IAP as a new diagnostic and effective therapeutic target molecule for prostate cancer

Review Article

Takeo Nomura*, Fuminori Satoh, Mutsushi Yamasaki, Hiromitsu Mimata
Department of Oncological Science (Urology), Oita University Faculty of Medicine, Japan

*Correspondence: Takeo Nomura, Department of Oncological Science (Urology), Oita University Faculty of Medicine, 1-1 Idaigaoka, Hasama-machi, Yufu, Oita 879-5593, Japan; Phone: 81975865893; Fax: 81975865899; e-mail: TAKE@med.oita-u.ac.jp

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Abbreviations: androgen-independent prostate cancer, (AIPC); androgen receptor, (AR); B-cell lymphoma-2, (Bcl-2); B-cell lymphoma-x long, (Bcl-xL); baculoviral IAP repeat, (BIR); caspase activation and recruitment domain, (CARD); cellular IAP, (cIAP); dihydrotestosterone, (DHT); epidermal growth factor, (EGF); human IAP, (hIAP); heat shock protein, (Hsp); high temperature requirement A2, (HtrA2); inhibitor of apoptosis protein, (IAP); immunohistochemistry, (IHC); insulin-like growth factor, (IGF); IGF binding protein, (IGFBP); c-jun N-terminal kinase, (JNK); keratinocyte growth factor, (KGF); prostate-specific antigen, (PSA); really interesting new gene, (RING); second mitochondrial derived activation of caspase/direct IAP binding protein with low pI, (Smac/DIABLO); sex hormone-binding globulin, (SHBG); small-interesting RNA, (siRNA), TGF β-activated kinase, (TAK); transforming growth factor β, (TGF β); tumor necrosis factor, (TNF); TNF receptor-associated factor, (TRAF); TNF-related apoptosis-inducing ligand, (TRAIL); XIAP-associated factor 1, (XAF1); X-chromosome-linked IAP, (XIAP)

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Summary

Prostate cancer is the second most common cause of cancer-related death among men in the United States. The conversion from androgen-dependent to androgen-independent state constitutes an important event in prostate cancer progression and is the main obstacle to improving the survival and quality of life in patients with advanced prostate cancer. Considerable progress has been made in the understanding of the molecular basis of prostate cancer. Prostate cancer progression and the development of the androgen-independent characteristics have been largely related to genetic abnormalities that not only androgen receptor (AR) but also crucial molecules involved in the regulation of survival or apoptotic pathways. One of these molecules including p53 and the B-cell lymphoma-2 (Bcl-2) family, the antiapoptotic protein, the inhibitor of apoptosis proteins (IAPs) have been associated with the promotion of tumorigenesis and drug sensitivity in prostate cancer due to their overexpression in prostate cancer cells treated with androgen ablation or chemotherapeutic agents. Therefore, IAPs may be of great value in clinical and prognostic markers in patients with prostate cancer and therapies that target IAPs may have the potential to improve outcomes for patients. In this review, we focus on the experimental evidence that associates IAPs expression with prostate carcinogenesis and cancer progression, and summarize the roles of IAPs in chemotherapy to develop a new target for the diagnosis and treatment of prostate cancer.

I. Introduction

Prostate cancer is the most frequently diagnosed disease and the second leading cause of cancer-related death in men in the United States (Greenlee et al, 2001). An estimated 240,000 newly diagnosed cases are expected to occur and close to 30,000 patients die from this disease in the United States in 2006. Worldwide, prostate cancer is the fourth most common male cancer with incidence and mortality rates that vary tremendously among countries or ethnic groups. Incidence and mortality rates are higher in Western countries than in Asian countries. Among Asian countries, Japan has the lowest prostate cancer incidence and mortality rates in the world, however, prostate cancer incidence and mortality in Japan have increased gradually as the country has become Westernized since 1990s (Landis et al, 1998). Since the early 1990s around the time a new screening prostate-specific antigen (PSA) test has been introduced and in widespread use among the world, prostate cancer incidence raised dramatically with a true increase in the number of patients with clinical prostate cancer rather than a simple result of increased detection (Brasso and Iverson, 1999; Hankey et al, 1999). In general, prostate cancer is a relatively slow growing and indolent malignancy with doubling times for local tumors...
estimated at 2 to 4 years. Although localized prostate cancer can be controlled successfully with surgery or radiation therapy, 15% of patients relapse after apparently successful treatment, eventually progress and metastasize (Hull et al, 2002). Prostate cancer is characterized by an initial stage during which the tumor growth is dependent on androgen receptor (AR) signaling triggered by dihydroteosterone (DHT). Therefore, in addition to surgical resection (prostatectomy) and radiation therapy in localized prostate cancer, the main effective treatment for local recurrence after surgery or after radiation therapy or advanced prostate cancer with distant metastasis is androgen deprivation procedures surgically with orchietomy or medically with estrogens or luteinizing hormone-releasing hormone analogues and by blocking the effects of residual androgen with competitive AR antagonists like flutamide, bicalutamide, and nilutamide. Since 1940s, hormone therapy has been the mainstay of treatment for advanced prostate cancer. The androgen withdrawal could produce a response rate of 70-80% with a median progression-free survival of 12-33 months and a median overall survival of 23-37 months (Hurtado-Coll et al, 2002). Androgen ablation induces rapid and dramatic responses with symptomatic relief by inducing apoptosis, but the effect is usually palliative and temporary. Despite the initial response to anti-androgen therapy, the disease recurs in androgen-independent state that is unresponsive to the existing treatments including additional androgen withdrawal and chemotherapy, as well as a combination of these therapies, within 12-18 months (Petrylak, 1999). The management of androgen-independent prostate cancer (AIPC) by current chemotherapeutic regimens can temporarily eliminate androgen-independent cells by inducing apoptosis, however, these chemotherapeutic agents are generally less effective. Overall median survival from first metastasis is typically 3 years from the time of diagnosis and is 2 years from androgen independence. Thus, AIPC constitutes potentially a serious life threat that accounts for the gross part of prostate cancer mortality. Therefore, to develop rationale alternative therapies and preventive treatments for AIPC, much attention has been directed to understanding the molecular basis for the progression to androgen independence in this process.

Although the specific causes of prostate cancer initiation and progression are unknown, it seems valid that both genetics and environment play an important role in the evolution of this disease. Two main potential mechanisms have been identified during the process of the development of AIPC. The first mechanism is hypersensitivity of AR signaling during the development of AIPC. This hypersensitive signaling may be caused by a variety of AR gene mutations (Taplin et al, 1995; Marcelli et al, 2000) or increased AR copy number (Koivisto et al, 1997) that result in a functionally altered expression of the AR. The second mechanism is based on the induction of a positive survival signaling independent of AR signaling pathway that can overcome the apoptosis induced by androgen ablation (Ruitter et al, 1999; Feldman and Feldman, 2001). It has been reported that insufficient apoptosis represents the explanation for the accumulation of prostate cancer cells (Carson and Ribeiro, 1993; Kerr et al, 1994). That is to say, progression of androgen-dependent tumor to hormone-refractory disease is related to genetic abnormalities that influence not only the AR but also crucial molecules involved in apoptosis. This aggressive stage of cancer is characterized by the appearance of apoptosis-resistant cells.

As previously mentioned, apoptosis is induced in prostate cancer responding to androgen ablation, radiation therapy, and chemotherapy. Molecularly, apoptosis is executed by the activation of caspasas, a family of intracellular cysteine proteases that cleave substrates at aspartic acid residues (Cryns and Yuan, 1998; Stennicke and Salvesen, 1998). Unfortunately, cancer fails to respond to treatment in varying degrees. In part, the failure of cell death is caused by failure of the apoptosis and caspase activation pathways. The inhibitor of apoptosis proteins (IAPs) are a family of antiapoptotic mediator that blocks cell death by inhibiting the downstream of the caspase activation pathway (Roy et al, 1997; Deveraux et al, 1999; Deveraux and Reed, 1999; Wright and Duckett, 2005). IAPs have been found to be involved in the molecular biology of a wide range of human cancers since their discovery as direct endogenous caspase inhibitors in baculovirus. It has been reported that there is a positive correlation between IAP expression and tumor progression in prostate cancer (Krajewska et al, 2003; Kishi et al, 2004). IAPs may play an important role in cancer progression, acquisition of androgen independence, and drug-sensitivity in prostate cancer.

Understanding the machinery of IAPs function can potentially allow for the development of novel therapeutic strategies targeting caspasas and IAPs for prostate cancer. Reviews of the actions of IAPs and their mechanism have already been well published. In this review, we will focus on the experimental evidence of the roles of caspasas and their negative regulators, IAPs in prostate cancer, and discuss how this evidence is being translated into the clinical field as the development of new diagnostic and prognostic markers and therapeutic target.

II. Prostate cancer and androgen-androgen receptor (AR) signaling

Because prostate cancer develops in aged men with low levels of androgen, a large number of studies have reported whether elevated levels of androgen are associated with an increased risk of prostate cancer. To date, the degree to which androgen or androgen metabolites such as DHT, contribute to risk of prostate cancer remains under discussion. Guess and colleagues reported in 1997 that there is no relationship between testosterone, sex hormone-binding globulin (SHBG), or 5α-reductase activity and risk of prostate cancer (Guess et al, 1997). In contrast, Gann et al observed increased testosterone levels, low levels of SHBG, and high levels of 5α-reductase activity as risk factors of prostate cancer (Gann et al, 1996). Although even well-designed clinical studies have presented conflicting views concerning the association of androgen with an increased risk of prostate cancer, it seems valid that prostate cancer initiation and
progression are strongly influenced by androgens in view of prostate cancer regression after surgical or medical castration. Androgen is a necessary growth factor for early-stage prostate cancer cells. The circulating androgen on male is composed of testosterone derived from testis and adrenal glands. Once inside prostate cells, 5α-reductatse converts androgen to DHT that is metabolically more active. The action of androgen is mediated by a specific receptor protein, AR, which is located on the human X-chromosome of epithelial and adjacent stromal prostate cells. DHT acts as the main ligand of AR and it is much more active than testosterone, having higher affinity for the AR. The AR, a transcription factor belonging to a classical nuclear receptor superfamilly, consists of a ligand-binding domain, an amino terminal activating domain, and DNA-binding domain (Gao et al, 2005). After activation of AR by phosphorylation, this activation promotes nuclear localization and binding of the steroid-AR complex to specific DNA target sequences located on androgen response elements of the androgen dependent genes such as PSA, leading to the initiation of transcription (Berger and Watson, 1989). Without AR binding, steroid hormones cannot exert their effects on prostate cancer growth.

In addition to androgens, AR also plays a crucial role in several stages of the progression of prostate cancer (Avila et al, 2001; Montgomery et al, 2001). The increased copy number of AR gene contributes to androgen-independent tumor progression (Koivisto et al, 1997). Likewise, mutations in the AR gene are identified in AIPC (Marcelli et al, 2000). These mutations in the AR gene have been found to make the AR responsive to different non-androgenic ligands, and may also contribute to the development of androgen independency. For this reason, urologists experience that AR antagonist, such as bicalutamide and flutamide, works like AR agonist suggesting that AR is stimulated by AR antagonist.

Strong evidence supports the relationship between prostate cancer progression and various peptide growth factors such as insulin-like growth factor (IGF), epidermal growth factor (EGF), and keratinocyte growth factor (KGF) (Culig et al, 1994). These growth factors released primarily by stromal cells can activate AR-related transcription on upstream of AR signaling. The cross talk between Her2/neu oncogene and AR signaling also results in the phosphorylation of AR, suggesting a mechanism whereby the pathways triggered by tyrosine kinase receptors could play a role in prostate cancer progression (Craft et al, 1999). In addition, Her2/neu also activates phosphatidylinositol 3-kinase (PI3K)/Akt survival pathway (Lin et al, 2001, Yakes et al, 2002). Although androgen is responsible for proliferative effects on prostate cancer and AR signaling, various complicated cellular regulation mechanisms that affect AR signaling are linked with the development of AIPC.

III. Regulation of apoptosis of prostate cancer cells

Even the patients with advanced prostate cancer potentially respond to androgen ablation, and serum PSA levels decrease in almost all patients. After treatment, proliferation of prostate cancer cells comes to a halt and cells fall into apoptotic cell death (Isaacs et al, 1992). The irreversible genomic DNA fragmentation takes place following activation of Ca2+/Mg2+ dependent endonuclease activity in the nucleus of apoptotic cells. However, for reasons that are only partly defined, the apoptotic process induced by androgen ablation fails to eliminate the entire cancer cells, because the threshold of apoptosis progressively drops to a point at which cell proliferation exceeds apoptosis as cancer progresses (Berges et al, 1995). This increase of proliferating cells is caused by the accumulation of androgen-independent cells that eventually relapse and metastasize. As previously mentioned, progression to androgen independence is multifactorial process by which cancer cells acquire the ability to both survive in the absence of androgens and proliferate with the use of androgen non-related stimuli for mitogenesis. In addition to the hypersensitivity of AR, inappropriate activation of AR, and alterations in the regulators of the cell survival pathway, this stage of cancer is also characterized by the emergence of apoptosis-resistant cells resulting from various genetic mutations and upregulation of antiapoptotic genes. In general, clusterin, AR, B-cell lymphoma-2 (Bcl-2), B-cell lymphoma-x long (Bcl-xl), heat shock protein 27 (Hsp27), IGF binding protein-2 (IGFBP-2), and IGFBP-5 are upregulated by androgen ablation and remain overexpressed in AIPC (Gleave et al, 2005). We previously reported the overexpression of cellular IAP-1 (cIAP-1) and cIAP-2 in prostate cancer tissue specimens treated with androgen ablation (Mimata et al, 2000), besides, it has been reported that AIPC cell lines PC3 and DU145 cells are highly resistant to drug-induced apoptosis due to the overexpression of cIAP-1, cIAP-2, X-chromosome-linked IAP (XIAP), and neuronal apoptosis inhibitory protein (NAIP) (McEleny et al, 2002). The aspect of AIPC cell biology of these antiapoptotic genes is developing rapidly, and IAPs may prove to be the importance of antiapoptotic action in prostate cancer progression.

IV. IAP: Cell survival gene

The IAPs have been identified as one of the most potent inhibitors of endogenous caspases and apoptosis. Unlike Bcl-2 protein, which blocks the mitochondrial pathway of apoptosis, the antiapoptotic function of IAPs is due to its ability to inhibit both intrinsic mitochondria-mediated and extrinsic death receptor-mediated pathways by directly binding to and inhibiting both initiator and effector caspases (Deveraux et al, 1997, 1998; Roy et al, 1997; Devi, 2004). To date, at least eight IAP-encoding genes have been recognized and reported in the human genome, including XIAP, human IAP-1 (hIAP-1, cIAP-2), hIAP-2 (cIAP-1), survivin, NAIP, apollon (BRUCE), livin (ML-IAP, KIAP), and IAP-like protein-2 (ILP-2), which are evolutionarily conserved with apparent homologies identified in flies, worms, yeast and several mammalian species including mice, rats, chickens, pigs, and humans (Roy et al, 1997; Deveraux et al, 1998; Tamam et al, 1998; Chen et al, 1999; Kasof and Gomes, 2001; Richter et al, 2001). All the IAPs show varying degrees of antiapoptotic effect, depending on the different mechanisms of action.
for each IAP homology. The IAPs are characterized and grouped together based on the presence of a highly conserved domain of ~70 amino acid motif termed the baculoviral IAP repeat (BIR) domain (Verhagen et al., 2001). In addition to BIR domains, IAPs possess caspase activation and recruitment domain (CARD) and really interesting new gene (RING)-zinc binding domains (Deveraux and Reed, 1999; Yang et al., 2000). IAPs can bind to and potently inhibit activated caspase-3, -7, and -9 through some of BIR domains, suggesting that the majority of IAPs activities are dependent on BIR domains (Deveraux and Reed, 1999). Both hIAP-1 and hIAP-2 inhibit caspase-3, -7, and -9, but they are less potent than XIAP (Zhivotovsky and Orrenius, 2003). A RING domain has ubiquitin protease activity; it can bind to ubiquitin-conjugating enzymes that promote autoubiquitination and degradation of IAP-caspase complexes after apoptosis stimulus, suggesting that RING domain-dependent proteasome caspase degradation may be another mechanism of the IAPs’ antiapoptotic activity (Yang et al., 2000). The requirement of RING domain for inhibition of apoptotic pathway seems to be dependent on the type of cells. The function of the CARD domain in hIAP-1 and hIAP-2 remains unknown. IAPs can protect cells from various triggers of intrinsic and extrinsic pathways. All the IAPs except NAIP can bind to and inhibit caspase-3 and -7 (Roy et al., 1997; Deveraux et al., 1998). XIAP, hIAP-1, hIAP-2, and survivin were also shown to bind to and inhibit caspase-9, but not caspase-1, -6, -8, or -10 (Roy et al., 1997). Although IAPs cannot bind to or inhibit caspase-8, they bind to and inhibit its substrate caspase-3, thus providing protection from death receptor-mediated apoptosis, because both death receptor-mediated and mitochondria-mediated pathways converge finally at the level of activation of caspase-3 (Roy et al., 1997; Deveraux et al., 1998). In contrast, XIAP, hIAP-1, and hIAP-2 bind to directly pro-caspase-9, and prevent its processing and activation induced by cytochrome c released from mitochondria, thus they can prevent the proteolytic processing of pro-caspase-3, -6, and -9 (Deveraux et al., 1998). It has been reported that XIAP mainly binds to active caspase-3, but also partially to the unprocessed pro-caspase-3 (Deveraux et al., 1997). We transiently cotransfected with XIAP and pro-caspase-3 cDNAs into LNCaP cells, and observed the strong interaction between XIAP and pro-caspase-3 by immunoprecipitation and immunoblot analysis (Nomura et al., 2003). XIAP may block a common downstream by directly inhibiting pro- and active caspase-9, and by interfering with caspase-3 activity and/or processing of pro-caspase-3. Interestingly, the activity of IAPs are controlled at various levels by the transcriptional factor, nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) signalling pathway. The hIAP-1 and hIAP-2 also bind to tumor necrosis factor receptor-associated factor (TRAF) heterocomplexes through their N-terminal BIR domain, interfering with the upstream activation of caspase-8 (Rothe et al., 1995; Wang et al., 1998). TRAF-1, TRAF-2, XIAP, hIAP-1, and hIAP-2 are identified as gene targets of NF-κB and the induction of XIAPs are an essential part in the process that protects cells from apoptotic signals caused by tumor necrosis factor-α (TNF-α). In addition, XIAP, NAIP, and ML-IAP bind to transforming growth factor β (TGF β)-activated kinase (TAK-1) and activate TAK-1/c-Jun N-terminal kinase (JNK) signaling cascade, with resulting inhibition of apoptosis (Sanna et al., 2002). TAK-1 dependent JNK activation also plays an important role in antiapoptotic efficacy of IAPs. In addition to regulation of apoptosis, IAP members such as survivin have been found to be a potent regulator of cell cycle progression and mitosis (Reed and Bischoff, 2000). Survivin was found in cytosolic fraction but it is also associated with chromatin expressed in G2/M phase and downregulated after cell cycle arrest, suggesting that survivin plays a role in monitoring chromosome replication and the inhibition of caspase activity in the nucleus (Ambrosini et al., 1997; Li et al., 1998). The evidence that survivin regulates apoptosis through a cyclin-dependent kinase inhibitor p21WAF1/Cip1 pathway is well documented (Beltrami et al., 2004; Fukuda et al., 2004). Interestingly, it has been also reported that survivin may contribute to tumor angiogenesis via angiopoietin-1 stabilization (Papapetropoulos et al., 2000). Survivin may regulate cell death by not only an antiapoptotic mechanism but also a caspase cascade-independent mechanism. Taken together, IAPs are classically regarded as caspase inhibitors, but the possibility exist that IAPs have multiple mechanisms of cancer growth and protection from cell death beyond the role as the direct inhibitors of caspases.

V. Endogenous IAP inhibitors

Currently, the following three endogenous regulatory proteins are known as blockers of IAPs; second mitochondrial derived activation of caspase/direct IAP binding protein with low pI (Smac/DIABLO), high temperature requirement A (HtrA2/Omi), and XIAP-associated factor 1 (XAF1). Smac/DIABLO and HtrA2/Omi are mitochondrial proteins first identified in Drosophila and subsequently recognized in humans (Srinivasula et al., 2002). Smac/DIABLO is released into the cytosol together with cytochrome c during mitochondrial disruption. Cleaved active form of Smac/DIABLO can inhibit IAPs through binding to some BIR domains of IAPs, resulting in degradation of IAPs protein by ubiquitin/ubiquosome pathway (Ekert et al, 2001). HtrA2/Omi, which belongs to the shock response serine protease-chaperone HtrA family, is released along with Smac/DIABLO from mitochondria (Gray et al, 2000; Hegde et al, 2002). XAF1 is known as the other endogenous antagonist of XIAP, which has the ability to directly interact with XIAP and exclusively blocks its antiapoptotic activity (Byun et al, 2003). Unlike Smac/DIABLO and HtrA2/Omi, XAF1 is located in the nucleus and affect a redistribution of XIAP from cytosol to the nucleus, resulting in inactivation of XIAP (Liston et al, 2001). Interestingly, XAF1 is mainly expressed in normal tissues but is low or missing in most cancer cells, which implies a tumor-suppressing function in the tumorigenic process. Taken together, these endogenous IAP inhibitors may play an important role as a potent tumor suppressor.
VI. IAP expression in prostate cancer

The upregulation of IAPs expression has been considered one of mechanisms for escape from elimination by apoptosis. Therefore, to investigate the IAPs expression in prostate cancer is essential for the development of novel therapeutic strategies targeting IAPs for prostate cancer. Overexpression of IAPs was observed in all the most common cancers by analysis of its transcript and protein. Evidence is accumulating that the levels of IAPs expression are related to progression and poor prognosis, including breast cancer (Tanaka et al, 2000), esophageal cancer (Kato et al, 2001), gastric cancer (Lu et al, 1998), colorectal cancer (Kawasaki et al, 1998), neuroblastoma (Adida et al, 1998), non-small cell lung cancer (Monzo et al, 1999), urinary bladder cancer (Swana et al, 1999), epithelial ovarian cancer (Sui et al, 2002), liver cancer (Ito et al, 2000), uterine cancer (Saitho et al, 1999), skin cancer (Grossman et al, 1999), and leukemia (Nakagawa et al, 1999), etc. These results suggest that IAPs may contribute to tumor progression and that detection of IAPs provides a specific and sensitive diagnostic marker. However, there have been few reports on the expression of IAPs in prostate cancer. Kishi and colleagues reported in 2004 that survivin mRNA expression was positively correlated with the progression (T-stage, lymph node metastasis, vessel invasion, surgical margin, and Gleason score) and aggressiveness (proliferative activity) in prostate cancer tissue specimens obtained from prostatectomy (Kishi et al, 2004). Shariat et al showed that survivin expression was associated with higher Gleason score and positive lymph node metastasis (Shariat et al, 2004). In contrast, Krajewska and colleagues reported in 2003 that expression levels of survivin, hIAP-1, hIAP-2, and XIAP by immunohistochemistry (IHC) on the microarrays elevated in prostate cancer, but the levels of these IAPs expression did not correlate with Gleason score and PSA levels (Krajewska et al, 2003). As previous reports on IAPs expression of many kinds of cancer have described, IAPs expression is thought to be a common event in prostate cancer.

The role of IAPs in the development of androgen independency has been controversial. IAPs overexpression in commonly used prostate cancer cell lines, including androgen-dependent LNCaP cells, androgen-independent DU145 and PC3 cells was observed (Tamm et al, 2000; McEleny et al, 2002). McEleny et al confirmed the expression of cIAP-1 and cIAP-2 and XIAP, and survivin in LNCaP, DU145, and PC3 cells at the level of the mRNA and the protein. They also showed an increased expression of hIAP-1, hIAP-2, and XIAP in DU145 and PC3 cells compared with LNCaP cells, and this expression is correlated with resistance to apoptosis (McEleny et al, 2002). Another study identified the expression of hIAP-2 and XIAP in DU145 and PC3 cells, but identified hIAP-1 expression only in DU145 cells, and did not identify the expression of cIAP-1 in these cell lines (Tamm et al, 2000). Interestingly, there are poor relationships between mRNA and protein expression for survivin (McEleny et al, 2002), hIAP-1, hIAP-2, and XIAP (Tamm et al, 2000), suggesting that these proteins are post-transcriptionally regulated. We previously showed that the expression of cIAP-1 and cIAP-2 was upregulated in patients treated with androgen ablation by IHC, suggesting that the advent of residual cancer cells after androgen ablation was due to the induction of these IAPs (Mimata et al, 2000). Upregulation of IAPs may develop androgen independency. Zhang et al indicated that androgen stimulation with DHT increased survivin expression and antiandrogen therapy with flutamide decreased its expression in LNCaP cells, suggesting that survivin played a potentially important role in androgen sensitivity and resistance to androgen ablation (Zhang et al, 2005). These results, therefore, indicated that prostate cancer cells induce IAPs expression during the progression under an androgen environment. In contrast, another study indicated that IAP expression in LNCaP cells was unaffected by charcoal-stripped medium (McEleny et al, 2002). They also confirmed that androgen-supplemented medium did not influence IAP expression in LNCaP cells (McEleny et al, 2002). These results suggest that IAP expression is unrelated to an androgen environment, then androgen ablation does not affect IAP expression and the acquisition of androgen independence is not due to the expression of IAP in prostate cancer. To show whether upregulation of IAPs expression results in androgen independence or not, the fact that IAPs belong to target genes of AR signaling needs to be explained.

Taken together, IAPs, particularly survivin, are thought to be important biomarkers for diagnosis, staging, and prognosis of prostate cancer, and may be useful as therapeutic response inducer for prostate cancer patients, but a relationship between IAP and androgen response remains to be elucidated.

VII. Rationale for IAPs as therapeutic targets

There appear to be more studies in the recent literature focusing on survivin and XIAP as potential therapeutic targets. The reason for this is that among IAPs, XIAP is the most potent inhibitor of caspases and apoptosis (Roy et al, 1997), and that survivin plays an important role in mitosis and angiogenesis as well as an inhibitor of caspases (Papapetropoulos et al, 2000; Reed and Bischoff, 2000; Adams et al, 2001). In addition, the most important feature of these two molecules is that there are upregulated in various cancers and high levels of survivin and/or XIAP are associated with poor prognosis. The antiapoptotic effects of survivin and XIAP on response to irradiation and chemotherapeutic agents have been extensively documented.

Radiation triggers the mitochondria-mediated pathway, resulting in apoptotic cell death in cancer (Zhivotovsky et al, 1999). It is reported that a low dose of γ-irradiation in non-small cell lung cancer resulted in upregulation of XIAP, and cancer cells acquired the resistance to γ-irradiation (Holcik et al, 2000). Another study showed that an inverse relationship between survivin expression and radiosensitivity in pancreatic cancer (Asanuma et al, 2000). Although radiation therapy is an
effective treatment for localized prostate cancer, the development of radioresistance may occur in some cases with advanced prostate cancer. Since there are no reports on the expression of IAPs in prostate cancer patients after radiation therapy, studies explaining the elusive mechanisms following radiation are expected to constitute a rational approach for molecular targeting treatment.

There have been no satisfactory chemotherapeutic strategies for the treatment of both hormone-sensitive and hormone-resistant prostate cancer. Recently, docetaxel (taxotere) or paclitaxel (taxol) based combination chemotherapy demonstrated significant antitumor activity and improvement in overall survival in advanced prostate cancer (Trivedi et al, 2000; Tannock et al, 2004). Although the relationship between IAPs expression and chemosensitivity is still unknown in prostate cancer, several experimental studies showed inhibition of apoptosis by IAPs in response to chemotherapeutic agents in various cancers (Tamm et al, 2000; Li et al, 2001; Nomura et al, 2003, 2004; Chandele et al, 2004). Overexpression of IAPs such as XIAP and survivin confers resistance to chemotherapy and stimuli that trigger the intrinsic and extrinsic pathways of caspase cascade. We previously showed that overexpression of XIAP by stable transfection in LNCaP cells inhibited taxol- and cisplatin-induced apoptosis (Nomura et al, 2003, 2004). Another study reported that XIAP suppressed apoptosis following treatment with some genotoxic agents or after irradiation in myeloid leukemia cells (Datta et al, 2000). In addition to experimental studies, the relationship between increased IAPs expression and chemosensitivity was clinically reported in several cancers (Kato et al, 2001; Schlette et al, 2004). Survivin expression is a useful as a prognostic and therapeutic response indicator for esophageal cancer (Kato et al, 2001) and lymphoma patients (Schlette et al, 2004). These results also suggest that IAPs have the potential as a novel determinant of chemosensitivity and therapeutic target.

Cisplatin, a most effective and widely used chemotherapeutic agent, is reported as a negative regulator of XIAP in several cancers including ovarian cancer (Sasaki et al, 2000; Li et al, 2001), oral cancer (Matsumiya et al, 2001), hepatic cancer (Notarbartolo et al, 2005), and glioblastoma (Roa et al, 2003). We reported that cisplatin induced apoptosis by the inhibition of XIAP expression and cisplatin sensitivity was dependent on the levels of XIAP protein expression in LNCaP cells (Nomura et al, 2004). We also reported that cisplatin-resistant LNCaP cells overexpressed hIAP-2, XIAP, and survivin, resulting in cross-resistance to several chemotherapeutic agents (Nomura et al, 2005). The most common reason for acquisition of resistance to a broad range of anticancer agents is expression of energy-dependent transporters that eject anticancer agents from cancer cells, but other mechanisms of resistance including insensitivity to drug-induced apoptosis by overexpression of IAPs probably play an important role in acquisition of chemo-resistance. Although the current regimens of chemotherapy for prostate cancer have no satisfactory advantage, therapeutic strategies interfering with XIAP expression by cisplatin may confer a novel insight to develop a XIAP-targeted therapy.

VIII. Future direction

Although the research works have revealed a molecular biology of IAPs, yet it is far from being satisfactory. Currently, two approaches to the management of IAPs activity are being investigated; antisense oligonucleotides and small molecule inhibitors. Antisense oligonucleotides against XIAP and survivin are in clinical phase I trials, but small molecule inhibitors are now under way in the laboratory. The antisense molecule in clinical trial is a mixture of DNA and RNA oligonucleotides. Antisense oligonucleotide can inhibit the protein expression by promoting the degradation of mRNA, therefore, this approach is more effective than by directly inhibiting translation from mRNA to protein. It has been reported that downregulation of XIAP expression by XIAP antisense induced apoptosis and enhanced sensitivity to cisplatin and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in DU145 cells (Amantana et al, 2004). Another study showed that an antisense to cIAP-1 sensitized PC3 cells to Fas antibody and TNF-mediated apoptosis (McEleny et al, 2004). Adenoviral vector of survivin antisense fragment induced apoptosis in DU145 cells and sensitized cancer cells to chemotherapeutic agents docetaxel and etoposide in vitro and in vivo (Hayashi et al, 2005). These results suggest that antisense strategy to downregulate IAPs provides an effective therapeutic approach to hormone refractory prostate cancer.

RNA interference (RNAi) refers to a group of related post-transcriptional gene silencing mechanisms whereby double-stranded short antisense RNA post-transcriptionally silences a specific gene. The small-interfering RNA (siRNA) technique has been broadly used to investigate gene function, gene regulation, and genespecific therapeutics because of its marked efficacy and specificity (Elbashir et al, 2001). Paduano and colleagues reported that silencing of survivin gene by siRNA induced apoptosis and enhanced the sensitivity to the heat shock protein 90 (Hsp90) inhibitor in DU145 cells (Paduano et al, 2006). We confirmed that LNCaP cells transfected with synthetic double-stranded siRNA against XIAP are enhanced to suppress cell growth by inducing apoptosis and sensitized to taxol (unpublished observation). It may be proven that the application of siRNA technique to gene therapy is effective.

Several novel approaches to interference of IAP expression have not only the potential for overcoming the antiapoptotic mechanism of IAP in prostate cancer but also an insight into the function of IAP in tumor progression and drug-resistance. However, before using these technologies in human, much work remains to be done to guarantee the specificity and to optimize safe and efficacious delivery system.

IX. Conclusions

In this review, we described the molecular mechanisms of androgen independence and discussed the regulators of apoptotic pathway including IAP family
proteins in prostate cancer cells. IAP overexpression occurs commonly in prostate cancer as an early event but it may cause progression and androgen independency. Thus, IAP may play an important role as a new diagnostic and prognostic marker of prostate cancer. Involvement of IAP in prostate cancer resistance to chemotherapeutic agents and other apoptotic maneuver has been investigated by use of IAP gene downregulation technique, supporting to validate IAP as potent therapeutic targets for prostate cancer. Various strategies to downregulate IAP including antisense, small molecules, and siRNA etc in cancer cells are currently under investigation with promising results. IAP family proteins thus may be candidate drug discovery target molecules for restoration of apoptosis sensitivity in prostate cancer. Besides targeting IAP, antagonists of IAPs may be also of great value as novel target genes. Overall, a better molecular knowledge of mechanisms that regulate IAP expression in prostate cancer contribute to developing a promising new approach for the treatment of prostate cancer.

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Takeo Nomura