



Roles of Renin-Angiotensin System in the Regulation of Prostate Cancer Bone Metastasis: A Critical Review

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Cite this article: Tran MT: Roles of Renin-Angiotensin System in the Regulation of Prostate Cancer Bone Metastasis: A Critical Review. *Ann Urol Oncol* 2021; 4(1): 31-41. <https://doi.org/10.32948/auo.2021.10.20>

Abstract

Metastatic prostate cancer cells (MPCCs) frequently metastasize to bone, which is a “favorite soil” for colonization and proliferation of MPCCs. Prostate cancer bone metastasis is tightly associated with tumor-induced bone lesions, most commonly caused from (1) the etiological imbalance between osteoblastic bone formation and osteoclastic bone resorption and from (2) the anti-tumor immune response. Therefore, understanding of prostate cancer biology and prostate cancer bone metastasis has led to the establishment of drug development programs for treatment of the patients with bone metastasis. The renin-angiotensin system (RAS) controls systemic body fluid circulation; nonetheless, the existence of a local RAS in tumors has been reported. Importantly, the local RAS has recently emerged as a potential regulator of tumorigenesis and cancer metastasis. This review summarizes and dissects the critical roles of the local RAS in promoting (1) progression of metastatic prostate cancer, and (2) development and progression of PCa bone metastasis, thereby providing multiple solutions for the potential therapeutic intervention.

Key words RAS, metastatic PCa, PCa bone metastasis, bone microenvironment

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Introduction

The RAS plays a pivotal role in the regulation of arterial blood pressure, volume and electrolyte balance and cardiovascular and renal structure and function [1]; moreover, it exerts its multiple biological effects on the regulation of tissue repair and remodeling, cognitive and autonomic functions, embryonic development and reproduction in other organs. Mechanistically, the cleavage of the single obligate precursor angiotensinogen (AGT), which is synthesized and released from the liver, to the inactive decapeptide angiotensin I (Ang I) is triggered by renin, a highly selective protease that is secreted from the juxtaglomerular cells of the renal afferent arterioles [2], following numerous physiological stimuli such as, for instance, reduction of (1) the arterial blood pressure, (2) the sodium reabsorption in the distal tubule, (3) the blood volume, and/or stimulation of β 1-adrenoreceptors [3]. Ang I is then cleaved by the angiotensin-converting enzyme (ACE), which are expressed on the luminal site of endothelial cells in the lungs [4], and ACE2 to produce Ang II and Ang1-7, respectively [5]. Ang II can be further processed by aminopeptidases A and N to generate Ang III and AngIV (Ang3-8) [6]. The binding of angiotensin peptides with type 1 receptor (AT1R) triggers the signaling transductions through both G protein-coupled and G protein-uncoupled mechanisms [7]. By coupling to *Gaq/11* (calcium mobilization), AT1R is activated to engage the other heterotrimeric G proteins, comprising *Gi/o*, *G12/13* and *Gs*, and classes of G proteins (*Rho*, *Ras* and *Rac*) [8]. Following the phosphorylation of AT1R, AT1R to bind to arrestins, which rapidly desensitizes protease-activated receptor-1 (PAR1) [9]. Besides, arrestins serve as the trafficking proteins recruiting various signaling molecules to the endosomal receptors, thereby dictating the alternative, compartmentalized signaling modalities.

Additionally, the activated AT1R could also be associated with the soluble and receptor tyrosine kinases (RTKs), the mitogen-activated protein kinases (MAPKs) such as extracellular-regulated kinases (ERK1/2, p38 MAPK and Jun N-terminal kinase) [10, 11], the JAK-STAT pathway [12]. Moreover, it also mediates the generation of reactive oxygen species (ROS) [13] and the modulation of various ion channels [14-16]. The mechanisms underlying the AT1R-triggered regulation of tyrosine kinase-related pathways are thought to initiate from (1) the *de novo* synthesis of cytokine and growth factor ligands, and/or (2) the binding of the AT1R with the upstream activators and scaffolds [17], (3) and/or the transactivation of growth factor receptors such as the epidermal growth factor receptor (EGFR) [18]. EGFR transactivation involves the capacity of GPCRs to activate metalloproteinases (MMPs) that cleave cell surface precursors of the EGF ligands, yielding the shed mature ligand that subsequently binds and activates the EGFR to pioneer various cellular signals [19], which are crucial for Ang II/AT1R-mediated hypertrophy and/or proliferation of cardiac, vascular and renal cells. The signaling pathways regulated by the other RAS receptors (AT2R, MASR, IRAP, renin receptor, ACE and ACE2) are poorly understood. However, several of the published reports have elucidated that AT1R, AT2R, MASR [20] and other members of GPCR family [21] could form dimerization, which declined the efficacy and the potency of ligand binding; however, the physiological effects of the dimerization of these receptors to the normal and disease states have been controversial. As the RAS plays a central role in (1) the elevation of blood pressure, (2) the maintenance of fluid homeostasis, the dysregulation of RAS has been tightly linked to hypertension and cardiovascular diseases.

Bone is a metabolically active tissue continuously remodeled throughout life. Bone remodeling is a process in which the old/damaged bone is removed and replaced with newly synthesized bone to maintain bone strength and mineral homeostasis [22].

Bone homeostasis is tightly controlled by (1) osteoblasts (OBs), which are responsible for synthesizing bone matrix proteins and promoting bone deposition and mineralization, and (2) osteoclasts (OCs), which are responsible for removing both the mineral and the organic matrix of bone [23]. Mechanical forces, proinflammatory cytokines, growth factors and hormones trigger an imbalance between osteoblastic and osteoclastic activities, which are one of the major causes of the bone-related diseases [24] such as postmenopausal osteoporosis, hyperparathyroidism, rheumatoid arthritis and osteopetrosis. In addition to the lungs and liver, bone is one of the most frequent sites of metastases. Prostate and breast cancers are responsible for the majority of bone metastases [25]. Patients with bone metastasis experience a very miserable quality of life as a consequence of severe pain, spinal cord compression, fractures, bone marrow aplasia and hypercalcemia, the latter being a principal cause of death. In fact, hypercalcemia can induce gastrointestinal dysfunctions, as well as constipation, polyuria and fatigue. In the advanced stage, it is the major cause of renal failure and cardiac arrhythmias. All these symptoms are usually classified as skeletal-related events (SREs) [26].

PCa arising in the prostate gland is the most common type of malignancy that has a tendency to develop in older men, aged 50 and over [27]. When PCa cells metastasize, they most frequently spread to bone. Advanced-stage PCa is characterized by tropism to bone with skeletal involvement present in approximately 90% of patients with metastatic disease. Metastatic PCa is characterized by a period of responsiveness to castration, and metastatic, castration-resistant prostate cancer (mCRPC) frequently leads to skeletal complications such as ineffective haematopoiesis, pain and skeletal related events (SREs) [28]. The term SRE is used to encompass common complications of bone metastases such as pathologic fracture secondary to a bone metastasis, spinal cord compression or the need for surgery or radiotherapy to bone [28]. Recent studies have shown several components of the RAS (renin, ACE, and Ang II receptors) were expressed in the bone marrow microenvironment, and play an important role in bone metabolism [29, 30]. Besides, the local bone RAS might participate in regulating the pathogenesis and progression of some metabolic bone diseases via two distinctive ways: (1) an imbalance in bone remodeling, characterized by the inhibition of osteoblastic activity, or (2) the stimulation of osteoclastic activity [31, 32]. Additionally, accumulating clues obtained from *in vitro*, animal and clinical studies, have substantiated a frequent dysregulation of RAS in metastatic tumor cells, in correlation with poor diagnosis in patients. Antagonism/blockade of the RAS by the specific antagonists/blockers suppress tumor growth, angiogenesis and metastasis in a broad range of experimental models of malignancy, and retrospective studies in humans, providing clues that a long-term use of angiotensin-converting enzyme (ACE) inhibitors may protect against cancer. In this article, I review and summarize the current knowledge on the specific roles of the RAS (1) in regulating the metastatic PCa progression in the primary (prostate) gland, and (2) in maintaining the functional interactions amongst the metastatic PCa cells, bone cells and immune cells.

Local prostate RAS in the regulation of metastatic PCa

PCa is the majority of malignancies arising from the peripheral portion of the gland surrounding the prostatic urethra just below the bladder [33]. Two disorders commonly affecting the prostate are (1) non-cancerous enlargement of the prostate, known as the benign prostatic hyperplasia (BPH), and (2) Pca. The initial feature of PCa bone metastasis is that PCa cells not only shed many of their epithelial characteristics, also known as mesenchymal-epithelial transition (EMT), but crucially acquire the metastatic capacities such as motility and invasiveness, to detach from the

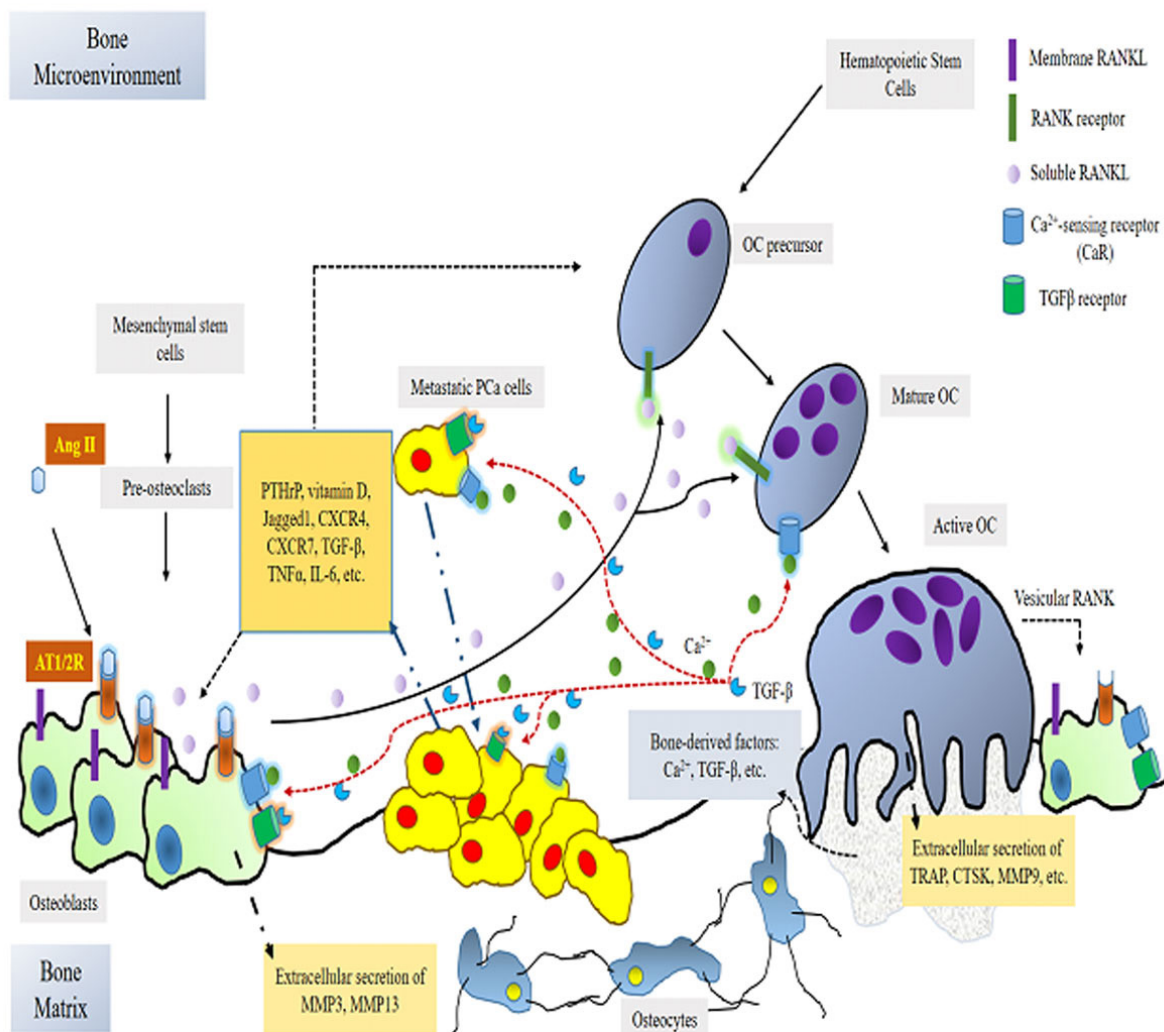


Figure 1. The possible mechanism underlying the RAS-mediated regulation of PCa bone metastasis. Ang II that is produced locally by the vascular endothelial binds to osteoblastic AT1R and AT2R, subsequently promoting OBs to the Cbfa1/Runx2-mediated secretion of the soluble RANKL into bone milieu. The OB-induced secretion of soluble RANKL binds and stimulates OC differentiation, thereby enhancing bone-resorbing activity of active OCs via the secretion of the specific enzymes (TRAP, CTSK and MMP9) into the bone matrix. Ca²⁺ ions and TGFβ, the bone elements, released from the OC-induced bone resorption, bind to CaRs and TGFβRs abundantly present in OBs, OCs and PCa cells, promoting the OB and OC differentiation and PCa bone metastasis. Several of the crucial factors, PTHrP, vitamin D, Jagged1, TGFβ, TNFα, etc., secreted by PCa cells, in turn, accelerate OB and OC differentiation. In addition, the active OC-secreted vesicular RANK further enhanced OB differentiation and then bone resorption.

prostate epithelium.

Many published reports have proven that a cancer incidence rate has been significantly reduced in cancer patients undergoing a long-term treatment with RAS-specific inhibitors. Perindopril-triggered blockade of ACE activity suppressed the VEGF-mediated tumor development and neovascularization in murine hepatocellular carcinoma cells [34]. Besides, Mamoru Fujita et al, found that blockade of ACE activity by a non-peptide AT1R antagonist (TCV-16) resulted in the (1) alleviation of the tumor angiogenesis and tumor growth in mice subcutaneously injected with sarcoma and fibrosarcoma cell lines [35], and (2) the impairment of the metastatic ability of Lewis lung carcinoma (3LL) cells, injected intravenously [35]. In addition, olmesartan-induced

blockade of ACE and AT1R activities suppressed the growth of subcutaneous tumors generated by the co-injection of pancreatic cancer cells with pancreatic stellate cells (PSCs) [36] whereas losartan-induced blockade of AT1R activity inhibited angiogenesis and tumor growth in mice subcutaneously injected with C6 rat glioma [37], which is the most frequent brain tumor [38]. Moreover, directly acting on AT1R, Ang II was demonstrated to be crucial for promoting (1) the invasive ability and VEGF secretion in SKOV-3 ovarian cancer cells [39], (2) promote the expression of MMP2 and MMP9 in MKN-28 cells. However, the stimulatory effects of Ang II on microvessel and VEGF expression could be abrogated by AT1R antagonist [40, 41]. Additionally, Ang (1-7) has an anti-metastatic action on lung adenocarcinoma cells [42, 43].

Table 1: The summary of the effects of the Ang II/AT₁R and AT₂R receptor antagonists/blockers in PCa cells.

Ang II/AT ₁ R and AT ₂ R receptor antagonists/blockers	Specific targets	PCa cell lines	Pharmacological effects
Candesartan	AT ₁ R antagonist	PC3, Du145, LNCaP	Inhibition of (1) cell growth and proliferation of PCa cells, following stimulation with either EGF or Ang II via the mechanism underlying the reduction of MAPK or STAT3 phosphorylation [58]. Alleviation of tumor angiogenesis in athymic nude mice [58]. Inhibition of growth of metastatic PC3 cell line [53].
Fimasartan, Losartan, Eprosartan and Valsartan	AT ₂ R blockers	PC3, DU-145 and LNCaP-LN3	Reduction of PCa cell viability [59]. Induction of autophagy-induced cell death in PC3 cell line [59]. Fimasartan-induced inhibition of the migratory capacity of DU-145 and PC-3 cell lines [59]. Losartan-induced suppression of the growth and the proliferation of DU-145 cell line, via abolishing the Ang-II-induced PAX2 expression [60].
Captopril	ACE blocker	Male patients aged 50-79 years old (clinical trials)	Showed a lower risk of developing and progressing PCa [61].
Telmisartan		PC3, DU-145 and LNCaP	Growth inhibition of PCa cells [62]. Induction of early apoptosis in PCa cells [62]. Suppression of PCa cell growth via inhibiting the Ang II-stimulated MAPK phosphorylation in LNCaP cell line [63].

Ultimately, AT₂R overexpression triggered (1) the cell cycle arrest at S phase, and (2) apoptosis via an extrinsic cell death signaling pathway in a manner dependent upon the activation of p38 MAPK, caspase-8 and caspase-3 in the PCa cell line, Du145 [44]. Besides, Ang II abundantly expressing in non-basal epithelial cells in prostate, directly stimulated the growth of prostate cancer cell lines, LNCaP and Du145, via AT₁R [45]. Ang II-mediated AT₂R stimulation diminished EGF-induced ERK2 phosphorylation in both LNCaP and PC3 cell lines. ERK2 activation is regulated by two independent pathways comprising (1) Ras/Raf-dependent pathway involving transactivation of the EGFRs mediated by heparin binding epidermal growth factor (EGF)-like growth factor (HB-EGF) [46], and (2) mediated by a PKC-dependent pathway [47]. The intracellular mechanisms of how AT₂R could inhibit ERK2 phosphorylation is poorly understood; however, Katarina Bedecs et al. once reported an important role of Ang II in rapidly activating the protein tyrosine phosphatase-1 (SHP-1), thereby terminating the cytokine and/or growth-factor receptor-mediated signaling pathways in the neuroblastoma cell line, N1E-115 and the Chinese hamster ovary cell line expressing recombinant human AT₂R [48]. Of greater potential significance, Ang II played a pivotal role in regulating the cell proliferation in LNCaP cells expressing both functional AT₁R and AT₂R whereas it served as a negative regulator of cell growth in PC3 cells expressing non-functional AT₁R, evidently indicating that the autocrine of Ang II on cell growth can vary dependently upon the functional state of both AT₁R and AT₂R [45]. Also, Ang II could act on the regulation of the signaling transducers such as MAPK and STAT3. Toshiaki Ishizuka et al. revealed (1) that stimulation of mouse induced pluripotent stem (iPS) cells with AT₁R enhanced LIF-induced DNA synthesis via mediating the JAK/STAT3 signaling pathway, and (2) that AT₁R stimulation enhanced mouse iPS

cell differentiation into the mesodermal progenitor cells via p38 MAPK activation [49]. Intriguingly, Kotha et al. had reported that resveratrol induced apoptosis in human PCa (Du145) cells by blocking STAT3 signaling pathway [50] before Mee-hyun Lee et al. revealed the suppressive effects of resveratrol on IL-6-induced androgen receptor (AR) transcriptional activity in LNCaP PCa cells partly via the inhibition of STAT3 reporter gene activity [51]. In addition, the elevated levels of EGF factor and its specific receptor, EGFR, were confirmed in PCa cells as well as the clinical specimens, suggesting that EGFR might have involved in the regulation of the prostate cancer progression. For instance, caveolin-1 (Cav-1), overexpressed in PCa and involved in the development and the progression of PCa, was involved in the regulation of the cellular signaling proteins within caveolae where it interacts with the receptor tyrosine kinases (RTKs), serine/threonine kinases (STKs), phospholipases (PLs), G protein-coupled receptors (GPCRs), Src family kinases (SFKs), and AT₁R. Cav-1 expression was increased, following Ang II treatment; nonetheless, candesartan (AT₁R blocker)-induced blockade of AT₁R strongly reduced the Cav-1 expression, subsequently inhibiting the growth of the AngII-treated metastatic prostate adenocarcinoma PC3 and Du145 cells [52, 53]. Although the IP3K/Akt pathway is the primary inducer of EMT, the Wnt/ β -catenin, Notch, Ras, integrin-linked kinase, and integrin signaling pathway are also involved [54-57]. Collectively, AT₁-R and/or AT₂-R blockers can be used as the potent inhibitors of MAPK, STAT3, IP3K/Akt pathways, thereby alleviating the PCa progression; accordingly, these blockers would be the potent anticancer drugs for treating patients with PCa (table 1).

Local bone RAS and metastatic PCa bone metastasis

1. Functional Interplay amongst metastatic PCa cells and bone cells

Osteoblastic bone formation is mediated by synthesizing the extracellular matrix (ECM) mostly including the dense lamellar type I collagen [58]. ECM, known as the osteoid, is sequentially deposited and mineralized via the accumulation of calcium phosphate as hydroxyapatite [59]. Multiple transcription factors such as RUNX2 and OSX and major developmental signals such as WNT signaling are reported to regulate OB differentiation and function [60]. Reversely, bone resorption is regulated by OCs, the large, multinucleated cells formed by the fusion of precursors from the monocyte-derived macrophage lineage. Functionally, OCs can dissolve minerals and digest bone matrix by secreting hydrochloric acid and proteolytic enzymes [61]. Similar to OBs, multiple factors are identified to regulate OC differentiation, function and survival, consisting of macrophage colony-stimulating factor (M-CSF), receptor activator of nuclear factor κ B ligand (RANKL), cytokines (for example, IL-1) and α V β 3 integrin [62]. RANKL, a transmembrane protein, is expressed by OBs, stromal cells, cancer cells and T lymphocytes. The proteolytic cleavage of RANKL generates its soluble form, subsequently binding and activating its RANK receptors that are abundantly expressed on cell surface of the osteoclast precursors [63]. In contrast, osteoprotegerin (OPG), also known as tumor necrosis factor receptor (TNFR) superfamily member 11B secreted by OBs [64], functions as a decoy receptor for RANKL, abolishes RANK-RANKL signaling. RANK-RANKL-OPG signaling is of critical importance to the maintenance of normal bone.

RAS plays an important role in bone metabolism, especially osteoporosis, a bone disease characterized by losses of bone mineral density and bone mass [65]. Previous studies have shown the expression of renin in bone marrow cells, and ACE in OBs and OCs [66]. In bone tissue, Ang II produced locally by the vascular endothelial cells exerts its action by binding to AT1R and AT2R, both of which are expressed in OBs. Etiologically, it was revealed that Ang II could stimulate the differentiation and the activity of OCs in vivo and in vitro and aggravate the loss of bone minerals in rats with osteoporosis induced by estrogen deficiency; furthermore, the AT1R knockout mice showed high bone mass. For instance, Sasa Gu et al. reported that the local bone RAS was involved in age-related osteoporosis of aging mice [30], bone deteriorations of mice with either obstructive nephropathy [67] or type 1 diabetes [68], indicating that the local bone RAS displays a pivotal role in the regulation of bone tissue homeostasis. Particularly, the interaction of Ang II with AT1R might be involved in the regulation of the OB-rich populations of cells through AT1R [32, 69]. Besides, both in vitro and in vivo studies have elucidated that Ang II was responsible for OC activation via promoting the OB-induced secretion of cytokines and/or RANK ligands [70]. The Ang II-mediated secretion of the matrix metalloproteinase (MMP)-3 and -13 via the AT1 receptor into the ECM in osteoblastic ROS17/2.8 cells accelerated bone resorption [71]. Notably, Ang II neither affected OC-induced bone resorption in the isolated OCs nor potentiate the RANKL-induced OC formation; however, it could stimulate RANKL-induced osteoclastogenesis in the OB and OC co-cultures [31, 72]. Moreover, Ang II-triggered activation of AT1R markedly inhibited OB differentiation and mineralization and alleviated the mineralized nodule formation of rat calvarial osteoblastic cells [31, 73].

PCa bone metastasis is commonly associated with osteoblastic lesions, generally arising from an imbalance between bone forming OBs and bone-resorbing OCs in prostate cancer. While bone metastasis from breast and lung cancers frequently induce osteolytic or the bone-lysing lesions, PCa bone metastasis uniquely triggers bone formation, suggesting OBs enables PCa

cells to metastasize to bone. More detailed, PCa bone metastasis is thought to originate from the functional interactions between MPCCs and the bone microenvironment, in general, bone cells, in particular. For instance, Li et al. showed that OBs promoted the growth of PCa cells in a co-culture model (androgen-independent C4-2 PCa cells and osteoblastic cells) [74]; besides, Gleave et al. demonstrated that co-inoculation of the athymic mice with human PCa cells (LNCaP) and with the human bone fibroblasts promoted the formation of LNCaP cell-derived tumor [75]. Later, Sung et al. reported that co-culture of human bone stromal cells with human PCa cells under 3D cultured condition enhanced tumor xenografts in mice [76]. Besides, hydrochloride-triggered blockade of bone morphogenetic protein 4 (BMP-4) receptor activation in OBs diminished the growth of PCa-18b tumors in mice [77], clarifying that BMP4-mediated osteoclastogenesis promotes the development and the progression of PCa bone metastasis in mice. Co-culturing human bone stromal cells with PCa cells in vitro, triggered the elevation of expression levels of ECM (versican and tenascin) and chemokines (BDFN, CCL5, CXCL5 and CXCL16) genes [76]; furthermore, using the bone metastasis exnograph models of human osteoinductive PCa cells (VCaP and C4-2B) to generate the osteoblastic bone metastasis-associated stromal transcriptome (OB-MDST), Berna C. O' zdemir et al. identified the up-regulation of PTN, EPHA3 and FSCN1 in mouse stromal cells [78]. However, how these stromal secreted factors provide a support for PCa bone metastasis needs to be further investigated.

OC activation has been associated with the development of PCa bone metastasis [79, 80]. Critically, it is worthy noting that OC differentiation might be originated from the Cbfa1/Runx2-induced secretion of RANKL, following Ang II-mediated OB activation. Cbfa1/Runx2 is an essential transcription factor for the differentiation of OBs from the mesenchymal precursors [81]. Ang II increased the [cAMP]_i either through interacting with AT1R in cultured OBs [31] or through facilitating the interaction between activated Gs and adenylyl cyclase (AC) in cultured rat vascular smooth muscle cells [82]. Elevation of [cAMP]_i results in the activation of the downstream signaling cascades, which enhance the RANKL secretion as well as down-regulate the transcriptional function of Cbfa1/Runx2, leading to the alleviation of OB activation and bone formation [70, 83]. Mature OCs subsequently adhere to the bone surface through α V β 3 integrin, form ruffled borders, and secrete acid to solubilize calcium phosphate crystals as well as secrete the collagenases and proteinases such as tartrate-resistant acid phosphatase (TRAP), matrix metalloproteinase 9 (MMP9), and cathepsin K (CTSK) that demineralize and degrade extracellular proteins such as type I collagen. The release of bone resorption-derived Ca²⁺ ions triggers activation of Ca²⁺-sensing receptor (CaR), a G protein-coupled receptors (GPCRs), on PM of OBs [84], OCs [85] and especially MPCCs [86, 87], subsequently stimulating the secretion of parathyroid hormone-related protein (PTHrP) from MPCCs (PC-3 cell line) [86, 87]. PTHrP secreted by PCa cells could then activate OCs and potentially contribute towards the skeletal invasiveness, bone pain, and/or pathological fractures. Additionally, the transforming growth factor beta, TGF β , a bone resorption-derived factor and a key mediator of bone metastasis, stimulates PTHrP expression in OBs [88] and tumor cells [89], thereby promoting osteolysis. Specifically, TGF β -mediated signaling pathway activating a couple of vital intracellular cascades such as MAPK, PI3K/Akt, and Rho-like GTPase signaling cascades, critically acts as a driver of tumor progression and bone metastasis. Besides, bone marrow produces the factors, such as CXCL12, with a chemotactic role on cancer cells, which, on the other hand, express the chemokine receptors, CXCR4 and CXCR7 [90], thereby facilitating the establishment of "fertile soil" that promotes the tumor cells to adhere to bone matrix and thrive in bone. Moreover, cancer cells, in turn, secrete

prostaglandins, parathyroid hormone (PTH), PTHrP, activated vitamin D, IL-6 and TNF that may lead to an elevation of RANKL expression on OBs and bone marrow stromal cells, resultantly stimulating the formation, survival and activity of OCs, thereby promoting osteolytic metastases. Notch-Jagged interactions in the bone marrow suggest direct activation of osteolysis by cancer cells through this unique interaction. In particular, Jagged1, which is a downstream mediator of the prometastatic TGF β , promotes tumor growth through stimulating IL-6 production from OBs, and directly it activates OC differentiation (Figure 1). Moreover, Jagged1 is overexpressed by the metastatic PCa cells with bone metastasis [91], whereas its Notch receptor is frequently expressed by progenitors and mature cells in the bone marrow [92]. Interestingly, metastatic PCa cells respond to growth factor stimulation via the activation of various osteoblastic transcription factors. This would suggest that bone lesions may also occur through the differentiation of the cancer cells towards an osteoblastic bone-forming phenotype, which is a phenomenon that has been observed in PCa bone metastasis.

2. Functional interplay amongst metastatic PCa cells and immune cells

Bone, immune and hematopoietic systems are tightly linked since bone cells and hematopoietic cells are in deep physical contact, are reciprocally regulated, are interconnected in their function, and share several common pathways. Indeed, bone cells express surface molecules regulating the expansion of hematopoietic stem cells (HSCs) from which all cells of the mammalian immune system derive, whereas many immunoregulatory cytokines directly act on bone cells. OBs and OCs both affect the maintenance and the mobilization of HSCs [93]. OBs control the proliferation of hematopoietic progenitors and the differentiation of all stages of B cell development. Indeed, *in vitro* production of B cell precursors from progenitors required contact with OBs and expression of CXCL12 and IL-7, which was induced by PTH [94]. Besides, OC precursors, T, B and NK cells are derived from the HSCs; accordingly, some of the receptors and ligands participating in regulating the immune processes also modulate the maturation of OC precursors and the bone-resorbing activity of active OCs. RANKL, its receptor RANK, and OPG form a crucial molecular link between the immune system and bone [95]. The membrane RANKL is expressed by stromal cells, OBs and metastatic PCa cells [96, 97]. The soluble RANKL was produced not only by the synovial fibroblasts, T helper 17 (Th17) cells [98, 99], but it is also expressed by B cells and PCa cells while RANK receptors are expressed by dendritic cells (DCs), monocytes, macrophages and PCa cells [100, 101]. Th17 cell- secreted RANKL directly regulates osteoclastogenesis and bone remodeling. Besides, the vesicular RANK secreted from OCs promotes osteoblastogenesis by activating Runx2 via RANK-RANKL interaction [102]. Many immune factors such as interferon- γ (IFN- γ) regulating osteoclastogenesis directly by inhibiting OC maturation [103] or indirectly by activating T cells, followed by the elevation of proosteoclastogenic factors [104].

Another important mediator of the interactions between T cells and bone cells is IL-7, a cytokine produced by stromal cells at the inflammatory sites, with different effects on hematopoietic and immunologic systems [105]. The main function of IL-7 is the control of B and T lymphopoiesis, but it significantly contributes towards the invasiveness of PCa cells by promoting EMT [106], and regulates bone homeostasis [107]. IL-7, produced by T cells, promotes osteoclastogenesis by upregulating T cell-derived cytokines, such as RANKL and TNF α [108]. However, OCs also plays a pivotal role in regulating the T cell function because it present the antigenic peptides to T cells and induce FoxP3

expression in CD8+ T cells [109], which regulate an inappropriate activation of the immune response. The interaction between T cells and OCs are tightly controlled by the reciprocal CD137/CD137L and RANKL/RANK interactions [110]. CD137 (4-1BB), a costimulatory member of the TNF receptor family, facilitates enhanced proliferation, cytokine production, and cytolytic activity of T and NK cells [111]. Ligation of its ligand, CD137L, constitutively expressed in OC precursors, inhibited RANKL-induced OC formation [110]; on the other hand, ligation of RANKL with OPG-Fc, the decoy receptor for RANKL, inhibited both CD4+ and CD8+ T cell proliferation [110].

T and NK cells are fully equipped with RAS components and are potentially capable of producing and delivering AngII to sites of inflammation. Ang II was shown to trigger significant leukocyte rolling, adhesion, and emigration, contributing not only to hypertension, but also to the onset and progression of the vascular damage associated with disease states [112]. Ang II, acting through the AT1R on immune cells, triggers the proliferation of splenic lymphocytes through a calcineurin-dependent pathway, which leads to the possibility of using RAS blockade as an anti-inflammatory and immunosuppressive therapy [113]. The stimulatory function of AngII on leukocyte proliferation seems to be mediated by both types of AngII receptors. The action of the RAS on T cells is mediated through several cytokines, particularly TNF α [114]. Inhibition of ACE diminished TNF α production in T cells [114]. Angiotensin receptor blockers (ARBs) have been shown to suppress lymphocyte proliferation and IFN- γ production, leading to the suppression of the antigen-specific Th1 and Th2 immune responses and suggesting another pathway for T cell activation [115]. In addition, Jon A Weidanz et al. reported that blockade of AT1R inhibited IFN- γ production in the cytotoxic T cells, suggesting that AT1R blockers may have a clinically relevant immunomodulatory role by blocking IFN- γ production in T cells [116].

However, very little is known about the roles of the RAS in mediating the functional interactions between immune cells and the metastatic PCa cells in bone milieu. Therefore, more works would be needed to verify whether the RAS is involved in the regulation of PCa bone metastasis.

3. RAS and mCRPC

The mCRPC, originating from metastatic hormone sensitive PC (mHSPC), is the advanced form not responding to initial treatments such as surgery and hormone therapy while mHSPC still responds to standard hormone treatment that is known as androgen deprivation therapy (ADT). The mCRPC is poorly diagnostic, and commonly associated with significant morbidity, and bone metastases along with the related complications that represent the major cause of death. To date, the mainstay treatment methods applied to patients with mHSPC is mostly based on ADT in combination with docetaxel chemotherapy and oral anti-androgens lead to a significant reduction of the level of circulating testosterone to “castrate level”, subsequently resulting in PCa cell apoptosis. However, PCa cells eventually become resistant to therapy with signs and/or symptoms of progression observable in most patients, in spite of good initial responses.

The recent results of phase III randomized trials showed that blockade of the androgen receptor (AR) through either inhibiting androgen synthesis or directly targeting AR can improve survival for patients with mCRPC by the use of gonadotropin-releasing hormone (GnRH) agents (agonists or antagonists) or orchiectomy [117]. The oncogenic roles of the ARs and AR signalings essential for CRPC development has been intensively investigated in recent years. Prostate-specific antigen (PSA) gene expression is regulated by the AR pathway as the androgen response elements (AREs) are

found in the promoter region of the PSA gene. In HSPC, ablation of testicular androgens resulted in alleviating the serum PSA levels, suggesting that AR signaling pathways were down-regulated in a castrate environment [118]. Nevertheless, it was confirmed that PSA levels were increased during disease progression in most patients, suggesting that AR signaling pathways might have been reactivated in a castration-resistant manner. Consequently, more studies will be required to further understand the signaling transduction mechanisms underlying castration resistance, thereby facilitating the development and approval of novel AR-directed drugs such as, for instance, abiraterone acetate plus prednisone (AAP), enzalutamide and taxanes [119]. The patients with mCRPC can be treated with docetaxel or AR-targeted therapies as first-line therapy [120]. However, Vincenza Conteduca et al. has reported that not only the circulating AR aberrations are associated with worse outcome on abiraterone/enzalutamide [121], but also plasma AR gain in docetaxel-treated patients was associated with significantly shorter overall survival (OS) [122]. More importantly, docetaxel can also be associated with toxic effects, comprising fatigue and myelosuppression, and many patients with CRPC are either not healthy sufficient to receive docetaxel or unwilling to receive docetaxel. Despite advances in the treatment of CRPC, patients ultimately progress; a need remains for effective and tolerable treatments, including those who are unsuitable for or decline docetaxel.

Radium-223 dichloride (radium-223), a novel targeted α -emitter, selectively binds to areas of increased metabolic activity in bone metastases. In principle, it emits the high-energy α -particles with ultra-short penetration (<100 μ m; 2-10 cell diameters) so as to efficaciously generate cytotoxicity in target areas, whereas reducing the damages to surrounding normal tissue, comprising bone marrow [123]. The phase 3 ALSYMPCA (ALpharadin in SYMptomatic Prostate Cancer patients) trial of radium-223 plus best standard of care versus placebo plus best standard of care in patients with CRPC and symptomatic bone metastases was unique as it is applicable for both groups of patients (1) who had received previous docetaxel treatment and/or (2) who were unsuitable for or unwilling to receive docetaxin [123]. From these, it is suggested that the treatment with Radium-223, which was based on a randomized controlled trial, might be useful for the patients with mCRPC, and that it is probable AT1/2R blockade might not be the best option for such patients. Intriguingly, although Hiroji Uemura et al. reported that blocking AT2R triggered the inhibition of proliferation of PCa cells [124], there was no randomized controlled trials reported as consequence. Furthermore, an ecologic study confirmed that there was no significant increase in the overall or site-specific cancer risk from ARBs, based on 15 trials enrolling 138 769 individuals [125]. Altogether, it is proposed that the alternative mechanisms of action might have played a key role in affecting the cancer risk; more importantly, it might perhaps explain the inconclusive findings on cancer risk in many trials of RAS blockade [43].

Conclusion

Bone metastasis accounts for a high degree of morbidity and mortality in patients with metastatic PCa. In spite of the considerable progress that has been made in understanding the osteotropic features of PCa, a huge number of questions still remain. To date, it is evident that PCa cells hijack the bone microenvironment and alter physiological processes to favor their survival, proliferation and metastasis. Indirect and direct crosstalk among the neoplastic cells, OBs and OCs has emerged as a central point of the regulation of PCa progression.

Accumulating clues has revealed the specific roles of the local RAS in regulating bone homeostasis and PCa bone metastasis;

nonetheless, its specific roles are sometimes controversial. As a consequence, more works are necessarily carried out to further understand the pathophysiological role of the local RAS in bone metastases and the mechanisms underlying the possible therapeutic and/or adverse effects of angiotensin-converting enzyme inhibitors (ACEIs) and ARBs observed in preclinical and, particularly, in clinical trials. Also, it is essential to identify the downstream signaling pathways that stimulate AT1R and AT2R in both the progression of metastatic PCa and PCa bone metastasis. In summary, many published reports have confirmed a central role of the RAS in the development and the progression of PCa bone metastasis as elaborately outlined in this review article. Notably, ACEIs and ARBs are first-choice drugs used to treat the patients with the PCa bone metastasis, a large number of the crucial tasks that need to be clarified as follow: which is the most effective way to alleviate the local bone RAS? What is the role of the ACE2-Ang (1-7) pathway? For improving the current knowledge of the role of RAS in augmenting the PCa bone metastasis, more works would probably be prerequisite for further elucidating its functional roles in mediating the interplay of metastatic PCa cells and immune cells.

Ethical policy

Not applicable.

Author contributions

The author contributed solely to the work.

Competing interests

The author declares that there are no conflicts of interest.

Funding

Not applicable.

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