Angiotensin II Receptor Blocker: Possibility of Antitumor Agent for Prostate Cancer

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Abstract: It is known that the renin-angiotensin system (RAS) plays a fundamental role not only as a vasoconstrictor in controlling blood pressure and electrolyte/fluid homeostasis, but also as a mitogenic factor through the Ang-II type-1 (AT1) receptor in smooth muscle cells and cardiac myocytes. Angiotensin II (Ang-II) is indeed thought to be a growth factor, and Ang-II receptor blockers (ARBs), a class of antihypertensive agent, suppress signal transduction pathways mediated by several growth factors or cytokines, through the AT1 receptor. There is increasing evidence that the RAS is implicated in the development of various cancers. We previously demonstrated that ARBs have the potential to inhibit the growth of prostate cancer cells and tumors through the AT1 receptor. This review highlights the possibility of ARBs as novel agents for prostate cancer as well as other cancers, and reviews the literature on this area.

Key Words: Angiotensin II, renin-angiotensin system, angiotensin II receptor blocker, prostate cancer, hormone-refractory cancer.

INTRODUCTION

Prostate cancer is the most common malignant disease in men and the second most frequent cause of cancer death in the United States [1]. For patients with early-stage disease, therapy such as radical prostatectomy and radiotherapy is beneficial. Since Huggins and Hodges first reported androgen ablation therapy for prostate cancer in 1941 [2], hormonal therapy with the concept of androgen ablation or blockade of androgen's action through the androgen receptor (AR) remains critical and universal, especially for advanced prostate cancer. However, although hormonal therapy for patients with advanced prostate cancer generally provides good efficacy initially, most patients develop resistance to treatment within several years, and the survival of those patients therefore remains poor.

Hormone-refractory prostate cancer (HRPC) has been especially attributed to amplification or point mutations of the androgen receptor (AR) [3], in addition to the existence of AR cofactors [4]. Other possible factors include various growth factors and cytokines acting in an autocrine or paracrine loop. To identify specific genes related to prostate cancer, we previously performed differential display PCR (DD-PCR) and GeneChip analysis using prostate cancer cells and tissue. To date, using DD-PCR analysis, we have identified several genes including liprin-α2 and nmt55; liprin-α2 gene expression was down-regulated by dihydrotestosterone (DHT) in prostate cancer cells, and nmt55 gene expression was up-regulated in human prostate cancer tissue [5, 6]. Using GeneChip analysis, we have found the gene neuroserpin (PI-12: a protease inhibitor-12), whose expression was higher in cancer than in normal tissue [7]. Currently, numerous studies seeking key genes related to prostate cancer have been performed in many laboratories.

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Angiotensin-II (Ang-II) is well known as a central factor in those factors constitutionally associated with hypertension, and also as a main effector peptide of the renin-angiotensin system (RAS), and its molecular mechanisms have recently been elucidated, especially in cardiovascular cells. Interestingly, Ang-II receptor blockers (ARBs) and angiotensin I converting enzyme (ACE) inhibitors have recently been reported to have antiproliferative activity [8]. Surprisingly, similar actions of Ang-II seem to occur in several kinds of cancer tissue, as we previously reported that Ang-II is a growth factor, and that an ARB could inhibit the proliferation of prostate cancer [9].

The fact that ARBs have the potential for a beneficial effect on various kinds of diseases prompted us to investigate the pathophysiological action of Ang-II. Consequently, it has been elucidated that RAS components, specifically Ang-II, play important roles in the development and progression of unexpected diseases. This review focuses on the role of Ang-II and the intrinsic RAS in the prostate gland, and discusses accumulated evidence concerning the pharmacological effects of ARBs on human cancer cells including prostate cancer.

ROLE OF ANDROGEN RECEPTOR IN DEVELOPMENT AND PROGRESSION OF PROSTATE CANCER

Generally, cancer development and progression are caused by many factors that are involved in DNA mutation and abnormal activity of oncogenes or tumor suppressor genes. Activation of growth factor receptors and many growth factors also contributes to cancer development and progression. Similarly to other cancers, these factors are involved in the development and progression of prostate cancer. In addition to these factors, prostate cancer has unique characteristics in that androgens and the AR signal play an important role in its development and progression (including in the normal prostate and in benign prostatic hypertrophy (BPH) progression) [10].

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Table 1. Evidence of Effect of Angiotensin II or ARB on Prostate Cancer

	Evidence	Reference
Positive	Secretion of Ang-II in rat prostate and LNCaP cells was confirmed. Angiotensin secretion in rat prostate was enhanced by DHT.	[46]
	ARB had potential biological effects including decreasing PSA and stabilizing performance status in HRPC patients.	[77]
	Ang-II activated the proliferation of prostate cancer cells, and ARB inhibited it by suppressing MAPK or STAT3 phosphorylation.	[9]
	Ang-II exerted mitogenic effects on cross-talk between stromal and cancer cells, and ARB inhibited tumor growth through actions on stromal cells.	[79]
	Prostatic RAS was overexpressed in HRPC tissue, and its components were influenced by DHT, E2, Dex or anti-androgen drugs.	[85]
Negative	Ang-II decreased the growth of DU145 cells as well as Ang-IV, and an inhibitor of aminopeptidase A abolished the effect of Ang-II.	[127]
	The anti-proliferative effect of ACE inhibitor was reversed by either Ang-II or Ang-IV, but the effect of angiotensins was not blocked by ARB.	[128]

Prostate cancer is initially hormone dependent, and growth and protection against apoptosis are controlled by androgen; however, hormone-dependent prostate cancer later becomes hormone independent. Although it is still unclear how prostate cancer growth changes from being hormone dependent to hormone independent, AR activation such as amplification, mutation and the related AR signaling is considered to play a key role [11-13].

As other factors related to AR activation, AR co-factors are considered to be important for AR transactivation. Some AR co-factors are known to be up- or down-regulators in prostate cancer. Deregulation of the expression of some AR co-factors or the interaction between AR and AR co-factors has been shown in many studies [14]. For example, ARA55 expression in HRPC is lower than that in BPH or untreated prostate cancer. Moreover, higher ARA55 expression was associated with shorter recurrence-free survival and overall survival in hormone-refractory prostate cancer patients [11].

GROWTH FACTORS AND CYTOKINES IN PROSTATE CANCER

In addition to the abnormal function of AR, many growth factors and cytokines have also been identified and characterized in prostate cancer progression, especially in the development of HRPC. For example, epidermal growth factor (EGF) and its related family members are expressed in prostate cancer cells. It is reported that EGF and its receptor (EGF-R) are expressed in prostate cancer tissue, and are associated with disease-free survival [15-17]. Furthermore, other growth factors, such as vascular endothelial growth factor (VEGF) [18, 19] and insulin-like growth factor (IGF) [20], are involved in prostate cancer progression. Inhibition of these growth factors and/or their signals is expected to provide new therapy for prostate cancer, especially HRPC.

Furthermore, various cytokines are also considered to be important for prostate cancer progression. In particular, among them, IL-6 can promote AR activity without androgen [21], through a mechanism involving STAT3 or MAPK activation [22, 23]. Another mechanism is that IL-6 induces AR expression itself and leads to enhancement of androgen-responsive gene expression [24]. The fact that IL-8 plays an important role in the proliferation of prostate cancer cells has been confirmed by *in vitro* and *in vivo* studies; IL-8 stimulation accelerates prostate cancer tumorigenesis, angiogenesis and metastasis, and IL-8 especially has the potential for promotion of HRPC progression [25, 26]. In addition to IL-6 and IL-8, other cytokines, e.g., IL-4 and IL-10, are also related to HRPC [27]. Taken together, many growth factor signals, cytokine signals and AR signals are not only independently regulated, but also interfere with each other.

CLASSICAL RENIN-ANGIOTENSIN SYSTEM (RAS)

The renin-angiotensin system (RAS) has classically been identified in reno-cardiovascular organs including the kidney, heart and vessel walls, where its enzymatic actions and produced peptides have been characterized mainly in terms of blood pressure regulation and electrolyte/fluid homeostasis. Physiologically, the main endocrine product of the RAS, Ang-II, is spliced from its liver-derived precursor angiotensinogen by renin and ACE. Renin is secreted from juxtaglomerular cells in the kidney, and cleaves circulating angiotensinogen, resulting in the formation of Ang-I (a decapeptide). Ang-I is continuously circulated in blood to the lungs, where ACE converts it to Ang-II by removing two amino acids from the C-terminus of Ang-I.

It is well known that Ang-II is a pivotal factor in the RAS, and increases blood pressure in the systemic and local blood circulation. In particular, this peptide affects the kidney and stimulates aldosterone release from the adrenal gland. These functions of Ang-II mainly induce systemic hypertension. Recently, Ang-II has been shown to play a key role in cardiac hypertrophy, heart failure, atherosclerosis and remodeling, which means that RAS exists locally and Ang-II directly functions as a pathophysiological factor in cardio-vascular and renal diseases.

Furthermore, other enzymes, aminopeptidase A (APA) and aminopeptidase N (APN), act on Ang-II or Ang-III, respectively, to yield active peptide fragments including Ang-III and Ang-IV, as shown in Fig. **1**. The biological effects of these peptides (Ang-II, Ang-III and Ang-IV) are mediated *via* specific receptors located on the cell membrane

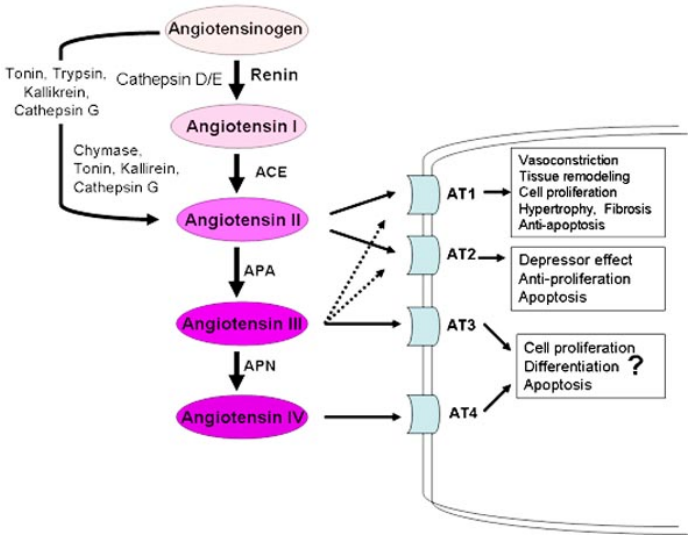

Fig. (1). Biological effects of converted Ang-II, Ang-III and Ang-IV. These are mediated *via* specific receptors; AT1 and AT2 receptors for Ang-II and Ang-III, AT3 receptor for Ang-III, and AT4 receptor for Ang-IV.

of target cells; AT1 and AT2 receptors for Ang-II and Ang-III, AT3 receptor for Ang-III, and AT4 receptor for Ang-IV.

LOCAL (TISSUE) RAS

Recently, discussion of the RAS has shifted from its endocrine role to its autocrine/paracrine role in specific tissues, associated with tissue growth and/or differentiation. Furthermore, current studies have elucidated that Ang-II has multifactorial effects including vasoconstriction, release of aldosterone, facilitation of sympathetic transmission, and trophic actions on vascular smooth muscle, cardiac myocytes and fibroblasts [28-32]. Besides the local RAS of the cardiovascular system, early studies have consolidated a body of evidence for an intrinsic RAS in other organs by identification of key RAS components - angiotensinogen, the AT1 receptor and renin. The association of the local RAS with cancer development has been recently elucidated in various organs [33-36].

Local RAS in Prostate

We expect that similar phenomena to the intrinsic RAS in many organs may also occur in the prostate gland. Dinh *et al*. reported that Ang-II immunoreactivity was markedly increased in acini in BPH compared with those in the normal prostate, while the AT1 receptor was significantly decreased in BPH [37]. Furthermore, they demonstrated that AT1 receptors were expressed abundantly on stromal smooth muscle cells, supporting the hypothesis that a local RAS in the prostate (prostatic RAS) may be strongly involved in the development of BPH. Another study indicated that ACE mRNA and protein were co-localized in the glandular epithelium [38]. Considering these findings together, it should be recognized that Ang-II exerts paracrine effects in the prostate, as in the kidney, heart, blood vessels, and pancreas [39-42].

Early reports revealed the existence of RAS in the prostate; all components of RAS, including angiotensinogen, renin, ACE, AT1 and AT4 receptors [37, 38, 43-45], were identified in the prostate. A 6 to 8-fold higher concentration of Ang-II in seminal fluid was confirmed in comparison with that in blood, which strongly supports the existence of RAS in the prostate gland. The structural locations of RAS components in the prostate have been characterized. Angiotensinogen and renin mRNA are detected in the prostate, and AT1 receptors were shown predominantly in periurethral stromal smooth muscle cells [44]. In contrast, ACE and renin are localized to the epithelium, and AT4 receptors are found in the glandular epithelium [43]. Intriguingly, Ang-II stimulated the release of noradrenaline from prostatic nerve endings, suggesting that it may play roles in anion secretion, tubular contractility and augmentation of sympathetic nerve activity. This implies that the prostatic RAS is necessary for the development of BPH.

We confirmed that AT1 receptor mRNA expression was higher in cancer tissue than in paired normal tissue [9]. In established prostate cancer cells, LNCaP cells highly expressed AT1 receptor mRNA compared to two other cancer cell lines, DU145 and PC3 cells [9]. Furthermore, a recent study demonstrated that rat prostate and human prostate cancer LNCaP cells have RAS components, which were confirmed by RT-PCR [46]. These results suggest that the prostate gland contains the intrinsic RAS, which might be involved in the pathophysiology of BPH or prostate cancer.

ANGIOTENSIN II RECEPTOR

The AT1 and AT2 receptors are functionally distinct polypeptides, with 30% sequence homology. In this section, we describe the biochemical traits of angiotensin II receptors, especially the AT1 receptor, and the molecular mechanisms of these receptors in cancer cells, including prostate cancer and various other cancers. Especially, although four receptors, the AT1 receptor, AT2 receptor, AT3 receptor, and AT4 receptor, have been identified, the biological functions of only the AT1 and AT2 receptors have been well elucidated.

AT1 Receptor

The AT1 receptor consists of 359 amino acids, and has a characteristic construction with seven transmembrane domains. The N-terminus is glycosylated at the surface of the cell, and the C-terminus is located in the cytoplasm [47, 48]. These receptors are ubiquitously present in humans, and are especially abundantly distributed in adult blood vessels and organs containing blood vessels - the heart, kidney, adrenal gland, liver, brain, and lung. This receptor binds to Ang-II with concentration-dependent affinity. It has been confirmed that the RAS is involved in the growth of several neoplasms including breast cancer, malignant glioma, hepatocellular carcinoma, renal cell carcinoma, melanoma, and pancreatic cancer. In prostate cancer, there have thus far been not so many reports besides our previous report in which AT1 receptor expression was shown to be higher in cancer tissue than in normal prostate tissue by RT-PCR analysis [9]. Besides in prostate cancer, comparative investigations of AT1 receptor expression have confirmed higher expression in breast cancer [49], laryngeal carcinoma [50], pancreatic cancer [51], and choriocarcinoma [52] than in normal tissue.

AT2 Receptor

The constructional characteristics show that the AT2 receptor is composed of 363 amino acids with seven transmembrane domains, similar to the AT1 receptor [53]. Interestingly, the antagonistic biological function of the AT1 receptor is mediated through the AT2 receptor. For instance, the AT2 receptor exerts vascular effects of vasodilation, proliferation, differentiation and apoptotic effects in endothelial cells, mesangial cells and pheochromocytoma cell lines. Furthermore, the expression of the AT2 receptor is notable, in that this receptor is highly present in fetal tissues, and after birth its expression decreases markedly. These phenomena imply that this receptor may be involved in growth and development of organs. In the adult human, the AT2 receptor has been demonstrated in many organs including adrenal cells, brain, myometrium, endothelial cells, pancreas, heart, kidney and female reproductive organs.

The role of the AT2 receptor in tumor growth and angiogenesis has been reported. Takagi *et al*. showed that the AT2 receptor increased chemical carcinogen-induced tumorigenesis in the colon through down-regulation of CYP2E1 expression in the liver [54]. With respect to stimulation of angiogenesis through the AT2 receptor, Rizkalla *et al*. reported that its antagonist (PD123319) attenuated Ang-II-associated increases in renal VEGF gene and protein expression to the same degree as an ARB. This finding suggests that the AT2 receptor, in addition of the AT1 receptor subtype, plays an important role in mediating the proliferative actions of Ang-II in the kidney [55]. It was reported that AT1 and AT2 receptor blockade attenuated renal injury and proteinuria as well as reducing cellular proliferation [56]. A similar phenomenon was observed in the retina, indicating that increased VEGF expression by Ang-II infusion was attenuated by AT1 and AT2 receptor blockade in diabetic rats [57]. Furthermore, *in vivo* studies demonstrated that the AT2 receptor activates nuclear transcription factor kappa B [58] and has trophic effects on blood vessels [58, 59]. These finding highlight the importance of the AT2 receptor in cellular proliferation and angiogenesis, and suggest that AT2 receptor blockade may confer an inhibitory effect on tumorigenesis.

AT4 Receptor

Binding of the AT4 receptor to Ang IV or Ang II (3-8) (Val-Tyr-Ile-His-Pro-Phe), a hexapeptide fragment of Ang II, has recently been characterized [43]. The AT4 receptor has been demonstrated in the brain, heart, bladder, spleen, kidney and prostate. Although the biological function of the AT4 receptor is obscure, some studies suggest that it may regulate blood flow, memory retention and neuronal development. Interestingly, in the normal human prostate, the AT4 receptor is localized to the glandular epithelium and is not present in the stroma, while its expression is significantly decreased in BPH tissue. This expression pattern is similar to that of the AT2/Ang-II system, and it is therefore suggested that the AT4/Ang-IV system may be implicated in ionic transport and glandular secretion in the prostate gland [43].

ANTIHYPERTENSIVE DRUGS AND CANCER RISK

Lever *et al*. performed a retrospective cohort study that raised the possibility of protection against cancer by the use of ACE inhibitors. Following that report, several studies concerning the association between antihypertensive medication and cancer risk have been presented. Regarding general cancer risk, the use of immediate-release calcium channel blockers (CCB) and thiazide diuretics may increase the risk of breast carcinoma among older women, while ACE inhibitors did not alter the risk of breast carcinoma [60]. On the other hand, a population-based cohort study in Denmark revealed that among users of ACE inhibitors, no risk reduction was observed for cancers including those of the breast and female reproductive tract, but not renal cell carcinoma [61]. Also, there was no difference between patients randomly assigned to conventional drugs, CCB or ACE inhibitors [62].

Regarding prostate cancer, Ronquist *et al*. found that users of captopril, an ACE inhibitor, showed a lower risk of subsequent prostate cancer, based on data from the General Practice Research Database in UK [63]. In contrast, a Canadian group reported that long-term use of β-blockers and α-blockers may prevent prostate cancer, whereas CCB or ACE inhibitors did not influence prostate cancer risk [64]. Thus, there has been some controversy on the association between antihypertensive agents and cancer risk. Further research is therefore needed to epidemiologically explore this association.

FUNCTION OF ARBS IN CANCER CELLS

Inhibitors of Angiotensin-I-Converting Enzyme (ACE Inhibitors)

Inhibitors of angiotensin-I-converting enzyme (ACE inhibitors), which inhibit stimulation by Ang-II by decreasing its production, were developed as first-line drugs for hypertension and are widely used clinically. Recently, much evidence has accumulated that ACE inhibitors have inhibitory potential against cancers, *in vitro* and *in vivo*. ACE

inhibitors retard the growth of a wide variety of cultured cancer cells *in vitro* [65, 66], and further have the potential to inhibit tumorigenesis and angiogenesis induced in cancer in animal models *in vivo* [66-68]. As mentioned above, some groups reported clinical evidence that long-term Ang-II blockade by an ACE inhibitor may have a protective effect against cancer, and suggested that it could prevent carcinogenesis. These reports support the hypothesis that Ang-II accelerates carcinogenesis, and blockade of Ang-II stimulation has inhibitory potential against carcinogenesis.

Angiotensin II Receptor Blockers (ARBs)

Numerous orally active, selective AT1 receptor antagonists (angiotensin receptor blockers, ARBs) have been synthesized and available for the treatment of hypertension since the 1990s [69, 70]. Losartan, valsartan, irbesartan, eprosartan, telmisartan, and candesartan cilexetil were approved by the Food and Drug Administration in 2000, and novel selective ARBs have now been developed. They have high affinity for AT1 receptors and almost no affinity for AT2 receptors. Their binding to AT1 receptors is competitive, with very slow dissociation. They dose-dependently block the response to exogenous Ang-II [71]. ARBs share the same mechanism of action; however, they have different pharmacokinetic profiles. Besides lowering elevated blood pressure, ARBs have further beneficial actions including amelioration of vascular diseases, post-myocardial infarction remodeling, and preservation of renal function in nephropathy [72-74].

ARBs and Prostate Cancer

We previously reported the potential of ARBs as novel therapeutic agents for HRPC [9]. First, we evaluated the expression of AT1 in prostate cancers, which showed that AT1 receptor mRNA was expressed in both prostate cancer and adjacent normal prostate tissue obtained from patients who underwent radical prostatectomy. RT-PCR analysis indicated that AT1 receptor mRNA level tended to be increased in tumors compared with normal tissue. AT1 receptor mRNA was also recognized in human prostate cancer cell lines, LNCaP and DU145. In LNCaP, Ang-II stimulation induced tyrosine-phosphorylation of proteins through stimulation by EGF, which has potency to accelerate tumorigenesis of prostate cancer. MAPK and STAT3 were also activated immediately after stimulation with Ang-II. Additionally, candesartan cilexetil, an ARB, suppressed not only activation of MAPK and STAT3 induced by Ang-II stimulation, but also their activation induced by EGF or IL-6 stimulation, as shown in Fig. **2**. Finally, candesartan cilexetil suppressed the growth of LNCaP and DU145 cells induced by EGF. As in vascular endothelial cells [75], the AT1 receptor, one of the G-protein-coupled receptors including the endothelin-1 receptor, can transactivate EGFR, leading to activation of MAPK, STAT3 and protein kinase C (PKC) in cancer cells [76]. Although activation of MAPK is not always caused by only cell growth stimulants, the inhibition of cell growth using anti-cancer drugs most commonly requires inactivation of the MAPK and STAT3 pathways. Considering that ARBs can inhibit activation of MAPK and STAT3 through the AT1 receptor, an ARB could be a molecular targeting agent for use as an anti-cancer drug.

In *in vivo* experiments, we investigated the effect of candesartan cilexetil on tumor xenografts of DU145 cells in athymic nude mice. We confirmed that there was a statistically significant difference in tumor relative volume between control (non-treated) and candesartan-treated mice

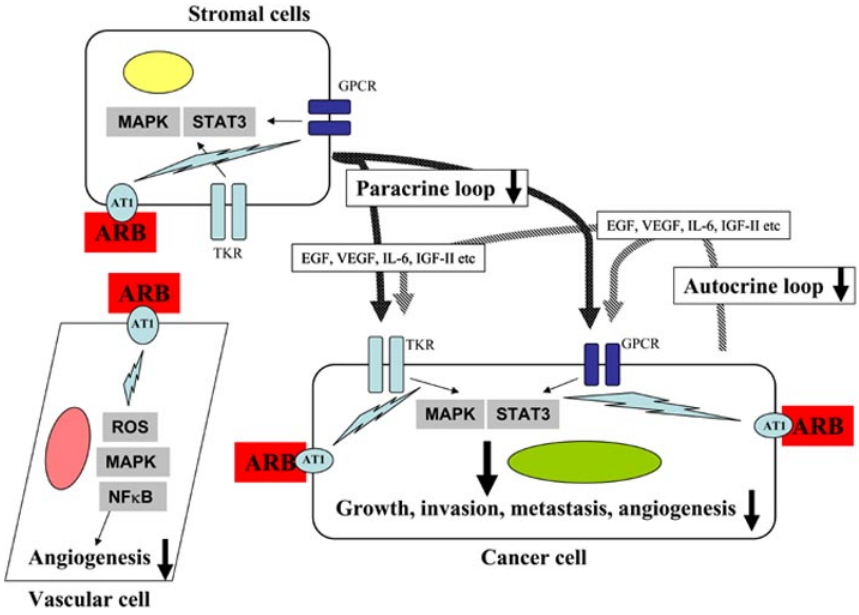

Fig. (2). Possible mechanism of ARB at multiple sites in prostate cancer cells. The ARB suppresses mitogenic actions by interaction with signal transduction *via* tyrosine kinase receptors (TKR) or G-protein coupled receptors (GPCR) in prostate cancer cells. Similarly, ARB influences stromal and vascular cells, resulting in suppression of signal transduction and transcriptional factors.

at 4 weeks. Immunohistochemical staining showed a highly statistically significant difference in microvessel number in xenografts between control and candesartan-treated mice [9].

Clinical Use of ARBs for Prostate Cancer

These experimental data prompted us to examine the clinical effects of an ARB in patients with HRPC. We conducted a pilot clinical study to examine whether an ARB was able to elicit an antiproliferative effect on HRPC clinically [77]. Surprisingly, a quarter of patients with a PSA decline of more than 50% showed an improvement in performance status. We experienced some cases in which the PSA response was delayed for several months after starting ARB treatment. Thus, we presume that the administered dose of candesartan was too low to overcome multiple metastases. Furthermore, a possible cause of the delayed PSA decline after treatment is thought to be that an ARB functions as a molecular targeting or cytostatic agent at many targeted points of signal transduction through membrane receptors such as G-protein coupled receptors. ARBs have the ability to interact negatively with the phosphorylation of MAPK or STAT3 activated by EGF or IL-6 stimulation [9]. They also have the potential to suppress the paracrine loop of growth factor or cytokine secretion from surrounding stromal tissue, which has been elucidated in vascular tissue and cardiac hypertrophy [78]. We also have confirmed a similar mechanism by which ARBs can suppress the cell growth and cytokine secretion of prostate stromal cells [79]. Based on these combined data, in advanced HRPC cases with widespread metastases, a low dose of ARB, as usually administered for hypertension, can not stop disease progression completely, but might delay it. PSA concentration rose coincident with the initiation of treatment, and several months later it declined or reached a stable state. Similar PSA kinetics were observed in a clinical trial using an angiogenesis inhibitor, TNP-470, for advanced HRPC [80]. Because both ARBs and TNP-470 are cytostatic rather than cytotoxic agents, they probably have slow PSA kinetics.

Some evidence suggesting that ARBs have antitumor potential against other solid tumors as well as prostate cancer has recently accumulated [6, 51, 67, 81]. If Ang-II and AT1 have positive potency for tumorigenesis, two possible reasons that ARBs would have a stronger anticancer effect than ACE inhibitors are expected. The first is that angiotensin I is activated not only by an ACE-dependent pathway, but also by other enzymes such as chymase, chymostatin-sensitive angiotensin II-generating enzyme (CAGE), cathepsin G, tissue type plasminogen activator (tPA), elastase, and tonin, as shown in Fig. **1**. ACE inhibitors do not inhibit all Ang-II production, whereas ARBs theoretically can inhibit all AT1 receptor activation stimulated by Ang-II. The second is that blockade of the AT1 receptor induced by ARBs increases the bioavailability of Ang-II by reducing the inhibitory effect on renin secretion, producing up-regulation and overstimulation of the AT2 receptor, which in turn potentiates antiproliferative effects [81-84].

At the end stage of HRPC, most patients develop a cachectic condition, in which they are constantly exposed to inflammatory cytokines produced by recurrent cancer cells. In other words, patients with HRPC suffer from severe chronic inflammation. Based on our clinical study using an ARB for patients with HRPC, ARBs may, therefore, be beneficial against inflammation caused by cancer. More interestingly, in our previous study, real-time RT-PCR analyses revealed that RAS components were expressed more highly in HRPC tissue than in normal and untreated prostate cancer tissue [85]. It is thus speculated that recurrent prostate cancer has greater susceptibility to ARBs compared to normal or untreated prostate cancer. Indeed, we confirmed the beneficial effect of an ARB to inhibit PSA progression in advanced HRPC patients [77]. Thus, these observations satisfactorily support the hypothesis of high expression of RAS components, especially the AT1 receptor, in recurrent prostate cancer tissue.

OXIDATIVE STRESS AND ANG-II IN PROSTATE CANCER

To date, a number of experimental and clinical studies have implicated oxidative stress in the development and progression of prostate cancer. A study of α-tocopherol (vitamin E) and β-carotene in male smokers showed chemoprevention of prostate cancer, with a 32% reduction in incidence and 41% reduction in mortality from prostate cancer in men who received supplementary α-tocopherol [86]. In another mega-study, skin cancer patients receiving selenium supplements showed a 60% decrease in the incidence of prostate cancer [87]. Substances such as α-tocopherol and selenium have the biological potential of anti-oxidant actions involving the quenching of reactive oxygen species (ROS) [88, 89]. An excess of ROS damages DNA adducts, associated with mutagenesis and carcinogenesis, and further, can increase the expression of transcription factors including oncogenes involved in neoplastic transformation [90]. Accordingly, agents such as antioxidants may be attractive for chemoprevention of prostate cancer.

Interestingly, Ang-II stimulates NADH and NADPH oxidase activity, resulting in an increase in intracellular superoxide anion formation in cultured vascular smooth muscle cells [91]. Furthermore, Ang-II-induced hypertensive rats showed an increase in vascular O^{2-} production through NADH/NADPH oxidase activation, and ARB (losartan) administration inhibited O^{2-} production and promoted vascular relaxation [92]. If these phenomena occur in the prostate, the hypothesis can be proposed that long-term administration of an ARB may reduce the incidence of prostate cancer through the biological mechanism of inhibiting oxidative stress in the prostate gland.

ANGIOTENSIN AND PROSTATE STROMAL CELLS

From the viewpoint of growth factors and cytokines in prostate cancer, it is important to understand the autocrine and paracrine mechanisms surrounding cancer cells. For instance, many reports revealed an elevated serum IL-6 level in patients with HRPC, and this cytokine is therefore thought to be involved in the progression of prostate cancer. Lee *et al*. reported that overexpression of IL-6 rendered androgen-sensitive prostate cancer cells more resistant to apoptosis induced by androgen deprivation [93]. As other growth factors and cytokines involved in the progression of prostate cancer, EGF, TNFα, HB-EGF and IGF [94] were shown to be expressed in stromal tissue.

Several recent studies have indicated that prostate stromal cells contain the AT1 receptor [95], and as demonstrated in this study, the prostate stromal cell number was increased by Ang-II treatment. It is well known that prostatic stromal cells, especially fibroblasts, are involved in the development of HRPC accompanied by the secretion of several growth factors [17, 96-98]. We have confirmed that Ang-II induced the secretion of IL-6 and other cytokines including IL-1α, IL-8 and MCP-1 from prostatic stromal cells [79]. IL-1α is required for *in vivo* angiogenesis and invasiveness of different tumor cells, and contributed to the production of VEGF and tumor necrosis factor (TNF) in tumor cells co-cultured with peritoneal macrophages [18]. IL-8, a chemokine involved in the metastasis and angiogenesis of some tumors, has been reported to be over-expressed in prostate cancer [19]. In particular, IL-8 confers androgen-independent growth and migration of LNCaP cells through activation of the androgen receptor, without androgen stimulation [20]. Therefore, IL-8 may play a role in the development of androgen-independent prostate cancer. Ohta *et al*. reported that MCP-1 mRNA was expressed in gastric carcinoma, and its expression was significantly correlated with VEGF level [99]. These factors secreted from PrSC stimulated by Ang-II treatment may contribute to the mechanisms underlying androgen independence through multiple pathways. It is, therefore, conceivable that Ang-II might induce neovascularization through activation of angiogenic factors *via* reactive prostate stroma, and specific ARBs possibly inhibit carcinogenesis through suppression of angiogenesis.

ARBS HAVE POTENTIAL AS PPAR-γ LIGAND

As well as the androgen receptor, peroxisome proliferation-activated receptor-γ (PPAR-γ) is recognized as another nuclear hormone receptor that influences prostate cancer growth. Activation of this receptor by various ligands induces apoptosis in several kinds of cancer. Established prostate cancer cell lines including LNCaP, PC-3 and DU145 cells express this receptor. Human prostate cancer tissue also expresses this receptor [100-102]; however, the molecular mechanism of the inhibition of prostate cancer cell proliferation has not been defined. Prostaglandin J2, and fibrates including thiazolindiediones, pioglitazone, and triglitazone have been reported as ligands of PPAR-γ.

Recently, Benson *et al*. reported an interesting structural resemblance between telmisartan and irbesartan, which are ARBs, and pioglitazone, a PPAR-γ ligand used for the treatment of type 2 diabetes [103]. This finding supports the hypothesis that this ARB not only blocks the Ang-II receptor, but also activates PPAR-γ, leading to regulation of carbohydrate and lipid metabolism. From the results of cellular assays of PPAR-γ activation, they found that telmisartan is also a partial agonist of PPAR-γ. In addition, accumulated evidence demonstrated that telmisartan influences the expression of PPAR-γ target genes; aP2 (FABP4), CD36, acetyl coenzyme A carboxylase (ACC), and adiponectin, in differentiating 3T3-L1 cells [103, 104]. Molecular studies suggest that telmisartan might influence PPAR-γ activity by interacting with regions of the ligand-binding domain that are not always engaged by a full agonist of the receptor. Nevertheless, other ARBs have no potential as a PPAR-γ ligand. A line of evidence has suggested that activation of RAS impairs the early steps of insulin receptor signaling such as tyrosine phosphorylation of insulin receptor substrate I or activation of PI3-kinase [105, 106], which indicates that the RAS may interact with IGF-IGFR signaling. Furthermore, ARBs showed the potential to improve insulin sensitivity in an insulin-resistant animal model [107]. It is not unreasonable to expect that a possible mechanism of the PSA decline may be related to PPAR-γ's function, especially with telmisartan and irbesartan. Therefore, agents with the function of a PPAR-γ-like ligand, such as these ARBs, could be more suitable for the treatment of HRPC patients.

ANG-II MEDIATES CALCIUM MOBILIZATION *VIA* AT1 RECEPTOR

As stated above, there is a body of evidence suggesting that Ang-II has potential not only as a steroidogenic and vasoactive peptide, but also as a growth factor-like substance able to induce hypertrophy and hyperplasia *via* the AT1 receptor [108-110]. These types of stimulation involving Ang-II require activation of several kinds of intracellular signal transduction pathways, which leads to the activation of protein kinase C [111] and Ca^{2+} mobilization. In particular, Ca^{2+} is an important mediator of the pressor and mitogenic actions of Ang-II [112, 113], and is associated with the expression of immediate early genes such as c-fos, c-jun and c-myc which regulate the transcription of target genes [114-116]. Greco *et al*. indicated that Ang-II increases intracellular calcium concentration ($[Ca^{2+}]i$) in both normal and cancerous human breast cells [117]. The greater Ang-II-evoked $[Ca^{2+}]i$ increase in cancer cells may be due to the higher expression of AT1 receptor mRNA compared to normal breast cells. Furthermore, the AT1 receptor mediates the Ang-II-dependent $[Ca^{2+}]i$ increase, and the ARB losartan blunted the $[Ca^{2+}]i$ increase induced by Ang-II in a dose-dependent manner, while an AT2 receptor inhibitor did not.

Wennemuth *et al*. confirmed, similarly to breast cells, an instantaneous linear rise in $[Ca^{2+}]i$ after local perfusion with Ang-II in a primary culture of human prostate stromal compartment (hPCPs) [118]. They demonstrated that a physiological concentration of Ang-II (10 nM) evoked a rise in $[Ca^{2+}]i$, a concentration at which Ang-II AT1 receptors are physiologically active in hPCPs. Furthermore, losartan was able to inhibit the Ca^{2+} response to Ang-II; however, PD123319, an AT2 receptor blocker, failed to have any effect. Similar results have been shown in other cells, such as mesothelial cells [119] and coronary artery smooth muscle cells [120]. In each cell type, Ang-II was observed not only to induce Ca^{2+} signaling, but also to have a mitogenic effect *via* the AT1 receptor, and ARBs inhibited it.

MICROENVIRONMENT OF PROSTATE CANCER TISSUE WITH HORMONAL THERAPY

Since the emergence of androgen ablation for prostate cancer following the report of Huggins and Hodges [2], castration therapy has been the gold standard thus far; however, hormonal therapy causes an abnormal state in men by lowering androgen. For example, androgen ablation induces apoptosis in prostate epithelium (cancer cells), leading to a hypoxic condition associated with fibrosis. It is

conceivable that stromal tissue containing fibroblasts may interact with residual cancer cells by secretion of various kinds of growth factors/cytokines including Ang-II. On the other hand, hormonal therapy induces obesity associated with expanded adipose tissue systemically. Recently, there has been a large amount of evidence that adipose tissue generates several kinds of growth factors (Fig. **3**).

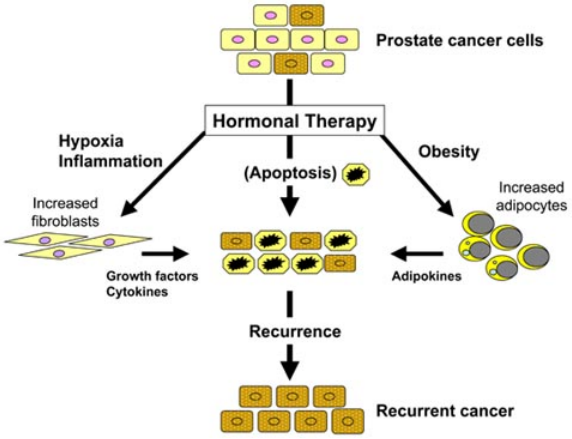

Fig. (3). Potential hormonal therapy-induced pathway to hormone-refractory prostate cancer. Hormonal therapy induces hypoxia and inflammation in prostate cancer tissue, and these sites secrete growth factors including Ang-II. Hormonal therapy increases adipocytes systemically, which also secrete various adipokines including Ang-II. Thus, chronic exposure to several growth factors or cytokines presumably induces the development of hormone-refractory cancer cells.

It is noteworthy that clinical problems attributable to increased adipose tissue include the metabolic syndrome comprising hyperlipidemia, high blood pressure, diabetes mellitus, and coronary atherosclerotic heart disease, gout, restrictive lung disease, gall bladder disease, and degenerative arthritis. Furthermore, it should be noted that obesity is a strong risk factor for some forms of cancers. Especially, evidence has emerged that adipose tissue is a source of growth factors such as IGF-I, IGF binding proteins, TNFα, Ang-II, and MCSF (so called "adipokines"). These substances have the potential to induce proliferation in surrounding cells, which indicates involvement of autocrine/paracrine factors [121]. It is noteworthy that Ang-II is also synthesized in adipocytes, and stimulates proliferation in an autocrine fashion in adipocytes themselves or in a paracrine fashion in other cell types including fibroblasts, endothelial cells and epithelial cells [122-128].

Taking these findings together, it is anticipated that hormonal therapy for prostate cancer can provide an abnormal microenvironment accompanied by exposure to various kinds of growth factors/cytokines generated by surrounding tissues (stromal and adipose tissue). Consequently, chronic exposure to growth factors/cytokines containing Ang-II may stimulate remaining prostate cancer cells, and subsequently, these cells become resistant to any kind of treatment and HRPC cells may re-grow.

CONCLUSION AND NEW DIRECTIONS

Ang-II receptor antagonists (ARBs) have the potential to inhibit the growth of prostate cancer and stromal cells. In particular, these drugs elicit multifactorial changes in cell proliferation, angiogenesis and fibrogenesis in cancer tissue (Fig. **4**). ARBs' action against tumor cells is likely to be cytostatic, not cytotoxic, indicating that these drugs are so-called molecular targeting medicine. Although the detailed molecular mechanisms of the development of hormone-independent prostate cancer remain to be understood, the fact that drugs such as ARBs have efficacy in HRPC may lead to elucidation of the mechanism of hormone-refractory cancer. Furthermore, ARBs have actions not only in cancer cells (epithelial cells), but also in stromal cells, suggesting the possibility that they are also effective for the chemo-prevention of prostate cancer or the development of benign prostatic hypertrophy.

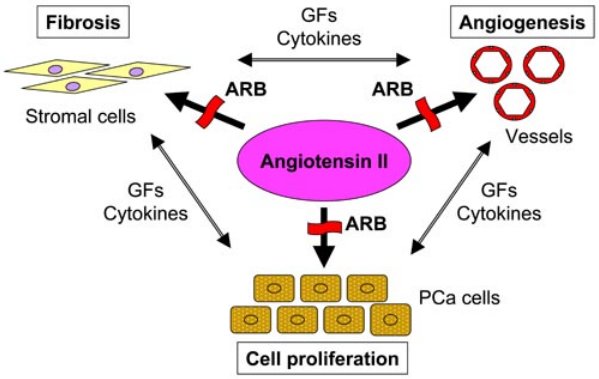

Fig. (4). Putative interaction between Ang-II and other molecules in tumor growth. Ang-II plays a pivotal role in cancer cell growth, angiogenesis and fibrosis in the development of prostate cancer. The ARB inhibits Ang-II's function, possible leading to inhibition of tumor growth.

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