-2. Preliminanry evaluation of a potential gene therapeutic agent for hormone-refractory human prostate cancer. Prostate 32, 246-258.

Dorai T., Perlman H., Walsh K., Shabsigh A., Goluboff E.T., Olsson C.A. and Buttyan R. (1999). A recombinant defective adenoviral agent expressing anti-bcl-2 ribozyme promotes apoptosis of bcl-2 expressing human prostate cancer cells. Int. J. Cancer 82, 846-85.

Dragovich S., Rudin C.M. and Thompson C.B. (1998). Signal transduction pathways that regulate cell survival and cell death. Oncogene 17, 3207-3213.

Eastham J.A., Hall S.J., Sehgal I., Wang J., Timme T.L., Yang G., Connell-Crowely L., Elledge S.J., Zhang W.-W., Harper J.W. and Thompson T.C. (1995). In vivo gene therapy with p53 or p21 adenovirus for prostate cancer. Cancer Res. 55, 5151-5155.

Eiseman J.L., Rogers F.A., Guo Y., Kauffman J., Sentz D.L., Klinger M.F., Callery P.S. and Kyprianou N. (1998). Tumor-targeted apoptosis by a novel spermine analogue, 1,12-diaziridinyl-4,9-diazadodecane, results in therapeutic efficacy and enhanced radiosensitivity of human prostate cancer. Cancer Res. 58, 4864-4870.

Esposito V., Baldi A., De Luca A., Groger A.M., Loda M., Giordano G.G., Caputi M., Baldi, F., Pagano M. and Giordano A. (1997). Prognostic role of the cyclin-dependent kinase inhibitor p27 in non-small cell lung cancer. Cancer Res. 57, 3381-3385.

Frost P., Ng C.P., Belldegrun A. and Bonavida B. (1997). Immuno-sensitization of prostate carcinoma cell lines for lymphocytes (CTL, TIL, LAK0-mediated apoptosis via the the Fas-Fas-ligand pathway of cytotoxicity. Cell. Immunol, 180, 70-76.

Fujiwara T., Grimm E.A., Mukhopadhyay T., Cai D.W., Owen-Schaub L.B. and Roth J.A. (1993). A retroviral wild-type p53 expression vector penetrates human lung cancer spheroids and inhibits growth by apoptosis. Cancer Res. 53, 4129-4133.

Furuya Y., Ludmo P., Short A.D., Gill D.L. and Isaacs J.T. (1994). The calcium. pH, and cell proliferation in the programmed (apoptotic) death of androgen-independent human prostate cancer cells induced by thapsigargin. Cancer Res. 54, 6167-6175.

Furuya Y., Lin X.S., Walsh J.C., Nelson W.G. and Isaacs J.T. (1995). Androgen ablation-induced programmed death of prostatic glandular cells does not involve recruitment into a defective cell cycle or p53 induction. Endocrinology 136, 1898-1906.

Gartel A.L., Serfas M.S. and Tyner A.L. (1996). p21-negative regulator of the cell cycle. Proc. Soc. Exp. Biol. Med. 213, 138-149.

Geiser A.G., Burmester J.K., Webbink R., Roberts A.B. and Sporn M.B. (1992). Inhibition of growth by transforming growth factor-ß following fusion of two non-responsive human carcinoma cell lines. J. Biol. Chem. 267, 2588-2593.

Gerwin B.I., Spillare E., Forrester K., Lehman T.A., Kispert J., Welsh J.A., Pfeifer A.M., Lechner J.F., Baker S.J., Vogelstein B. and Harris

C.C. (1992). Mutant p53 can induce tumorigenic conversion of human bronchial epithelial cells and reduce their responsiveness to a negative growth factor, transforming growth factor beta 1. Proc. Natl. Acad. Sci. USA 89, 2759-2763.

Gleave M., Tolcher A., Miyake H., Nelson C., Brown B., Beraldi E. and Goldie J. (1999). Progression to androgen independence is delayed by adjuvant treatment with antisense bcl-2 oligonucleotides after castraton in the LNCaP prostate tumor model. Clin. Cancer Res. 5, 2891-2898.

Glick A.B., Lee M.M., Darwiche N., Kulkarni A.B., Karlsson S. and Yuspa S.H. (1994). Targeted deletion of the TGF-beta 1 gene causes rapid progression to squamous cell carcinoma. Genes Dev. 8, 2429-2440.

Green D.R. and Reed J.C. (1998). Mitochondria and apoptosis. Science 281, 1309-1312.

Guo Y. and Kyprianou N. (1998). Overexpression of transforming growth factor (TGF) ß1 type II receptor restores TGF-ß1 sensitivity and signaling in human prostate cancer cells. Cell. Growth Diff. 9, 185-193.

Guo Y. and Kyprianou N. (1999). Restoration of transforming growth factor ß signaling pathway in human prostate cancer cells suppresses tumorigenecity via induction of caspase-1-mediated apoptosis. Cancer Res. 59, 1366-1371.

Guo Y., Jacobs S.C. and Kyprianou N. (1997). Down-regulation of protein and mRNA expression for transforming growth factor-ß (TGF-ß) type I and type II receptors in human prostate cancer. Int. J. Cancer 71, 573-579.

Hakem R., Hakem A., Duncan G.S., Henderson J.T., Woo M., Soengas M.S., Elia A., de la Pompa J.L., Kagi D., Khoo W., Potter J., Yoshida R., Kaufman S.A., Lowe S.W., Penninger J.M. and Mak T.W. (1998). Differential requirement for caspase 9 in apoptotic pathways in vivo. Cell 94, 339-352.

Hall M. and Peters G. (1996). Genetic alterations of cyclins, cyclin dependent kinases, and Cdk inhibitors in human cancer. Adv. Cancer Res. 68, 67-108.

Hanada M., Aime-Sempe C., Sato T. and Reed J.C. (1995). Structure-function analysis of bcl-2 protein: identification of conserved domains important for homodimerization with bcl-2 and heterodimerization with bax. J. Biol. Chem. 270, 11962-11969.

Hannon G.J. and Beach D. (1994). p15INK4B is a potential effector of TGF-beta-induced cell cycle arrest. Nature 371, 257-261.

Harris C.C. and Hollstein M.C. (1993). Clinical implications of the p53 tumor suppressor gene. N Engl. J. Med. 328, 1318-1327.

Hartwell L.H. and Kastan M.B. (1994). Cell cycle control and cancer. Science 266, 1821-1828.

Heidenberg H.B., Bauer J.J., McLeod D.G., Moul J.W. and Srivastava S. (1996). The role of the p53 tumor suppressor gene in prostate cancer: a possible biomarker? Urology 48, 971-979.

Henke R.P., Kruger E., Ayhan N., Hubner D., Hammerer P. and Huland H. (1994). Immunohistochemical detection of p53 protein in human prostatic cancer. J. Urol. 152, 1296-1301.

Herrmann J.L., Bruckheimer E.M. and McDonnell T.J. (1996). Cell death signal transduction and bcl-2 function. Biochem. Soc. Trans. 24, 1059-1066.

Hockenbery D., Nunez G., Milliman C., Schreiber R.D. and Korsmeyer S.J. (1990). Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature 348, 334-336.

Hollstein M., Sidransky D., Vogelstein B. and Harris C.C. (1991). P53 mutations in human cancers. Science 253, 492-495.

Hsing A.Y., Kadomatsu K., Bonham M.J. and Danielpour D. (1996). Regulation of apoptosis induced by transforming growth factor-beta1 in non-tumorigenic rat prostatic epithelial cell lines. Cancer Res. 56, 5146-5149.

Isaacs J.T., Furuya Y. and Berges R. (1994). The role of androgen in the regulation of programmed cell death/apoptosis in normal and malignant prostatic tissue. Semin. Cancer Biol. 5, 391-400.

Jiang W., Zhang Y.J., Kahn S.M., Hollenstein M.C., Santella R.M., Lu S.H., Harris C.C., Montesano R. and Weinstein I.B. (1993). Altered expression of the cyclin D1 and retinoblastoma genes in human esophageal cancer. Proc. Natl. Acad. Sci. USA 90, 9026-9030.

Johnson M.I. and Hamdy F.C. (1998). Apoptosis regulating genes in prostate cancer. Oncol. Rep. 5, 553-557.

Joshi B., Li L., Taffe B.G., Zhu Z., Wahl S., Tian H., Ben-Josef E., Taylor J.D., Porter A.T. and Tang D.G. (1999). Apoptosis induction by a novel anti-prostate cancer compound, BMD1888 (a fatty acid-containingt hydroxamic acid), requires the mitochondrial respiratory chain. Cancer Res. 59, 4343-4355.

Kastan M.B., Onyekwere O., Sidransky D., Vogelstein B. and Craig R.W. (1991). Participation of p53 protein in the cellular response to DNA damage. Cancer Res. 51, 6304-6311.

Knudson M.C. and Korsmeyer S.J. (1997). Bcl-2 and bax function independently to regulate cell death. Nature Genet.16, 358-363.

Ko S.-C., Gotoh A., Thalmann G.N., Zhau H.E., Johnston D.A., Zhang W.-W., Kao C. and Chung L.W.K. (1996). Molecular therapy with recombinant p53 adenovirus in an androgen-independent, metastatic human prostate cancer model. Human Gene Ther. 7, 1683-1691.

Korsmeyer S.J., Shutter J.R., Veis D.J., Merry D.E. and Oltvai Z.N. (1993). Bcl-2/Bax: a rheostat that regulates an anti-oxidant pathway and cell death. Sem. Cancer Biol. 4, 327-332.

Korsmeyer S.J. (1995). Regulators of cell death. Trends Genet. 11, 101-105.

Krajewska M., Krajewski S., Epstein J.I., Shabaik A., Sauvageot J., Song K., Kitada S. and Reed J.C. (1996). Immunohistochemical analysis of bcl-2, bax, bcl-X, and mcl-1 expression in prostate cancer. Am. J. Pathol. 148, 1567-1576.

Kroemer G. (1997). The proto-oncogene bcl-2 and its role in regulating apoptosis. Nature Med. 3, 614-620.

Kroemer G., Zamzani N. and Susin S.A. (1997). Mitochondrial control of apoptosis. Immunol. Today 18, 44-45.

Kuida K., Haydar T.F., Kuan C.Y., Gu Y., Taya C., Karasuyama H., Su M.S., Rakic P. and Flavell R.A. (1998). Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. Cell 94, 325-337.

Kyprianou N. (1994). Apoptosis: therapeutic significance in the treatment of androgen-dependent and androgen-independent prostate cancer. World J. Urol. 12, 299-303.

Kyprianou N. and Isaacs J.T. (1988). Activation of programmed cell death in the rat ventral prostate after castration. Endocrinology 122, 552-562.

Kyprianou N. and Isaacs J.T. (1989). Expression of transforming growth factor-beta in the rat ventral prostate during castration-induced programmed cell death. Mol. Endocrinol. 3, 1515-1522.

Kyprianou N., English H. and Isaacs J.T. (1990). Programmed cell death during regression of PC-82 human prostate cancer following androgen ablation. Cancer Res. 50, 3748-3753.

Kyprianou N., King E.D., Bradbury D. and Rhee J.G. (1997). Bcl-2 over-expression delays radiation-induced apoptosis without affecting the

clonogenic survival of human prostate cancer cells. Int. J. Cancer 70, 341-348.

Laiho M., DeCaprio J.A., Ludlow J.W., Livingston D.M. and Massague J. (1990). Growth inhibition by TGF-beta linked to suppression of retinoblastoma protein phosphorylation. Cell 62, 175-185.

Landis S.H., Murray T., Bolden S. and Wingo P.A. (1999). Cancer statistics, 1999. CA Cancer J. Clin. 49, 8-31.

Lazebnik Y.A., Kaufmann S.H., Desnoyers S., Poirier G.G. and Earnshaw W.C. (1994). Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties like ICE. Nature 371, 346-347.

Lezefsky M.J., Leibel S.A., Bruman C.M., Kuthener G.J., Harrison A., Happerest L. and Fuks Z. (1994). Neoadjuvant hormonal therapy improves the therapeutic ration in patients with bulky prostatic disease treated with three dimensional conformal therapy. Int. J. Rad. Oncol. Biol. Phys. 29, 755-761.

Levine A.J., Momand J. and Finlay C.A. (1991). The p53 tumor suppressor gene. Nature 351, 453-456.

Li H., Zou H., Slaughter C. and Wang X. (1998). Cleavage of BID by caspase-8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell 94, 491-502.

Lin H.Y., Wang X., Ng-Eaton E., Weinberg R.A. and Lodish H.F. (1992). Expression cloning of the TGF-ß type II receptor, a functional transmembrane serine/threonine kinase. Cell 68, 775-785.

Loda M., Cukor B., Tam S.W., Lavin P., Fiorentino M., Draetta G.F., Jessup J.M. and Pagano M. (1997). Increased proteasome-dependent degradation of the cyclin-dependent kinase inhibitor p27 in aggressive colorectal carcinomas. Nat. Med. 3, 231-234.

MacCorkle R.A., Freeman K.W. and Spencer D.M. (1998). Synthetic activation of caspases: artificial death switches. Proc. Natl. Acad. Sci. USA 95, 3655-3660.

Mackey T.J., Borkowski A., Amin P., Jacobs S.C. and Kyprianou N. (1998). bcl-2/bax ratio as a predictive marker for therapeutic response to radiotherapy in patients with prostate cancer. Urology 52, 1085-1090.

Marcelli M., Cunningham G.R., Haidacher S.J., Padayatty S.J., Sturgis L., Kagan C. and Denner L. (1998). Caspase-7 is activated during lovastatin-induced apoptosis of the prostate cancer cell line LNCaP. Cancer Res. 58, 76-83.

Marcelli M., Cunningham G.R., Walkup M., He Z., Sturgis L., Kagan C., Mannucci R., Nicoletti I., Teng B. and Denner L. (1999). Signaling pathway activated during apoptosis of the prostate cancer cell line LNCaP: overexpression of caspase-7 as a new gene therapy strategy for prostate cancer. Cancer Res. 59, 382-390.

Markowitz S.D., Myeroff L., Cooper M.J., Traicoff J., Kochera M., Lutterbaugh J., Swiriduk M. and Willson J.K. (1994). A benign cultured colon adenoma bears three genetically altered colon cancer oncogenes, but progresses to tumorigenicity and transforming growth factor-beta independence without inactivating the p53 tumor suppressor gene. J. Clin. Invest. 93, 1005-1013.

Martikainen P., Kyprianou N. and Isaacs J.T. (1990). Effect of transforming growth factor-beta 1 on proliferation and death of rat prostatic cells. Endocrinology 127, 2963-2968.

Massague J., Attisano L. and Wrana J.L. (1994). The TGF-ß family and its composite receptors. Trends Cell Biol. 4, 172-178.

McDonnell T.J., Deane N., Platt F.M., Nunez G., Jaeger U., McKearn J.P. and Korsmeyer S.J. (1989). Bcl-2-immunoglobin transgenic mice demonstrate extended B cell survival and follicular lymphoproliferation. Cell 57, 79-88.

McDonnell T.J. and Korsmeyer S.J. (1991). Progression from lymphoid hyperplasia to high-grade malignant lymphoma in mice transgenic for the t(14;18). Nature 349, 254-256.

McDonnell T.J., Troncoso P., Brisbay S.M., Logothetis C., Chung L.W., Hsieh J.T., Tu S.M. and Campbell M.L. (1992). Expression of the protooncogene bcl-2 in the prostate and its association with emergence of androgen-independent prostate cancer. Cancer Res. 52, 6940-6944.

Minn A.J., Swain R.E. and Thompson C.B. (1998). Recent progress on the regulation of apoptosis by bcl-2 family members. Adv. Immunol. 70, 245-279.

Miyake H., Tolcher A. and Gleave M.E. (1999). Antisense bcl-2 oligonucleotides inhibit progression to androgen-independence after castration in the Shionogi tumor model. Cancer Res. 59, 4030-4034.

Miyashita T., Krajewski S., Krajewska M., Wang H.G., Lin H.K., Liebermann D.A., Hoffman B. and Reed J.C. (1994). Tumor suppressor p53 is a regulator of bcl-2 and bax gene expression in vitro and in vivo. Oncogene 9, 1799-1805.

Montenarh M. (1992). Biochemical properties of the growth suppressor/oncoprotein p53. Oncogene 7, 1673-1680.

Morana S.J., Wolf C.M., Li J., Reynolds J.E., Brown M.K. and Eastman A. (1996). The involvement of protein phosphatases in the activation of ICE/CED-3 protease, intracellular acidification, DNA digestion, and apoptosis. J. Biol. Chem. 271, 18263-18271.

Mori M., Mimori K., Shiraishi T., Tanaka S., Ueo H., Sugimachi K. and Akiyoshi T. (1997). p27 expression and gastric carcinoma. Nat. Med. 3, 593-597.

Nagata S. (1996). Apoptosis: telling cells their time is up. Curr. Biol. 6, 1241-1243.

Navone N.M., Labate M.E., Troncoso P., Pisters L.L., Conti C.J., von Eschenbach A.C. and Logothetis C.J. (1999). p53 mutations in prostate cancer bone metastases suggests that selected p53 mutants in the primary site define foci with metastatic potential. J. Urol. 161, 304-308.

Nakayama K., Ishida N., Shirane M., Inomata A., Inohe T., Shishido N., Hori I., Loh D.Y. and Nakayama K. (1996). Mice lacking $p27^{Kip1}$ display increased body size, multiple organ hyperplasia, retinal hyperplasia and pituitary tumors. Cell 85, 733-736.

Newman R.M., Kramer A., Borkowski A. and Kyprianou N. (1999). Loss of caspase-1 protein expression in human prostate cancer. (Submitted).

Nigro J.M., Baker S.J., Preisinger A.C., Jessup M.J., Hostetter R., Cleary K., Bigner S.H., Davidson N., Baylin S., Devilee P., Glover T., Collins F.S., Weston A., Modali R., Harris C.C. and Vogelstein B. (1989). Mutations in the p53 gene occur in diverse human tumor types. Nature 342, 705-708.

Nunez G., Benedict M.A., Hu Y. and Inohara N. (1998). Caspases: the proteases of the apoptotic pathway. Oncogene 17, 3237-3245.

Ohtsubo M., Theodoras A.M., Schumacher J., Roberts J.M. and Pagano M. (1995). Human cyclin E, a nuclear protein essential for the G1-to-S phase transition. Mol. Cell. Biol. 15, 2612-2624.

Oltvai Z.N., Milliman C.L. and Korsmeyer S.J. (1993). Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell 74, 609-619.

Oyama T., Kashiwabara K., Yoshimoto K., Arnold A. and Koerner F. (1998). Frequent overexpression of the cyclin D1 oncogene in invasive lobular carcinoma of the breast. Cancer Res. 58, 2876-2880.

Palayoor S.T., Bump E.A., Teicher B.A. and Coleman C.N. (1997). Apoptosis and clonogenic cell death in PC3 human prostate cancer

cells after treatment with gamma radiation and suramin. Radiat. Res. 148, 105-114.

Phillips S.M., Barton C.M., Lee S.J., Morton D.G., Wallace D.M., Lemoine N.R. and Neoptolemos J.P. (1994). Loss of the retinoblastoma susceptibility gene (RB1) is a frequent and early event in prostatic tumorigenesis. Br. J. Cancer 70, 1252-1257.

Polyak K., Kato J.Y., Solomon M.J., Sherr C.J., Massague J., Roberts J.M. and Koff, A. (1994). $p27^{Kip1}$, a cyclin-Cdk inhibitor, links transforming growth factor-beta and contact inhibition to cell cycle arrest. Genes Dev. 8, 9-22.

Porter P.L., Malone K.E., Heagerty P.J., Alexander G.M., Gatti L.A., Firpo E.J., Daling J.R. and Roberts J.M. (1997). Expression of cell-cycle regulators $p27^{Kip1}$ and cyclin E, alone and in combination, correlate with survival in young breast cancer patients. Nat. Med. 3, 222-225.

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. 55, 4438-4445.

Raghavan D. (1988). Non-hormone chemotherapy for prostate cancer: principles of treatment and application to the testing of new drugs. Semin. Oncol. 15, 371-389.

Reed J.C. (1998). Bcl-2 family proteins. Oncogene 17, 3225-3236.

Reiss M., Vellucci V.F. and Zhou Z.L. (1993). Mutant p53 tumor suppressor gene causes resistance to transforming growth factor beta 1 in murine keratinocytes. Cancer Res. 53, 899-904.

Roklin O.W., Glover R.A. and Cohen M.B. (1998). Fas-mediated apoptosis in human prostatic carcinoma cell lines occurs via activation of caspase-8 and caspase-7. Cancer Res. 58, 5870-5875.

Roth J.A., Nguyen D., Lawrence D.D., Kemp B.L., Carrasco C.H., Ferson D.Z., Hong W.K., Komaki R., Lee J.J., Pisters K.M., Putnam, J.B., Schea R., Shin D.M., Walsh G.L., Dolormente M.M., Han C.I., Yen N., Xu K., Stephens L.C., McDonnell T.J., Mukhopadhyay T. and Cai D. (1996). Retrovirus-mediated wild-type p53 gene transfer to tumors of patients with lung cancer. Nature Med. 2, 985-991.

Roy N., Quinn D., Takahashi R., Salvesen G.S. and Reed J.C. (1997). The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. EMBO J. 16, 6914-6921.

Rupnow B.A., Murtha A.D., Alarcon R.M., Giaccia A., J. and Knox S.J. (1998). Direct evidence that apoptosis enhances tumor responses to fracrionated radiotherapy. Cancer Res. 58, 1779-1784.

Schellhammer P.F. (1994) Radiation therapy for localized prostate cancer: what has been and still remains to be learned. In: Monographs in Urology. Vol. 15 (5). Stamey T. (ed). Medical Directions Publishing Company. Stanford, CA.

Scherr D.S., Vaughan E.D., Wei J., Chung M., Fielsen D., Allbright R. and Knudsen B.S. (1999). Bcl-2 and p53 expression in clinically localized prostate cancer predicts response to external beam radiotherapy. J. Urol. 162, 12-21

Sedlak T.W., Oltvai Z.N., Yang E., Wang K., Boise L.H., Thompson C.B. and Korsmeyer S.J. (1995). Bcl-2 family members demonstrate selective dimerizations with bax. Proc. Natl. Acad. Sci. USA 92, 7834-7838.

Serrano M., Hannon G.I. and Beach D. (1993). A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature 366, 704-707.

Sgambato A., Doki Y., Schieren I. and Weinstein I.B. (1997). Effects of cyclin E overexpression on cell growth and response to transforming growth factor beta depend on cell context and $p27^{Kip1}$ expression. Cell Growth Differ. 8, 393-405.

Shabaik A.S., Krajewski S., Burgan A. and Reed J.C. (1995). Bcl-2 protooncogene expression in normal, hyperplastic, and neoplastic prostate tissue. J. Urol. Pathol. 3, 17-27.

Sherr C.J. (1996). Cancer cell cycles. Science 274, 1672-1677.

Singh S.P., Lipman J., Goldman H., Ellis F.H. Jr, Aizenman L., Cangi M.G., Signoretti S., Chiaur D.S., Pagano M. and Loda M. (1998). Loss or altered subcellular localization of p27 in Barrett's associated adenocarcinoma. Cancer Res 58, 1730-1735.

Sklar G.N., Eddy H.A., Jacobs S.C. and Kyprianou N. (1993). Combined antitumor effect of suramin plus irradiation in human prostate cancer cells: the role of apoptosis. J. Urol. 150, 1526-1532.

Slawin K.M., Spenser D.M., Song W., Bergamaschi F. and Yamamoto T. (1998). Apoptosis induction in BPH-derived smooth muscle cells: Preclinical studies of a novel gene therapeutic approach to BPH. J. Urol. 159, 110A.

Slee E.A., Harte M.T., Kluck R.M., Wolf B.B., Casiano C.A., Newmeyer D.D., Wang H.G., Reed J.C., Nicholson D.W., Alnemri E.S., Green D.R. and Martin S.J. (1999). Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner. J. Cell. Biol. 144, 281-292.

Soussi T. and May P. (1996). Structural aspects of the p53 protein in relation to gene evolution: a second look. J. Mol. Biol. 260, 623-637.

Srikanth S. and Kraft A.S. (1998). Inhibition of caspases by cytokine response modifier A blocks androgen ablation-mediated prostate cancer cell death in vivo. Cancer Res. 58, 834-839.

Srivastava S., Katayose D., Tong Y.A., Craig C.R., McLeod D.G., Moul J.W., Cowan K.H. and Seth P. (1995). Recombinant adenovirus vector expressing wild-type p53 is a potent inhibitor of prostate cancer cell proliferation. Urology 46, 843-848.

Stapleton A.M.F., Timme T.L., Gousse A.E., Li Q.-F., Tobon A.A., Kattan M.W., Slawin K.M., Wheeler T.M., Scardino P.T. and Thompson T.C. (1997). Primary human prostate cancer cells harboring p53 mutations are clonally expanded in metastases. Clin. Cancer. Res. 3, 1389-1397.

Steller H. (1998). Artificial death switches: induction of apoptosis by chemically induced caspase multimerization. Proc. Natl. Acad. Sci. USA 95, 5421-5422.

Strickler H.J., Jay J.K., Linder M.D., Tamboli P. and Amin M.B. (1996). Determining prognosis of clinically localized prostate cancer by immunohistochemical detection of mutant p53. Urology 47, 366-369.

Sun X.M., MacFarlane M., Zhuang J., Wolf B.B., Green D.R. and Cohen G.M. (1999). Distinct caspase cascades are initiated in receptor-mediated and chemical-induced apoptosis. J. Biol. Chem. 274, 5053-5060.

Sutkowski D.M., Fong C.J., Sensibar J.A., Rademaker A.W., Sherwood E.R., Kozlowski J.M. and Lee C. (1992). Interaction of epidermal growth factor and transforming growth factor beta in human prostatic epithelial cells in culture. Prostate 21, 133-143.

Tanaka S., Saito K. and Reed J.C. (1993). Structure-function analysis of the Bcl-2 oncoprotein. Addition of a heterologous transmembrane domain to portions of the Bcl-2 beta protein restores function as a regulator of cell survival. J. Biol. Chem. 268, 10920-10926.

Thomas D.J., Robinson M.. King P., Hasan T., Charlton R., Martin J., Carr T.W. and Neal D.E. (1993). p53 expression and clinical outcome in prostate cancer. Br. J. Urol. 72, 778-781.

Thomas G.V., Szigeti K., Murphy M., Draetta G., Pagano M. and Loda M. (1998). Down-regulation of p27 is associated with development

of colorectal adenocarcinoma metastases. Am. J. Pathol. 153, 681-687.

Tsujimoto Y., Finger L.R., Yunis J., Nowell P.C. and Croce C.M. (1984). Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. Science 226, 1097-1099.

Troy C.M., Stefanis L., Prochiantz A., Greene L.A. and Shelanski M.L. (1996). The contrasting roles of ICE family proteases and interleukin-1beta in apoptosis induced by trophic factor withdrawal and by copper/zinc superoxide dismutase down-regulation. Proc. Natl. Acad. Sci. USA 93, 5635-5640.

Tu H., Jacobs S.C., Borkowski A. and Kyprianou N. (1996). Incidence of apoptosis and cell proliferation in prostate cancer: relationship with TGF-ßeta1 and bcl-2 expression. Int. J. Cancer 69, 357-363.

Van Veldhuizen P.Z., Sadasivan R., Cherian R., Dwyer T. and Stephens R.L. (1993). p53 expression in incidental prostatic cancer. Am. J. Med. Sci. 305, 275-279.

Vaux D.L., Cory J. and Adams J.M. (1988). Bcl-2 gene promotes haematopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. Nature 335, 440-442.

Vogelstein B. and Kinzler K.W. (1992). p53 function and dysfunction. Cell 70, 523-526.

Wang H., Grand R.J., Milner A.E., Armitage R.J., Gordon J. and Gregory C.D. (1996). Repression of apoptosis in human B-lymphoma cells by CD40-ligand and Bcl-2: relationship to the cell-cycle and role of the retinoblastoma protein. Oncogene 13, 373-379.

Wang J.D., Takahara S., Nonomura N., Ichimaru N., Toki, K., Azuma H., Matsumiya K., Okuyama A. and Suzuki S. (1999). Early induction of apoptosis in androgen-independent prostate cancer cell line by FTY720 requires caspase-3 activation. Prostate 40, 50-55.

Weinberg R.A. (1995). The retinoblastoma protein and cell cycle control. Cell 81, 323-330.

Westin P., Lo P., Marin C.M., Fernandez A., Sarkiss M., Tu S.-M., Brisbay S.M., von Eschenbach A.C. and McDonnell T.J. (1997). Bcl-2 expression confers androgen independence in androgen sensitive prostatic carcinoma. Int. Nat. J. Oncol. 10, 113-118.

Wieser R., Attisano L., Wrana J.F. and Massague J. (1993). Signaling activity of transforming growth factor type II receptors lacking specific domains in the cytoplasmic region. Mol. Cell. Biol. 13, 7239-7249.

Wilding G., Zugmeier G., Knabbe C., Flanders K. and Gelmann E. (1989). Differential effects of transforming growth factor beta on human prostate cancer cells in vitro. Mol. Cell. Endocrinol. 62, 79-87.

Williams R.H., Stapleton A.M., Yang G., Truong L.D., Rogers E., Timme T.L., Wheeler T.M., Scardino P.T. and Thompson T.C. (1996). Reduced levels of transforming growth factor beta receptor type II in human prostate cancer: an immunohistochemical study. Clin. Cancer Res. 2, 635-640.

Wolf B.B. and Green D.R. (1999). Suicidal tendencies: apoptotic cell death by caspase family proteinases. J. Biol. Chem. 274, 20049-20051.

Wrana J., Attisano L.,Wiesert R., Ventura F. and Massague J. (1994). Mechanism of activation of the TGF-ß receptor. Nature 370, 341-347.

Yang C., Cirielli C., Capogrossi M.C. and Passaniti A. (1995a). Adenovirus-mediated wild-type p53 expression induces apoptosis and suppresses tumorigenesis of prostatic tumor cells. Cancer Res. 55, 4210-4213.

Yang E., Zha J., Jockel J., Thompson C.B. and Korsmeyer S.J. (1995b). Bad, a heterodimeric partner for bcl-XL and bcl-2, displaces bax and promotes cell death. Cell 80, 285-291.

Yang G., Stapleton A.M.F., Wheller T.M., Truong L.D., Timme T.L., Scardino P.T. and Thompson T.C. (1996). Clustered p53 immunostaining: a novel pattern associated with prostate cancer progression. Clin Cancer Res. 2, 399-401.

Yin X.M., Oltvai Z.N. and Korsmeyer S.J. (1994). BH1 and BH2 domains of bcl-2 are required for inhibition of apoptosis and heterodimerization with bax. Nature 369, 321-323.

Yin X.M., Oltvai Z.N. and Korsmeyer S.J. (1995). Heterodimerization with Bax is required for Bcl-2 to repress cell death. Curr. Top. Microbiol. Immunol. 194, 331-338.

Zhang Y., Fujita N. and Tsuruo T. (1999). Caspase-mediated cleavage of $p21^{Waf1/CIP1}$ converts cancer cells from growth arrest to undergoing apoptosis. Oncogene 18, 1131-1138.

Zou H., Li Y., Liu X. and Wang X. (1999). An APAF-1.cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9. J. Biol. Chem. 274, 11549-11556.

Accepted May 15, 2000