The role of angiotensin II in proliferation and fibrosis of peritoneal dissemination in gastric cancer

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Abstract

The renin-angiotensin system (RAS) plays an important role not only in homeostasis, but also in carcinogenesis. Recent studies have shown that a local RAS can exist in malignant tumor tissue, with angiotensin II potentially acting as a key factor for promotion of tumor growth and metastasis. We discuss the role of angiotensin II in proliferation and fibrosis of peritoneal dissemination in gastric cancer. We previously demonstrated the presence of a local angiotensin II/AT1 receptor generating system in human gastric cancer. Furthermore, three types of cascade for tumor progression with accompanying extensive stromal fibrosis in response to angiotensin II stimulation were identified: antiapoptosis pathway through NF-κB activation, cell proliferation through phosphorylation of ERK1/2, and fibrosis through TGF-β1-induced EMT. AT1 receptor blockers (ARBs) suppress tumor proliferation and fibrotic changes by impairing angiotensin II stimulation, and thus the above cascades, in a xenograft mouse model of fibrotic tumor. ARBs have been widely used as clinical antihypertensive agents without serious side effects, and recent study were reported that ARBs was associated with longer progression-free survival and overall survival in malignancy patients. ARB are expected to offer a new repositioning drug strategy for peritoneal dissemination in gastric cancer.

Introduction

Gastric cancer is the fourth most commonly diagnosed cancer and the second leading cause of cancer death worldwide [1]. Peritoneal dissemination is a critical indicator for poor prognosis and the most frequent metastatic pattern in gastric cancer [2]. Although clinical outcomes for patients with gastric cancer with peritoneal dissemination have improved with advances in systemic and/or intraperitoneal chemotherapy, sufficiently satisfactory outcomes have not yet been achieved [3–7]. Peritoneal dissemination is characterized by cancer cell infiltration and proliferation accompanied by extensive stromal fibrosis [8]. This results in the development of chemoresistance and obstructive disorders including ileus, obstructive jaundice, and hydronephrosis. Therefore, new strategies for the treatment of tumor proliferation and fibrosis in peritoneal dissemination of gastric cancer are required.

Angiotensin II, a multifunctional bioactive octapeptide of the renin–angiotensin system (RAS), plays a fundamental role as a vasoconstrictor in controlling cardiovascular function and renal homeostasis [9]. It was previously thought that RAS is present only in the circulatory system (circulating RAS); however, recent studies have shown that a local RAS can exist in malignant tumor tissue, with angiotensin II potentially acting as a key factor for promotion of tumor growth and metastasis via the angiotensin II type 1 (AT1) receptor [10]. Furthermore, locally synthesized angiotensin II stimulates fibrosis through the AT1 receptor and TGF-β1 signals [11].

Our previous studies have shown that local tissue RAS is present in gastric cancer tissues and that angiotensin II can promote cell proliferation [12]. Additionally, we have reported that AT1 receptor blockers (ARBs) have the potential to suppress proliferation and fibrosis in gastric cancer [13].

In this review article we will discuss the role of angiotensin II in proliferation and fibrosis of peritoneal dissemination in gastric cancer and the potential efficacy of a treatment strategy targeting the angiotensin II signaling pathway.

Local RAS exists in gastric cancer tissues

Recent studies have focused on measuring the concentration of angiotensin II to confirm the local angiotensin II/AT1 receptor generating system. Previous studies demonstrated that trypsin generates angiotensin II from circulating angiotensinogen in the absence of angiotensin converting enzyme (ACE) at a weakly acidic pH of 5.5 [14]. In acidic tissues, such as those found in gastric cancer, tryptase and trypsinogen derived from migrating mast cells may convert angiotensinogen to angiotensin II. We have further suggested that circulating angiotensinogen in the blood is converted directly to angiotensin II by trypsin in the tumor microenvironment at the weakly acidic pH found in conditions of anaerobic glycolysis [15] (Fig. 1).

We previously demonstrated that tissue angiotensin II concentrations within

pancreatic ductal cancers were significantly higher than those of normal pancreas [16]. This study also showed that AT1 receptor protein was overexpressed in pancreatic ductal cancer cells by immunohistochemical staining [16]. In gastric cancer, there was a sharp contrast between cancer and normal regions with respect to the concentration of angiotensin II (Fig. 2A) [12]. Furthermore, we demonstrated that AT1 receptor was highly expressed in both gastric cancer cell lines and gastric cancer tissues (Fig. 2B) [12,13]. To our knowledge, this was the first demonstration of a local angiotensin II/AT1 receptor generating system in human gastric cancer.

The influence of angiotensin II and ARB on cellular proliferation

Lever and colleagues reported the first clinical evidence that a long-term angiotensin II blockade may be protective against carcinogenesis [17]. We have previously reported that angiotensin II has the potential to impair apoptosis induced by NF-κB activation and overexpression and promote tumor proliferation induced by ERK1/2 activation (Fig. 3) [12]. Proliferation of MKN45 gastric cancer cells was significantly increased after treatment with angiotensin II (Fig. 4A) [13]. Furthermore, pretreatment of cells with ARB (candesartan) for 1 h completely inhibited the angiotensin II-induced proliferative response (Fig. 4B) [13]. Therefore, blockade of angiotensin II has been considered a potential target for antiproliferative therapy in tumorigenesis.

The influence of angiotensin II and ARB on TGF-β1 and EMT marker expression

Many studies suggest that fibrous tissue in various organs is influenced by the epithelial–mesenchymal transition (EMT), which is characterized by a loss of epithelial cell characteristics and gain of extracellular matrix–producing myofibroblast characteris -tics [18]. TGF-β1 signals play an important role in the progression of EMT and contribute to the metastatic spread of cancer cells by influencing migration and invasion [19]. Therefore, targeting the effects of TGF-β1–induced EMT is important to attenuate both metastasis and fibrosis. Angiotensin II induces expression of the TGF-β1 activator thrombospondin-1 via the AT1 receptor, thereby mediating activation of latent TGF-β1 [11]. The angiotensin II/AT1 receptor axis has been shown to contribute to fibrosis through endogenous production of TGF-β1 in chronic renal disease [20, 21]. Our studies demonstrated that treatment of MKN45 cells with angiotensin II increased the expression of TGF-β1, whereas pretreatment of cells with ARBs effectively inhibited this response (Fig. 5) [13].

Recent developments have shown that tumor progression results from interactions between cancer cells and various stromal cells, including endothelial cells, immune cells, and fibroblasts, in the tumor microenvironment [22]. We have previously reported that TGF-β1–mediated activation of human peritoneal mesothelial cells (HPMCs) induces an EMT-like process whereby these cells adopt a fibroblast or

myofibroblast-like phenotype [23]. HPMCs are one of the origins of carcinoma-associated fibroblasts (CAFs) [23]. HPMCs cultured with serum-free conditioned media (SF-CM) from MKN45 cells that were pretreated with angiotensin II promoted EMT-like changes, with increased expression of the mesenchymal marker α-SMA and decreased expression of the epithelial marker E-cadherin [13]. Conversely, HPMCs cultured with SF-CM from MKN45 cells that were pretreated with ARB markedly suppressed these EMT-like changes (Fig. 6) [13]. Therefore, the angiotensin II/AT1 receptor axis contributes to an EMT-like process through endogenous production of TGF-β1.

ARB suppresses proliferation and fibrosis in gastric cancer

Several studies have detected ARB in carcinomas of the larynx, lung, liver, bladder, prostate gland, breast, ovary, and cervix [24-31]. Our studies showed that angiotensin II can promote cell proliferation during cancer development, and that ARBs may suppress this effect by antagonizing the AT1 receptor in gastric cancer and intrahepatic cholangiocarcinoma [12, 32]. Furthermore, ARBs have recently attracted attention for their direct antifibrotic activity. In particular, ARBs may have the potential to inhibit fibrotic change in chronic kidney disease by reducing TGF-β1 expression [20]. Additionally, ARB treatment in patients with Marfan's syndrome significantly slowed the rate of progressive aortic root dilation, which is caused by excessive TGF-β1 signaling [33].

We previously reported that fibrotic tumors demonstrating advanced progression can be established in a subcutaneous xenograft model using the gastric cancer cell line MKN45 in co-culture with HPMCs [23]. Thus, the interaction between cancer cells and HPMCs in the tumor microenvironment contributes to tumor proliferation and fibrotic change. To evaluate whether ARBs exhibit antiproliferative and antifibrotic activity in vivo, ARB (candesartan 10 mg/kg) was delivered orally to female nude mice with tumor xenografts. Tumors derived from MKN45 cells co-cultured with HPMCs were significantly larger than those derived from MKN45 cells alone when measured at day 28. Furthermore, tumors derived from MKN45 cells co-cultured with HPMCs were significantly smaller in the candesartan treatment group compared with those in the untreated group as early as day 20 (Fig. 7). In histologic examination, fibrotic areas in tumors derived from MKN45 cells co-cultured with HPMCs were significantly larger than those in tumors from MKN45 cells alone. Tumors from MKN45 cells co-cultured with HPMCs also exhibited increased α-SMA expression and decreased E-cadherin expression. Conversely, tumors from the candesartan treatment group exhibited increased E-cadherin expression and decreased α-SMA expression compared with untreated co-culture tumors (Fig. 8). This suggests that candesartan suppressed tumor proliferation and fibrotic changes by impairing angiotensin II stimulation.

The above results suggest three types of cascade for tumor progression with accompanying extensive stromal fibrosis. First, angiotensin II-induced NF-κB activation may have antiapoptotic activity in cancer cells. Second, angiotensin II transduces mitogenic signals leading to cell proliferation of gastric cancer cells through ERK1/2 activation. Third, angiotensin II induces an EMT-like change in HPMCs by TGF-β1 expression and subsequently promotes stromal fibrosis. Thus, locally synthesized angiotensin II has the potential to enhance cellular proliferation and fibrosis through AT1 receptor activation and TGF-β1 signaling. As a result, peritoneal dissemination progresses rapidly with extensive stromal fibrosis, contributing to the poor prognosis associated with disseminated gastric cancer. ARBs may suppress tumor cell proliferation and stromal fibrosis by blocking the effects of AT1 receptor on these cascades (Fig. 9).

Possible chemoprevention and anticancer effects of ARB

This comprehensive literature review implicates blockade of angiotensin II stimulation as a potential target for antiproliferative and antifibrotic therapy in tumorigenesis. Previous reports have shown that TGF-β1 neutralizing antibodies and TGF-β1 receptor kinase inhibitors can suppress EMT and reduce stromal fibrosis [34,35]. However, these agents cannot be administered to patients with various cancers and fibrotic diseases because TGF-β1 and its receptors are almost ubiquitously expressed in normal tissues. This is a major conceptual problem with the long-term clinical use of these agents as there is a high likelihood of adverse side effects resulting from disruption of the many important roles played by TGF-β1 in normal tissues [36]. However, ARBs, including candesartan, have been widely used as clinical antihypertensive agents without serious side effects and could potentially be safely used as anticancer agents. This current review supports the hypothesis that ARBs offer a new repositioning drug strategy for peritoneal dissemination through suppression of both tumor proliferation and fibrosis.

Candesartan was effective at a dosage of 10 mg/kg/day, which is close to the maximal clinical dose [37]. Targeting the angiotensin II signaling pathway may not only impair tumor proliferation, but might also be a novel efficient strategy for treating associated tissue fibrosis such as that associated with chemoresistance. In fact, Nakai et al. showed that the use of ACE inhibitors or ARBs was associated with longer progression-free survival and overall survival in patients with advanced pancreatic cancer receiving gemcitabine monotherapy [38]. Therefore, combination therapy using ARBs and cytotoxic antineoplastic agents could potentially improve the prognosis for patients with peritoneal dissemination of gastric cancer.

In conclusion

Most patients with peritoneal dissemination of gastric cancer suffer from

uncontrollable disease progression and have a dismal prognosis. We believe that our literature review presents a very clear and compelling case for intensive research on RAS and its relationship to gastric cancer. Understanding the mechanism of the angiotensin II/AT1 receptor axis and TGF-β signaling is necessary for development of a molecular approach to combat peritoneal dissemination of gastric cancer .

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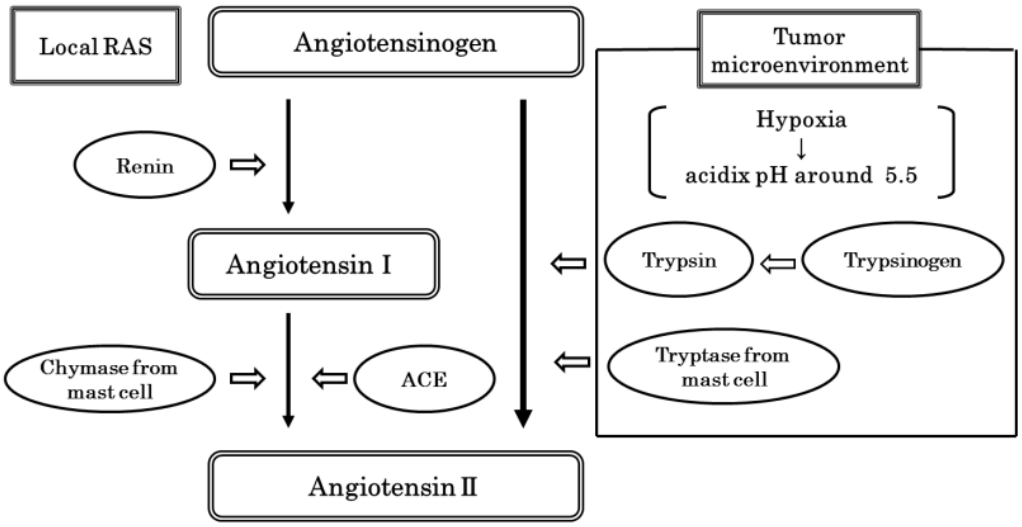
Figure. 1

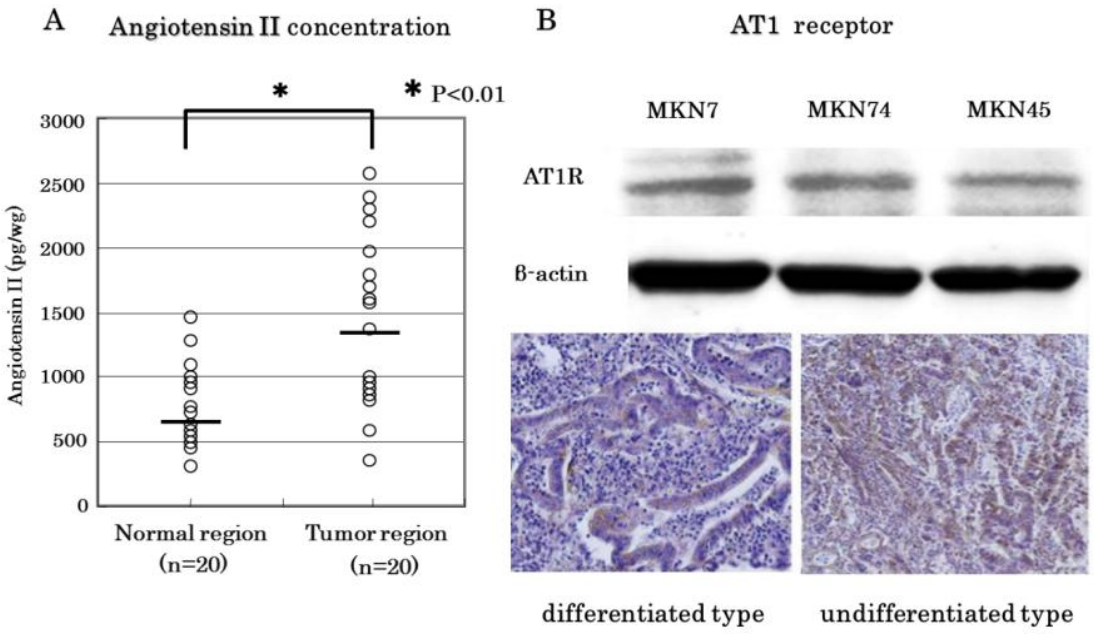
Local RAS exists in gastric cancer tissues.



RAS, renin-angiotensin system; ACE, angiotensin converting enzyme

Figure. 2

Angiotensin II concentration and AT1 receptor expression in gastric cancer[12,13] .



A, Angiotensin II concentration in tissue; B, AT1 receptor protein expression in cancer cells

Figure. 3

Angiotensin II stimulated phosphorylation of ERK1/2 and NK-κB binding activity[12].

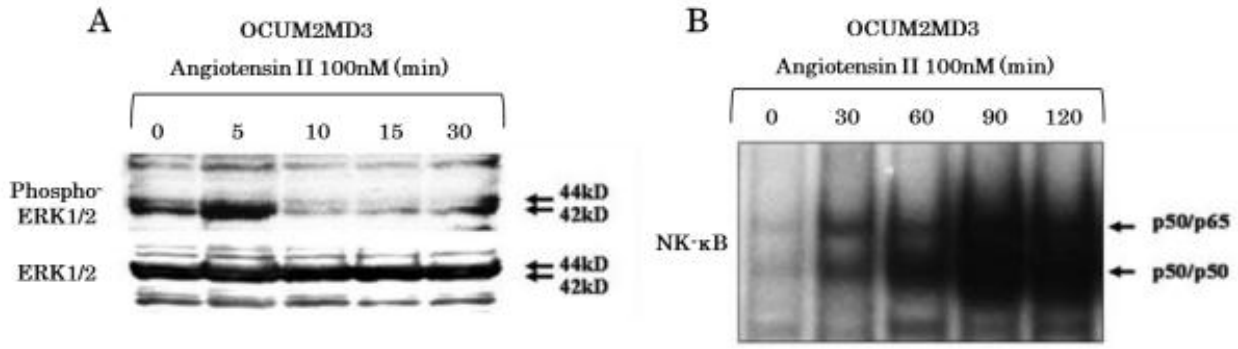


Figure. 4

Angiotensin II-induced proliferation of MKN45 cells is inhibited by ARBs[13].

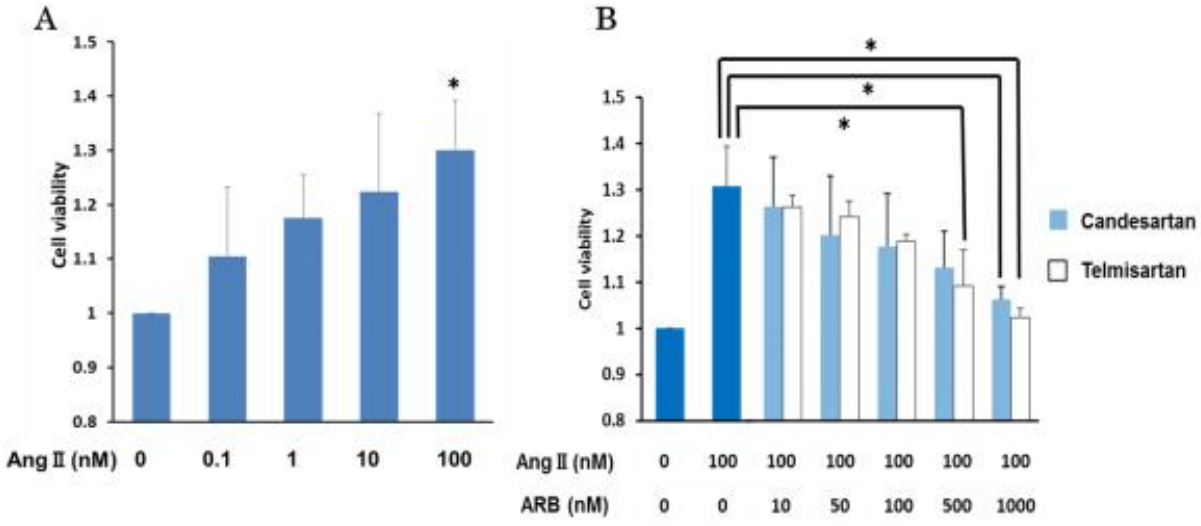
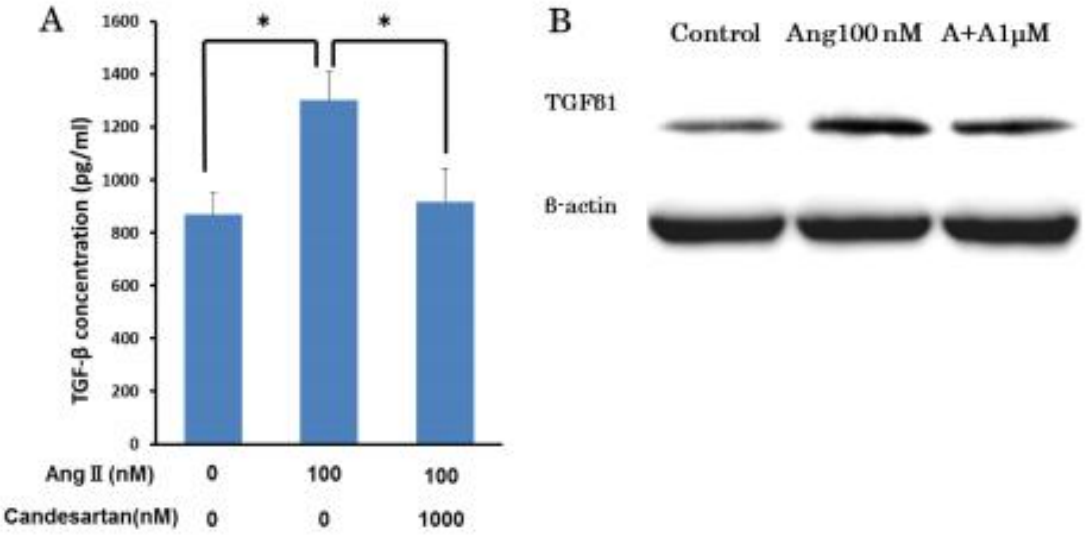


Figure. 5

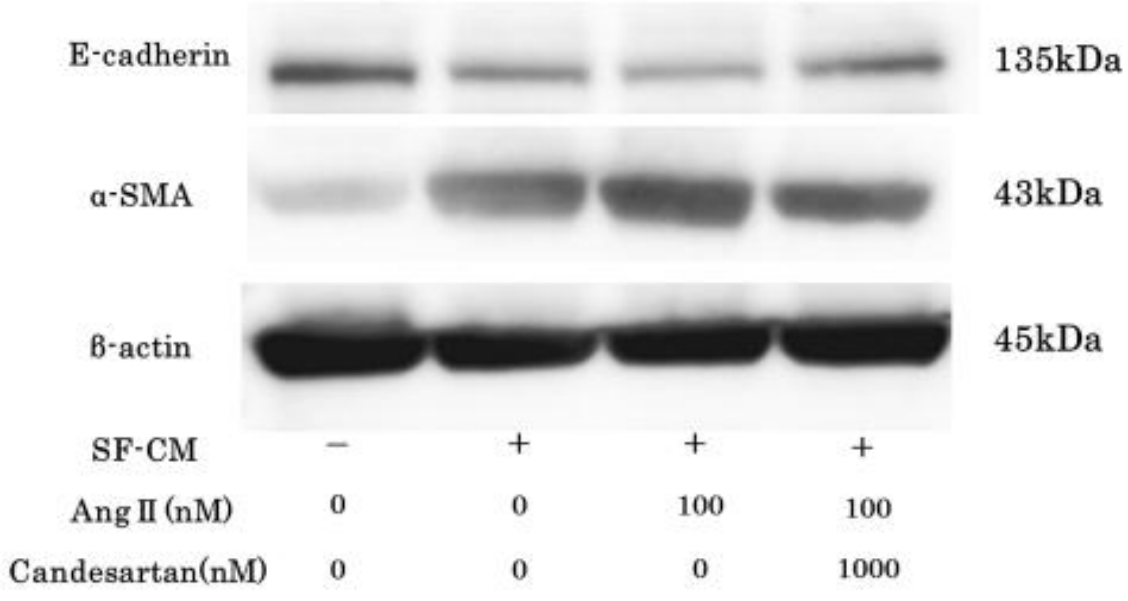
Results of the effect of Angiotensin II and ARB in TGF-β level [13].



A, ELISA; B, Western blot

Figure. 6

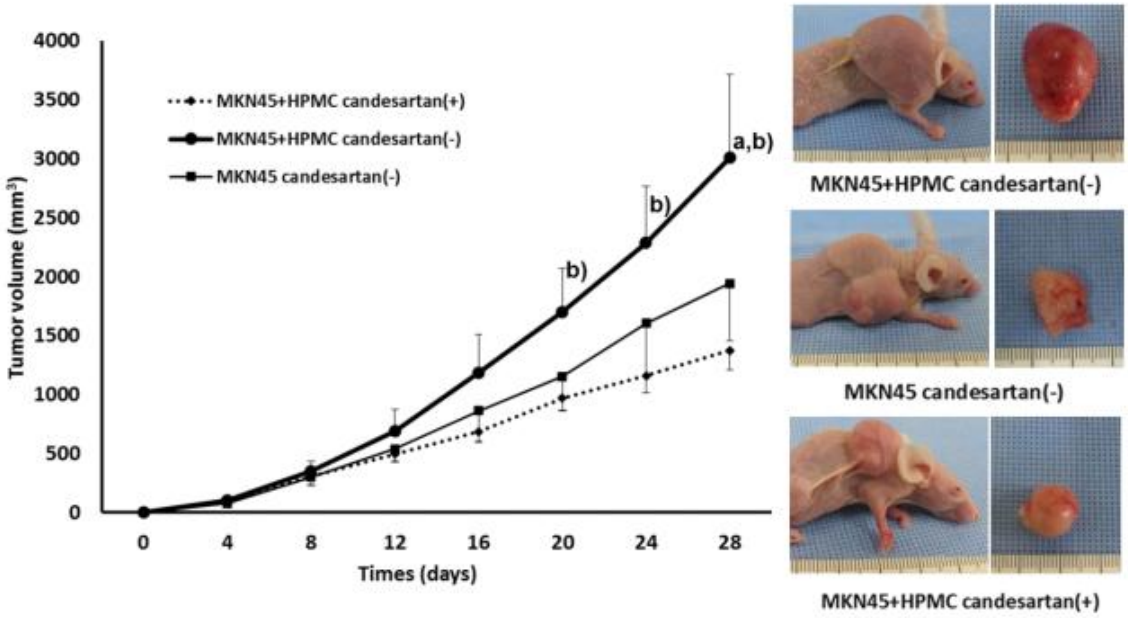
Results of the western blot analysis assaying for EMT marker[13].



SF-CM, serum-free conditioned media; Ang II, Angiotensin II

Figure. 7

Candesartan inhibits growth of tumors derived from MKN45 and HPMC co-cultures[13].



a)p<0.01 vs. MKN45 candesartan(-) b) p<0.01 vs.MKN45+HPMC candesartan(+)

Figure. 8

Histological examination of subcutaneous xenograft[13].

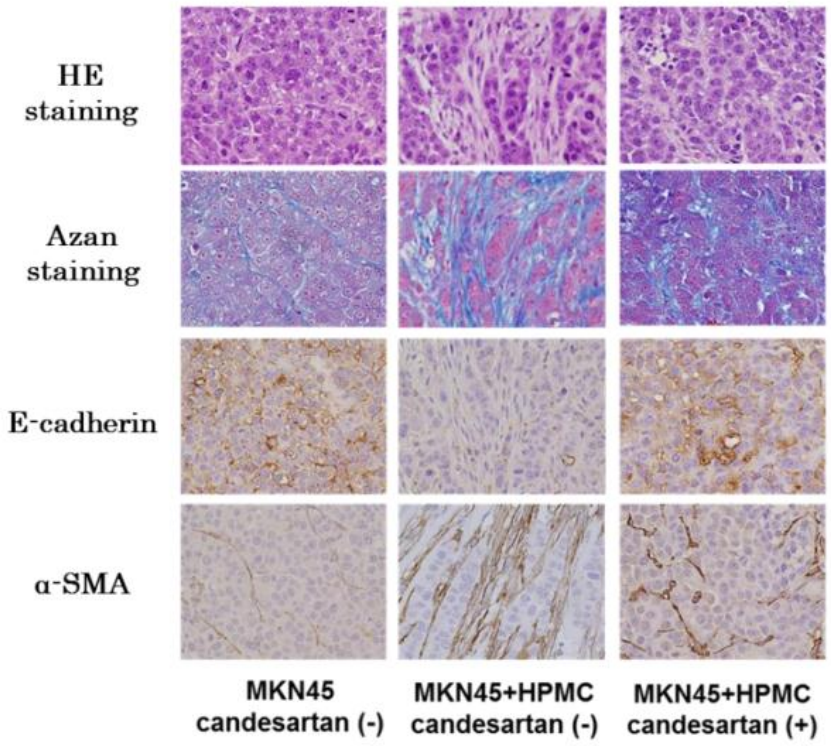
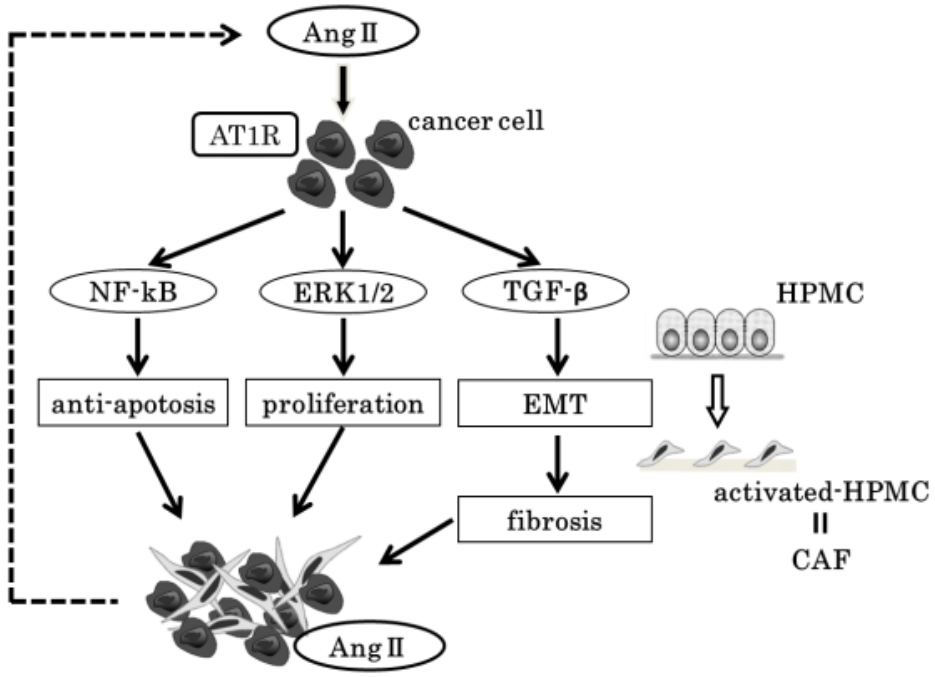


Figure. 9

Mechanism of cellular proliferation and fibrosis through Angiotensin II stimulation



Ang II , Angiotensin II ; AT1R, Angiotensin II type 1 receptor; EMT, epithelial–mesenchymal transition; HPMC, human peritoneal mesothelial cell; CAF, carcinoma-associated fibroblast

