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PROSPECTS

Apoptosis Evasion: The Role of Survival Pathways in Prostate Cancer Progression and Therapeutic Resistance

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Abstract The ability of a tumor cell population to grow exponentially represents an imbalance between cellular proliferation and cellular attrition. There is an overwhelming body of evidence suggesting the ability of tumor cells to avoid programmed cellular attrition, or apoptosis, is a major molecular force driving the progression of human tumors. Apoptotic evasion represents one of the true hallmarks of cancer and appears to be a vital component in the immunogenic, chemotherapeutic, and radiotherapeutic resistance that characterizes the most aggressive of human cancers [Hanahan and Weinberg, 2000]. The challenges in the development of effective treatment modalities for advanced prostate cancer represent a classic paradigm of the functional significance of anti-apoptotic pathways in the development of therapeutic resistance. J. Cell. Biochem. 97: 18–32, 2006. © 2005 Wiley-Liss, Inc.

Key words: prostate growth; apoptosis; survival signaling; Bcl-2; cancer therapy; molecular targets

Prostate cancer is the most commonly diagnosed cancer in American males and is second only to lung cancer in cancer-related mortality within this patient population [Jemal et al., 2005]. Most prostate tumors are initially responsive of androgen ablation therapy, which acts to devoid these tumor cells of their primary growth stimulus [Arnold and Isaacs, 2002]. Unfortunately, prostate cancer, under the physiologic stress of hormone ablation, ultimately progresses to androgen-independent disease that has proven resistant to both hormone ablation, as well as other systemic chemotherapies [Debes and Tindall, 2004]. Apoptosis appears to be the predominant form of tumor cell demise caused by both androgen ablation and chemotherapeutic agents and plays a role in prostate cancer radiosensitivity [Arnold and Isaacs, 2002; Debes and Tindall, 2004]. It is the acquisition of anti-apoptotic signal transduction that ultimately leads to the characteristic treatment resistance that typifies advanced prostate cancer.

Execution of apoptosis can occur via two distinct signaling pathways. The intrinsic and extrinsic apoptotic pathways leading to cellular death are summarized in Figure 1. The extrinsic pathway is initiated by the binding of apoptosis-inducing ligands to cell surface death receptors associated with Fas-associated death domain (FADD) [Debatin and Krammer, 2004]. Activated FADD then interacts with initiator enzymes of the caspase cascade, notably caspase 8 and 10, resulting in further downstream effector caspase activation (caspases 3 and 7) and ultimately programmed cellular destruction through proteolytic cleavage of caspase substrates [Debatin and Krammer, 2004]. The intrinsic pathway is initiated by intercellular stress, lack of growth factors, or overwhelming DNA injury and subsequently targets the mitochondrial membrane [Okada and Mak, 2004]. Loss of mitochondrial membrane potential and increased membrane permeability leads to the release of cytochrome c [Newmeyer and Miller, 2003]. Release of this protein into the cytosol

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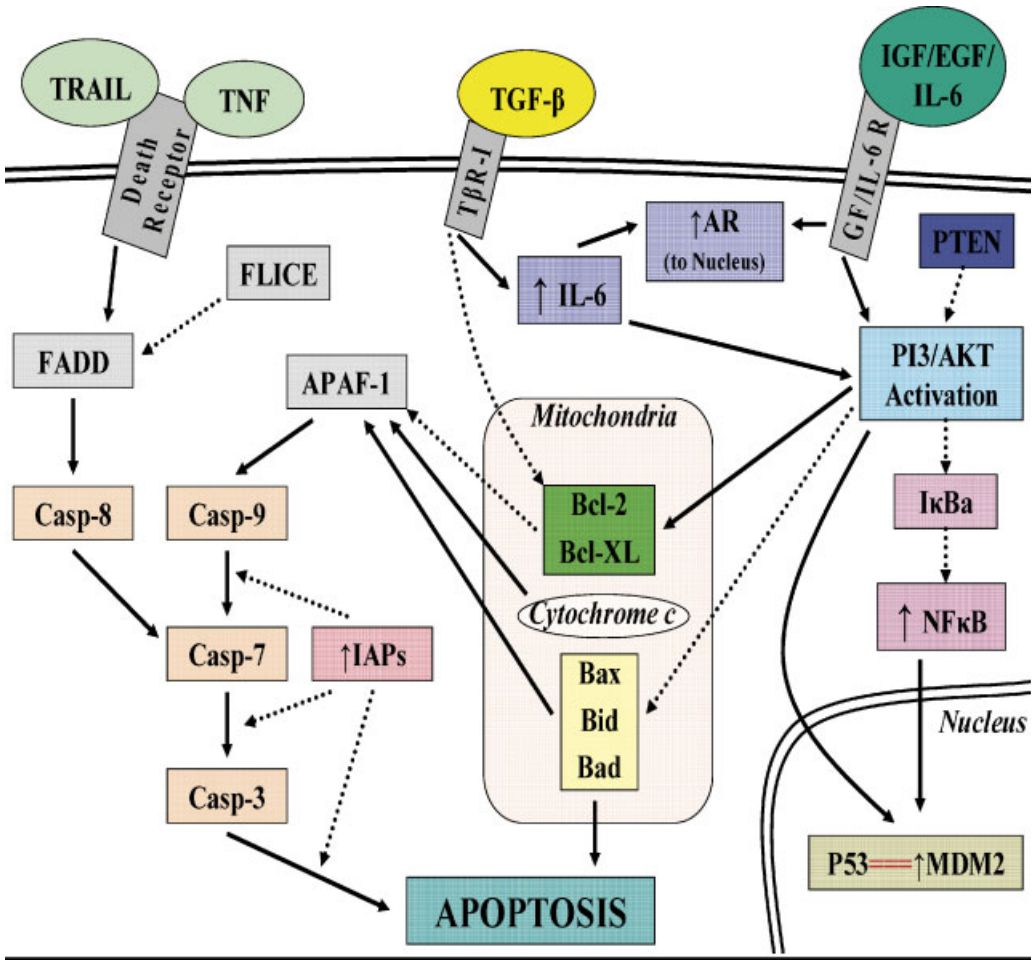

Fig. 1. Signaling cross-talk between survival and apoptosis pathways in prostate cancer cells. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

results in activation of apoptotic protease activating factor-1 (APAF-1) and caspase 9 recruitment [Okada and Mak, 2004]. These proteins form a functional apoptosome that activates the effector caspase cascade again resulting in programmed cellular destruction [Debatin and Krammer, 2004]. While these two pathways are often described as separate pathways, significant cross-talk is known to exist, most notably through caspase-8 directed Bid activation leading to cytochrome c release [Kulik et al., 2001a]. Prostate cancer cells have shown the ability to acquire both intracellular survival pathways and alterations in chemokine and growth factor signal transduction that allow them to circumvent either apoptotic pathway and ultimately contribute to the androgen-independent and classically aggressive phenotype that is resistant to any form of conventional chemotherapy.

THE APOPTOTIC PLAYERS

The Bcl-2 Family of Proteins

The Bcl-2 family proteins include a heterogeneous group of both pro-apoptotic and anti-apoptotic molecules that exert their effect on mitochondrial function [DiPaola et al., 2001]. Many Bcl-2 family members contain a hydrophobic stretch of amino acids near their carboxyl-terminus that anchors them in the outer mitochondrial membrane. In contrast, other Bcl-2 family members, such as Bid, Bim, and Bad, lack these membrane anchoring-domains, but target mitochondria. Anti-apoptotic members, most notably Bcl-2 and Bcl-xL, inhibit the release of cytochrome c from the mitochondria, consequently inhibiting mitochondrial-induced apoptosis. Their action is antagonized by proapototic members of the Bcl-2 family such as Bax, Bad, and Bid, allowing for mitochondrial

cytochrome c release and caspase cascade activation [DiPaola et al., 2001]. It is the ratio of pro-apoptotic to anti-apoptotic family members that ultimately determines the survival of tumor cells.

Both in vitro and in vivo studies have established that Bcl-2 and other anti-apoptotic members of its family are significantly upregulated in aggressive prostate cancer phenotypes [Kajiwara et al., 1999; McCarty, 2004]. Overexpression of Bcl-2 and Bcl-xL has been shown in prostate cancer and other malignancies to confer resistance to both chemotherapy and radiation therapy [McCarty, 2004]. Furthermore, the Bcl-2 family plays a critical role in the androgen-signaling axis operating in prostate cancer cells. In androgen-responsive prostate cancer cells, androgens downregulate expression of pro-apoptotic Bcl-2 family members such as Bax [Coffey et al., 2002]. Increased Bcl-2 and Bcl-xL expression in androgen-independent tumors [Furuya et al., 1996] is directly linked to the ability of prostate cancer cells to survive in androgen-free environments [Kajiwara et al., 1999], evidence highlighting the functional as well as predictive significance of Bcl-2/Bcl-xL over-expression as one of the key regulators allowing for selection of androgen-independent recurrences in prolonged androgen ablation therapy [McCarty, 2004]. While recognizing that expression changes in the Bcl-2 family can contribute the emergence of therapeutic resistance, the dynamic cross-talk between this "powerful" family and other anti-apoptotic pathways influenced by exogenous ligand-receptor signaling must be acknowledged and will be discussed.

The NF-κB Intracellular Connection

The NF-κB/Rel protein family are transcription factors that regulate a multitude of immunologic and inflammatory responses as well as individual cell growth, differentiation, and apoptosis [Suh and Rabson, 2004]. While the oncogenic properties of NF-κB include augmenting angiogenesis, invasion, and metastasis formation, the most important mechanism driving the carcinogenic effect of this transcription factor is its antiapoptotic pathway [Chen, 2004; Suh and Rabson, 2004]. In the majority of cell types, NF-κB is kept inactive through compartmentalization to the cytosol via binding with inhibitors of κBs (IκBs). In response to the appropriate stimuli, IκB is phosphorylated through interaction with the IκB kinase complex (IKK), allowing nuclear translocation of NF-κB and subsequent NF-κB driven signal transduction (Fig. 1) [Chen, 2004; Suh and Rabson, 2004]. The antiapoptotic effect of NF-κB has been attributed to its ability to directly upregulate Bcl-2 and Bcl-xL expression in prostate cancer cells leading to inhibition of mitochondrial apoptosis [Shukla and Gupta, 2004]. However, dissection of the prostate apoptosis response-4 protein (PAR-4) pathway has shown that NF-κB inhibition is required for the proapoptotic effect of PAR-4 on the FAS ligand extrinsic apoptotic pathway to occur [Chakraborty et al., 2001]. Furthermore, NF-κB has been shown to upregulate FLICE inhibitory protein (c-FLIP-L) expression in prostate cancer cells; c-FLIP-L upregulation directly interferes with recruitment of caspase-8 to FADD [Zhang et al., 2004]. Downregulation of c-FLIP-L appears to restore sensitivity to Fas-mediated apoptosis in aggressive prostate cancer cells [Hyer et al., 2002]. Downstream effector caspase activation is not immune to the inhibitory effects of NK-κB either, as it has been shown to upregulate several members of the inhibitors of apoptosis proteins (IAPs) which directly inhibit caspases -3, -7, and/or -9 [McEleny et al., 2001].

Constituitive activation of NF-κB is widely in many human malignancies, and while NF-κB activity occurs in low levels in androgen-dependent prostate tumors, it appears in high levels in androgen independent tumor cell lines and in highly aggressive prostate tumors and metastatic lesions [Ismail et al., 2004; Ross et al., 2004]. In a recent immunohistochemical evaluation of prostatectomy specimens, elevated NF-κB immunoreactivity correlated directly with advanced tumor stage, tumor grade, and tumor recurrence [Ross et al., 2004]. In lymph node metastasis, nuclear localization of NF-κB was significantly greater in lymph node containing tumor cells when compared to local tumor controls [Ismail et al., 2004] Interestingly, elevated NF-κB activation was also seen in tumor cell surrounding lymphocytes [Ismail et al., 2004] suggesting possible cross-talk between prostate cancer cells and surrounding lymphocytes, leading to oncogenically favorable release of paracrine prostate cancer mediators such as Il-6 and TNF-a. This apparent upregulation of NF-κB seen in aggressive prostate cancers has been correlated with resistance to

both chemotherapy and radiation therapy [Suh and Rabson, 2004]. While there appears to be a direct correlation between increased NF-κB activity and androgen independence, the interaction between the NF-κB pathway and the androgen receptor (AR) pathway appears to be pleomorphic [Coffey et al., 2002; Suh and Rabson, 2004] and requires further elucidation.

p53: Guardian of Genomic Integrity

The p53 tumor suppressor gene regulates both cell cycle and apoptosis in response to numerous cellular stresses such as DNA injury, hypoxia, free radical injury, and damage to the mitotic machinery [Hernandez et al., 2003]. It is believed that the oncolytic responsibility of the p53 gene product is to invoke cell-cycle arrest and stimulate apoptosis in cells that have acquired overwhelming genetic aberrations to avoid mutation propagation [Hernandez et al., 2003]. p53 action is inhibited by MDM2, through binding to the p53 gene product and relegating it to ubiquinylation and proteasomal degradation [McCarty, 2004]. Mechanistically the ability of p53 to induce apoptosis in tumor cells results from its upregulation of BAX, leading to mitochondrial driven apoptosis [Hernandez et al., 2003]. Furthermore, recent analysis of specific p53 mutations p53 revealed that altered p53 expression can also adversely affect Fas-mediated apoptosis [Gurova et al., 2003].

Loss or mutation of p53 has been identified in over 10,000 different types of tumors analyzed and mutations of this tumor suppressor gene are found in 45%–50% of all human cancers [Soussi et al., 2000]. In prostate cancer, while mutations in p53 are uncommon in early, well-differentiated disease, mutations become abundant in both metastatic disease as well as hormone-independent tumors [Navone et al., 1993]. Moreover, upregulation of MDM2 expression has been found in up to 40% of prostatectomy specimens and correlated with advanced disease [Leite et al., 2001]. In hormone-responsive prostate cancer cells, loss of wild-type p53 leads to development of a hormone-resistant phenotype, thus implicating altered p53 function in the development of hormone refractory disease [Scherr et al., 1999; Burchardt et al., 2001]. Significantly enough altered p53 has been shown to influence chemotherapeutic response, with most mutations leading to resistance, while certain select mutations lead to increased sensitivity to specific agents such as paclitaxel [DiPaola et al., 2001]. Growing evidence indicates a direct correlation between altered p53 expression with prostate cancer resistance to radiotherapy [Pisters et al., 2004], while resolution of functional p53 status with specific p53 mutants restores the apoptotic signaling and ultimately therapeutic sensitivity in experimental models of prostate cancer [Hernandez et al., 2003; Pisters et al., 2004].

PTEN/P13K/AKT: The Downstream Intracellular Players

Phosphatase and tensin homolog deleted on chromosome 10 (*PTEN*) is a highly conserved tumor supressor gene that induces cellular apoptosis through its modulation of the P13K/AKT signal transduction pathway. Specifically, PTEN inhibits phosphorylation of AKT, which is necessary for its activation and targeting of its many downstream effectors [Wang et al., 2003]. Loss of PTEN, a common event in treatment resistant and poorly differentiated prostate cancers, leads to constituative activation of the P13K/AKT pathway and subsequent apoptotic resistance [Davies et al., 1999]. Restoration of PTEN activity in PTEN deficient prostate cancer cell lines has been shown to increase sensitivity to FADD mediated caspase-8 driven apoptosis as well as to facilitate BIDD cleavage allowing for cytochrome c release and subsequent mitochondrial driven apoptosis [Yuan and Whang, 2002]. AKTs are activated by second messengers via phosphatidylinositol 3′-kinases (P13Ks). This phosphorylation is counterbalanced by the activity of PTEN phosphatases [Stern, 2004]. In prostate cancer, AKT phosphorylation can occur constitutively through loss of PTEN activity, or be stimulated and upregulated in PTEN positive tumors through autocrine and paracrine cell membrane receptor-ligand interactions [Pfeil et al., 2004]. The ability of phosphorylated AKT to inhibit prostate cancer cellular apoptosis appears to be the result of the powerful crosstalk that exists between this effector and multiple other anti-apoptotic pathways (Fig. 1). Activated AKT has been shown to activate MDM2 leading to proteolysis of p53 and subsequent inhibition of p53 mediated apoptosis with stimulation of cell-cycle progression [Gao et al., 2003; Stern, 2004]. Activated AKT also inactivates Bad and caspase-9, allowing for Bcl-2 release and inhibition

of mitochondrial apoptosis [Ghosh et al., 2003; Wang et al., 2003; Stern, 2004]. Furthermore, upregulation of P13K/AKT activity leads to phosphorylation of IκB, allowing for nuclear translocation of NF-κB and subsequent NF-κB driven suppression of apoptosis [Wang et al., 2003; Stern, 2004]. Phosphorylated AKT can induce phosphorylation of the AR, as well as upregulate AR expression, which can lead to inhibition of androgen-deprivation-induced apoptosis [Lin et al., 2001; Ghosh et al., 2003].

An expanding body of recent evidence indicates that both PTEN inactivation and AKT phosphorylation are hallmarks of aggressive prostate cancer. While PTEN inactivation is present in only 10%–15% of primary prostate cancers, PTEN loss is detected in 30%–50% of hormone-refractory tumors, as well as 60% of xenograft models derived from metastatic prostate cancer cell lines [Wang et al., 2003; Pfeil et al., 2004]. Furthermore, loss of PTEN has correlated with aggressive local disease (T3b-T4 tumors) and Gleason score >6 [McMenamin et al., 1999]. AKT phosphorylation has been shown to be a marker for aggressive disease, but also an independent prognostic indicator. High levels of AKT phosphorylation are exclusive associated with prostatic adenocarcinoma, compared to benign tissues [Ayala et al., 2004]. AKT phosphorylation has been shown to correlate with Gleason score [Liao et al., 2003], and, in poorly differentiated tumors (Gleason 8–10), strong presence of phosphorylated AKT is observed in over 90% of specimens examined [Malik et al., 2002]. In prostate cancer specimens with Gleason scores of 5–6, a notoriously difficult patient population to predict prognosis, elevated AKT phosphorylation proved to be an indicator for recurrence [Liao et al., 2003]. Furthermore, it was recently established that phosphorylation of AKT was a more effective prognostic indicator of recurrence than both mitotic index and Gleason score [Kreisberg et al., 2004]. Not surprisingly, loss of PTEN and phosphorylation of AKT are associated with resistance to chemotherapy and been implicated in the progression of refractory prostate cancer after long term androgen ablation therapy [Yuan and Whang, 2002; Ghosh et al., 2003]. Novel targeting strategies inhibiting AKT phosphorylation or restoring PTEN activity appear to cause profound apoptosis and restore sensitivity to chemotherapy in vitro and in xenograft models [Yuan and Whang, 2002; Shaw et al., 2004].

The Antagonists: Inhibitors of Apoptosis Proteins (IAPs)

Recently, a new family of apoptosis inhibitors has been identified and appears to have a role in prostate cancer treatment resistance. The IAPs are a group of caspase inhibitors that directly inhibit the effector caspases 3, 7, and 9 resulting in decreased cellular apoptosis [Schimmer, 2004]. Currently, eight human IAPs have been identified with the most studied being X-linked inhibitor of apoptosis protein (XIAP), inhibitor of apoptosis protein 1 (IAP1), inhibitor of apoptosis protein 2 (IAP2), and survivin [Krajewska et al., 2003]. While all appear capable of inhibiting effector caspases, IAP1 and IAP2 can upregulate NF-κB expression pointing to a possible positive feedback loop between these two pathways (Fig. 1) [McEleny et al., 2001]. Elevated expression of these four IAPs has been shown in both animal models of prostate cancer and prostatectomy specimens from cancer patients, and this elevation appears to be present early in prostate cancer development [Krajewska et al., 2003]. The ability of IAPs to inhibit apoptosis in response to multiple chemotherapeutic agents has been established in several tumor models [Debatin and Krammer, 2004], although the significance of IAPs in prostate cancer therapeutic resistance is an area of recently "active" investigations. Indeed recent evidence suggests that XIAP inhibition enhances chemotherapy sensitivity in otherwise resistant prostate cancer cell lines [Amantana et al., 2004]. Another small study of 23 patients revealed that IAP1 and IAP2 expression were dramatically upregulated in patients receiving neoadjuvant androgen ablation suggesting a potential role of IAPs in androgen independence [McEleny et al., 2001]. As the role of IAPs in prostate cancer treatment resistance continues to be discerned, manipulation of IAP pathways may become a valuable way to circumvent the apoptotic resistance present in the upstream intracellular apoptotic escape mechanisms such as AKT and Bcl-2.

Tumor microenvironment: extracellular forces driving intracellular resistance. Recently, the focus of tumor biology has embraced a crucial paradigm shift implicating not only the intracellular pathophysiology inherent

to carcinoma cells, but also the critical role that the tumor microenvironment plays in developing aggressive cancer phenotypes. It has become apparent that the cross-talk that exists between prostate epithelial tumor cells and their surrounding stromal and endothelial partners, as well as the localized inflammatory cells attracted to the neoplastic region, is a driving force towards both androgen independence and subsequent treatment resistant recurrence [Arnold and Isaacs, 2002; Chung et al., 2005]. The ability of tumor microenvironment to alter the apoptotic outcomes of prostate cancer cells is exemplified by recent advances in the understanding of tumor hypoxia. The ability of solid organ tumors, including prostate adenocarcinoma, to outgrow their own blood supply coupled with their innate propensity for disordered neovascularization creates an intratumoral environment with often severely diminished oxygen tension. Consequently, tumor hypoxia has been shown to correlate significantly with prostate cancer stage, aggressiveness, androgen independence, and treatment resistance [Movsas et al., 2000; Cvetkovic et al., 2001; Hochachka et al., 2002; Ghafar et al., 2003]. Furthermore, this hypoxia driven aggressive behavior can, at least in part, be attributed to enhanced apoptotic resistance in hypoxic prostate cancer cells due to suppression of p53 activity and upregulation of AKT [Skinner et al., 2004; Liu et al., 2005]. Along with tumor hypoxia, the paracrine and subsequent autocrine release of both growth factors and cytokines has also been shown to affect apoptotic sensitivity and, ultimately, tumor aggressiveness.

Growth factor signaling pathways. Mechanistic dissection of the pathways leading to the emergence of hormone independent-prostate cancer identified the dynamic contribution of an array of growth factors, in addition to the androgen-signaling axis. While a full understanding of the pro-survival characteristics of these growth factor pathways is still evolving, the impact that growth factors such as epidermal growth factor, insulin-like growth factor 1, and transforming growth factor-β can be appreciated by the robust development of new cancer therapies targeting their signal transduction. As the medical and scientific community enthusiastically witnessed the development of the therapeutically promising tyrosine kinase inhibitor Iressa, the role of the epidermal growth factor (EGF) system in apoptosis evasion and prostate cancer progression has been exposed. EGF can be secreted in a paracrine followed by autocrine manner in prostate tumors [Mimeault et al., 2003]. Upregulation of its membrane receptor in invasive prostate carcinoma cells has also been well characterized [Di Lorenzo et al., 2002; Hernes et al., 2004; Shuch et al., 2004]. While upregulation of this pathway has been associated with increased cellular proliferation and increased invasion [Mimeault et al., 2003], its role in prostate cancer therapeutic resistance and androgen independent status can be attributed to its ability to protect prostate cancer cells from apoptosis. Indeed, the EGF-EGFR system can activate Pi3K leading to AKT phosphorylation and subsequent inhibition of proapoptotic BAD (Fig. 1) [Mimeault et al., 2003]. Interestingly, while disruption of the EGF-EGFR pathway leads to robust prostate cancer cell apoptosis [Harper et al., 2002; Farhana et al., 2004], the enhanced apoptotic sensitivity can be only partially explained by downregulation of the PI3K/AKT pathway. Recent mechanistic analysis of this pathway has exposed the ability of the EGF-EGFR system to rescue prostate cancer cells from the proapototic effects of PI3k/AKT inhibition [Torring et al., 2003]. The ability of the EGF-EGFR system to provide apoptotic evasion in a PI3K/AKT independent manner can be attributed, at least in part, to its effects on AR signaling. While the ability of epidermal growth factor to induce AR transcriptional activity alone has been a topic of debate [Orio et al., 2002; Mellinghoff et al., 2004], the ability of this system to, at minimum, coactivate the AR, sensitize it to the low levels of androgen characteristic of hormone ablation therapy, and synergize androgenic stimulation of AR transcriptional activity has been established [Orio et al., 2002; Bonaccorsi et al., 2004; Gregory et al., 2004]. These characteristics predict the clinical experience with EGF-EGFR signal transduction and its relationship with prostate cancer progression and treatment resistance.

Tissue analysis of tumor specimens from both mouse xenografts and human patients has shown EGFR expression to be a predictor of aggressive disease. Elevations of EGFR expression occur in prostate cancer cells and associated endothelial cells from bony metastases, as opposed to other metastatic sites in experimental xenograft models [Kim et al., 2003].

Furthermore, elevated EGFR expression in human tumor specimens has been correlated with increased stage, Gleason grade, PSA, invasiveness and metastatic disease [Di Lorenzo et al., 2002; Shuch et al., 2004]. Elevated receptor expression is also associated with the molecular switch to androgen independence [Hernes et al., 2004] and has been implicated in the racial disparities that exist in prostate cancer disease behavior and outcomes [Shuch et al., 2004]. This constellation of data provided a strong molecular basis for the development of inhibitors of the EGF-EGFR pathway. Both in vitro and in vivo experimental models firmly established that EGF-EGFR pathway disruption leads to increased apoptosis and growth inhibition in prostate cancer cells [Harper et al., 2002; Kim et al., 2003; Vicentini et al., 2003; Farhana et al., 2004]. This has led to clinical trials with compounds, such as the EGFR tyrosine kinase inhibitor IRESSA. Although early results in prostate cancer have met with a certain degree of variability, one has to recognize that disruption of this pathway emerges as an attractive target with therapeutic promise [Blackledge, 2003].

Like the EGF-EGFR pathway, the insulin-like growth factor (IGF) axis has proven to be a critical player in the progression of prostate cancer. Unlike EGF, the IGF pathway may be equally important in the development of early prostate cancer. The IGF signaling pathway is a complex balance of interactions between IGF-1 ligand, multiple IGF binding proteins (IGFBPs), IGF receptor (IGFR), and IGFBP proteases. IGF-1 is synthesized in nearly every human tissue, but in the prostate, appears to exert its action on prostate cancer cells through paracrine release from the prostate stroma [Moschos and Mantzoros, 2002]. There are six known IGFBPs described in humans, and these compounds determine both the bioavailability of IGF-1 as well as guide its effects on target tissue [Djavan et al., 2001; Moschos and Mantzoros, 2002]. Ninety-nine percent of the circulating IGF-1 is bound to IGFBPs with 75% of IGF-1 bound specifically to IGFBP-3 [Djavan et al., 2001; Moschos and Mantzoros, 2002]. IGFR is also constituitively expressed in human tissues but quantitative receptor expression can be altered and will affect tissue response to IGF-1 [Djavan et al., 2001; Moschos and Mantzoros, 2002; Krueckl et al., 2004]. IGF-1 function can be further regulated by IGFBP proteases of which PSA is included. In prostate cancer cells, binding of IGF-1 to IGFR initiates two predominant apoptotic resistance pathways: the P13K/AKT pathway and, to a lesser extent, the NF-κB pathway (Fig. 1) [Djavan et al., 2001; Moschos and Mantzoros, 2002; Bogdanos et al., 2003]. In a pattern to the EGF-EGFR signaling events, in the presence of elevated IGFR, which is common in advanced disease, IGF-1 is able to rescue prostate cancer cells from apoptosis induced by P13K/AKT pathway disruption [Miyake et al., 2000]. IGF-1 has also been shown to stimulate the AR [Moschos and Mantzoros, 2002] and has been directly implicated in the progression to androgen independence [Krueckl et al., 2004]. Furthermore, the differential expression of IGFBPs by prostate cancer cells also influences apoptotic sensitivity. While several of the IGFBPs have been implicated in prostate cancer progression, IGFBP 3 appears to be the most influential player [Djavan et al., 2001; Hong et al., 2002; Moschos and Mantzoros, 2002; Li et al., 2003]. IGFBP 3 binding to IGF-1 attenuates the upregulation of the P13K/AKT pathway leading to increased prostate cancer cell apoptosis [Djavan et al., 2001; Moschos and Mantzoros, 2002]. Recent evidence shows that IGFBP 3 is able to sensitize prostate cancer cells to apoptosis in the absence of IGF-1 binding [Hong et al., 2002]. It is not surprising that downregulation of IGFBP 3 is common in prostate cancer and is also degraded by the known IGFBP protease PSA [Djavan et al., 2001].

The clinical impact of the IGF/IGFB/IGFR/PSA axis cannot be overemphasized. While there is some debate whether this axis is more important during the development of prostate cancer or during the progression to metastatic, treatment refractory disease, it is clear that IGF signal transduction is crucial to the pathogenesis of prostate cancer from initiation through to metastatic formation. Increased IGFR expression is common in androgen-independent metastatic lesions and increased IGFBP 2 and 5 levels in prostate cancer specimens correlate with increased Gleason grade [Djavan et al., 2001]. Furthermore, elevated serum IGF-1 levels as well as an elevated IGF/IGFBP 3 ratio has been found in multiple clinical studies to be an independent predictor of prostate cancer risk and to also improve the diagnostic yield of PSA screening [Chan et al., 1998; Li et al., 2003; Stattin et al., 2004]. Recent insight into the bone

microenvironment has implicated the IGF1 axis as the predominant survival factor pathway responsible for the androgen ablation and chemotherapy refractoriness seen in prostate cancer bony metastasis [Bogdanos et al., 2003]. As metastatic prostate cancer cells release urokinase-type plasminogen activator (uPA), hydrolosis of IGFBPs occurs, resulting in a local increase in IGF-1 bioavailability and subsequent apoptotic resistance and osteoblastic reaction [Bogdanos et al., 2003]. As both uPA and IGF-1 have promoter region binding sites for the glucocorticoid receptor, glucocorticoid therapy has become a component of rescue therapy in the case of skeletal metastatic disease [Bogdanos et al., 2003]. Several similar treatments aimed at disrupting IGF signal transduction including GNRH antagonism, somatostatin analogs, and IGFBP protease inhibition, are currently under active investigation [Djavan et al., 2001]. To date, while quality of life measurements with such therapeutic strategies are encouraging, no significant changes in survival have been appreciated [Bogdanos et al., 2003].

The role of transforming growth factor-β1 (TGF-β1) in prostate cancer pathogenesis represents a classic ability of cancer cells to alter signal transduction in the presence of an abundant apoptosis-inducing ligand in order to evade cell death and promote disease progression. TGF-β1 signaling in normal prostate epithelium and in early prostate cancer can be characterized by proliferation inhibition and tumor suppression [Bello-DeOcampo and Tindall, 2003]. The ability of TGF-β1 to suppress early prostate cancer tumorigenesis requires intact signal transduction via interaction with the TGF-β1 receptors TGFβR-I and TGFβR-II and subsequent downstream targeting through regulation of the SMAD family of protein effectors [Bello-DeOcampo and Tindall, 2003]. Upregulation of this pathway from TGF-β1 and receptor binding leads to caspase-1 activation, upregulation of BAX, and downregulation of Bcl-2, ultimately resulting in tumor cell apoptosis [Guo and Kyprianou, 1999; Kyprianou, 1999]. Furthermore, the enhanced expression of TGF-β1 and its receptors that occurs after medical or surgical castration has been implicated as the main driving force for the pronounced prostate cancer cell apoptosis seen with such therapy [Wikstrom et al., 1999]. Unfortunately, TGF-β1's ability to induce prostate cancer apoptosis eventually gives way to disease promotion and metastatic formation.

Increased TGF-β1 ligand expression directly correlates with prostate cancer progression, while there is loss expression of its receptors [Wikstrom et al., 1999]. This disruption of normal TGF-β1 signaling tips the axis in favor of enhanced angiogenesis, extracellular matrix remodeling favorable for invasion and, most importantly, immununosuppression [Matthews et al., 2000; Tuxhorn et al., 2002; Bello-DeOcampo and Tindall, 2003]. TGF-β1 overexpression, common in advanced prostate cancer, has been shown to directly inhibit the ability of tumor specific cytotoxic T lymphocytes (CTLs) and NK cells to induce prostate cancer cell apoptosis; downregulation of TGF-β1 can restore immunogenicity of prostate cancer cells and suppress metastasis formation [Matthews et al., 2000; Teicher, 2001; Shah et al., 2002], potentially via activation of Il-6 expression (Fig. 1), a powerful inhibitor of prostate cancer cell apoptosis and metastasis promoter [Park et al., 2003]. Bcl-2 overexpression, another common finding in prostate cancer, is also able to inhibit TGF-β1 induced apoptosis [Bruckheimer and Kyprianou, 2002]. Moreover TGF-β1 can synergize with AR transactivation in response to androgen and upregulate downstream targets, such as PSA, which have been implicated in apoptotic evasion [Kang et al., 2001].

TGF-β1 ligand overexpression coupled with the downregulation of TGβR-I and TGFβ R-II are hallmarks of advanced prostate cancer. Numerous clinical studies of both prostatectomy specimens as well as serum analysis of patients both before and after prostatectomy have revealed that upregulation of TGF-β1 along with downregulation of TGFβR-I and TGFβR-II is associated with invasive disease, increased Gleason grade, and treatment refractory disease [Shariat et al., 2004a,b; Zeng et al., 2004]. The prognostic power of TGF-β1 is exemplified by its inclusion in current preoperative nomograms that have proven more effective at predicting recurrent disease than standard parameters used today such as preoperative PSA or Gleason grade [Kattan et al., 2003]. Attempts to target TGF-β1 signaling have included quinazoline-based α1-adrenoceptor antagonists, restoration of TGF-β receptor expression through gene delivery, and antisense inhibition of TGF-β1 expression [Guo and

Kyprianou, 1999; Matthews et al., 2000; Partin et al., 2003]. The encouraging results in the laboratory have yet to be translated to the clinical setting.

The role of cytokines and inflammatory response in prostate tumor progression. Our current understanding of the contribution of inflammation to the tumorigenic process points to an enticing question: could it be possible that the immune response generated to combat cancer initiation and progression, provides yet another opportunistic interaction within the tumor microenvironment? While a connection between inflammation and the development and progression of cancer has been suspected for some time, new insights into the importance of this relationship are emerging. Two recent experimental models have convincingly implicated the inflammatory response, occurring both in intestinal colitis and chronic hepatitis, as a key mediator not only in the development of solid tumors but also in tumor progression [Balkwill and Coussens, 2004]. In both the colitis and hepatitis model, the NF-κB system appears to be the intracellular pathway link that allows the inflammatory response to be a potential co-conspirator in tumor progression [Viatour et al., 2005]. Further dissection by Greten and colleagues revealed the importance of secretion inflammatory mediators such as TNF-a, IL-1, IL-6, and IL-8 in driving the NF-κB pathway towards apoptotic resistance and tumor progression [Greten et al., 2004]. While the NF-κB link between cancer and inflammation has been proposed in other tumor systems [Viatour et al., 2005], it has yet to be validated in prostate cancer. Considering the evidence linking prostate cancer development to chronic inflammation [Konig et al., 2004; Nelson et al., 2004], one must recognize the immediate need for molecular exploration of this relationship. Perhaps the most important lesson learned so far from the experimental and clinical studies on prostate cancer, is the vital role of cytokines in the host inflammatory response during tumor progression. The two cytokines most often implicated in this dual capacity, TNF-a and IL-6, will be examined.

Tumor necrosis factor-α. TNF-a is a pleiomorphic cytokine involved in both inflammation and cancer biology. The cellular response to TNF-α ligand-receptor binding can invoke either the apoptotic cascade or promote tumor cell survival. There are two well-described TNF-α receptors, TNFRI and TNFRII. Ligand binding to TNFRI usually results in FADD recruitment and subsequent caspase 8 activation, which ultimately results in apoptosis [Guseva et al., 2004]. Ligand binding to TNFRII leads to activation in the MAPK and NF-κB pathways resulting in proliferation and apoptotic resistence [Guseva et al., 2004]. However, receptor expression alone does not dictate the tumor cell's fate, as TNFRI binding can also stimulate NF-κB activation through TRAF2 activation of IKK mediated IκB-α phosphorylation [Chopra et al., 2004; Guseva et al., 2004]. In prostate cancer cells, the response to TNF-α appears to be linked to androgen responsiveness. Data from both in vitro and in vivo model experimental studies confirm that androgen responsive prostate cancer cell lines are sensitive to TNF-α induced apoptosis via both p53 accumulation, as well as BID cleavage and subsequent caspase cascade initiation leading to cytochrome c release [Rokhlin et al., 2000; Kulik et al., 2001b]. Prolonged exposure of androgen sensitive prostate cancer cells to TNF-α leads to increased AR activity and hypersensitivity to low-androgen levels [Harada et al., 2001]. Furthermore, in metastatic androgen-insensitive prostate cancer cells, exposure to TNF-α can actually promote apoptotic resistance rather than sensitivity. This axial shift towards tumor promotion rather than apoptotic sensitivity has been attributed to the high levels of constitutive NF-κB expression in androgen insensitive prostate cancer cells, as well as TNF-α mediated upregulation of IKK activity and subsequent NF-κB activation through PI3K-AKT dependent and independent pathways (Fig. 1) [Sumitomo et al., 1999; Gustin et al., 2001; Dhanalakshmi et al., 2002; Chopra et al., 2004]. Moreover, fibronectin can protect aggressive prostate cancer cells from TNF-α induced apoptosis via the Akt/survivin pathway, with surviving maintaining a critical anti-apoptotic threshold [Fornaro et al., 2003].

Examination of prostate cancer specimens reveals increased TNF-α expression in both epithelial tumor cells and tumor-associated macrophages [Muenchen et al., 2000; Michalaki et al., 2004]. Furthermore, serum levels of TNF-α taken from patients prostate cancer correlated with both bulky local disease and metastatic progression, and elevated levels were shown to be an independent prognostic indicator for survival [Michalaki et al., 2004]. Due to the

pleiomorphic response to TNF-α in prostate cancer cell lines it has been widely used as a therapeutic target via signal transduction inhibition. The majority of the therapeutic exploration has centered around the radiation sensitizing effects of TNF-α on multiple tumor systems including prostate cancer [Chung et al., 1998; Kimura et al., 1999]. However, recent studies have targeted inhibition of the NF-κB pathway, driving the response to TNF-α downstream in the apoptotic pathway [Dhanalakshmi et al., 2002; Papandreou and Logothetis, 2004]. A recent weapon in the cancer armamentarium, proteosome inhibition, has been shown to target numerous signaling pathways, most notably NF-κB activation, with promising results in patients with hormone refractory disease [Papandreou and Logothetis, 2004].

Interleukin-6. Similar to TNF-α, IL-6, while traditionally described as a key mediator in the inflammatory response, has proven to be an integral part of prostate cancer biology. The ability of IL-6 to affect the intracellular apoptotic machinery can be attributed to its effects on both the PI3K/AKT pathway and the AR pathway (Fig. 1). Elevations in IL-6 lead to activation in the PI3K/AKT pathway with subsequent increases in Bcl-xl [Pu et al., 2004; Xie et al., 2004] and resistence to standard chemotherapy-induced apoptosis. This increase in AKT activation has also been associated with neuroendocrine differentiation, a common phenotype of treatment resistant prostate cancer cells [Xie et al., 2004]. Additional evidence demonstrated that IL-6 stimulates AR activity in the absence of androgen via the STAT3 pathway [Lee et al., 2004]. This ability to bypass the receptor ligand interaction of androgen and its receptor allows IL-6 to protect hormone sensitive prostate cancer cells from apoptosis induced by androgen deprivation [Lee et al., 2004]. Interestingly, there is an inversely proportional correlation between IL-6 expression and androgen expression, in aging, healthy male subjects, and the effects of IL-6 on hormone responsive cell lines can be blunted with the addition of androgen [Kim et al., 2004; Xie et al., 2004]. IL-6 overexpression in the prostate cancer microenvironment is due to both autocrine and paracrine feedback loops. The constitutive overexpression of IL-6 in hormone resistant prostate cancer cell lines has been attributed to an autocrine loop governed by the NF-κB activity [Zerbini et al., 2003]. Within the bone microenvironment IL-6 plays a critical role in osteoblast paracrine interactions with metastatic prostate cancer cells [Garcia-Moreno et al., 2002; Lu et al., 2004].

The clinical significance of IL-6 is exemplified in its prognostic capabilities. Studies including serum measurements of IL-6 with or without the addition of its soluble receptor have shown both to be powerful predictors of PSA failure, disease progression, and mortality [Shariat et al., 2001; George et al., 2005]. Furthermore, preoperative serum IL-6 measurements appear to be an effective screening tool for occult metastatic disease at the time of resection [Shariat et al., 2001] and may prove valuable in the development of adjuvant therapy protocols. Attempts to directly target IL-6 expression with monoclonal antibody therapy have had moderate success in animal models [Smith and Keller, 2001]. One could easily argue that as autocrine IL-6 production is apparently NF-κB driven, NF-κB targeting and proteosome inhibition may ultimately inhibit this cytokine's prosurvival activity as well.

CONCLUSION

As one dissects the mechanisms underlying prostate cancer progression to hormone independence and treatment resistance during the clinical course of the disease, the role of apoptotic evasion takes center stage. The ability of prostate cancer cells to activate intracellular survival pathways coupled with the critically dynamic intracellular cross-talk and anti-apoptotic pathway redundancy leaves a formidable opponent for the most powerful of cytotoxic therapies, much less hormone withdrawal. Furthermore, the ability of these cells to adapt to their extracellular microenvironment by alterations in: (1) epithelial-stromal interactions; (2) pathophysiologic cellular stress responses; (3) growth factor-receptor pathways; or (4) the inflammatory response; allows the most hostile of tumor microenvironments to promote rather than inhibit cancer cell survival and, ultimately, encourage aggressive phenotypes. Whether it is the upregulation of intracellular survival pathways or the extracellular influence that upregulates intracellular anti-apototic signal transduction that allows for such aggressive adaptation remains a subject

of debate. It is becoming increasingly apparent however that our ability to positively improve the therapeutic response and survival of patients with hormone-refractory metastatic prostate cancer will ultimately require a therapeutic arsenal that targets multiple and often functionally overlapping signal transduction pathways rather than the current, frequently ineffective attempts at monotherapy for advanced disease.

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