Angiotensin II, Cell Proliferation and Angiogenesis Regulator: Biologic and Therapeutic Implications in Cancer

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Abstract: Angiotensin II (ANG II) is the main effector peptide in the renin-angiotensin system. It is generated by the activation of Angiotensin I through the Angiotensin II Converter Enzyme (ACE II). ANG II has multiple physiologic effects that regulate vascular tone, hormone secretion, tissue growth and neural activity. It has systemic (endocrine) and local (paracrine and autocrine) effects, favoring cell growth and differentiation through four types of receptors from which types 1 and 2 (AT_1 and AT_2) are the most important. Stimulation of AT_1 leads to the activation of intracellular pathways that finally lead to vasoconstriction, inflammation and proliferation. The AT_2 receptor is mainly expressed in fetal tissue and scantly in the cardiovascular system under different circumstances. Its effects are opposite to those of the AT_1. The stimulation of AT_1 activates second messengers that lead to a rapid production of diacylglycerol and 1-4-5-inositol triphosphate, as well as to the activation of C protein. Several reports indicate that ANG II can induce neovascularization in experimental systems due to the expression of different growth factors such as angiopoietin 2, vascular endothelial factor, and its receptor, fibroblast growth factor, platelet derived growth factor, transforming growth factor β and epidermal growth factor. Other mechanisms associated with ANG II induced angiogenesis are nitric oxide synthase and metalloproteinase expression, as well as inflammation induction. Angiogenesis is a fundamental process to tissue repair and development, and it participates in several pathologic processes. In addition, the AT_1 receptor is expressed in many malignant neoplasms and its blockade through ECA II inhibitors and ANG II antagonists has shown antineoplastic activity as well as angiogenesis inhibition in tumoral experimental models. This review discusses the mechanisms by which ANG II participates in neoplastic and non-neoplastic tissue angiogenesis and its possible therapeutic implications.

Keywords: Angiotensin, cancer, angiogenesis, apoptosis, AT_1, growth factors.

1. OVERVIEW OF THE ANGIOTENSIN II SYSTEM

ANG II is a peptidic hormone. It is the main effector in the renin-angiotensin-aldosterone system (RAAS), one of the most powerful vasoconstrictor systems of the body [1]. The activity of this system starts with the release of renin from the juxtaglomerular cells in the kidney, where renal ischemia is one of the main activators of the system. Renin acts as an enzyme on angiotensinogen or plasmatic substrate, an α globulin synthesized by the liver. This catalytic reaction cleaves the amino terminal residue of the decapeptide generating ANG I, an unstable peptide that is quickly cleaved by ECA II into ANG II [2-4].

RAAS has an important role in the regulation of blood pressure and liquid and electrolyte balance; it participates in the pathophysiology of hypertension and its complications (cardiac hypertrophy, remodeling and nephropathy), coronary artery disease, heart failure and renal failure [1, 5]. The final responses to the activation of this system are different in every tissue, inducing vasoconstriction in smooth vascular muscle, release of aldosterone in adrenal glands, gluconeogenesis in the liver, sodium absorption in intestine and kidneys, increased fibroblast growth in the heart, increased β-adrenergic activity in the nervous system and increased thirst [6].

ANG II was initially described as a vasoconstrictor peptide but recent studies have revealed that ANG II acts also as a growth factor. ANG II plasmatic levels modulate contraction, cell growth, apoptosis and cell differentiation, and it has a role in cell migration, extracellular matrix conformation, inflammation and stimulation of the production of several growth factors as the platelet derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor beta β (TGFβ), insulin like growth factor 1 (IGF-1), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) [7-13] and platelet activator factor (PAF). It also stimulates the production of some vasoconstrictors as endothelin 1 (ET-1) and it helps to transactivation of growth factor receptors (EGF receptor and IGF receptor) [14]. Besides, it induces the expression of several proto-oncogenes in smooth vascular muscle in rats and humans, including c-fos, c-jun, c-myc, erg-1, VL-30, and the activator of the protein 1 complex [15, 16].

At present time, ANG II is known as a regulator of proliferation and hypertrophy of vascular smooth muscle cells, acting as a bifunctional apoptosis modulator through its receptors: AT_1 (antiapoptotic) and AT_2 (proapoptotic) [17].

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All actions of ANG II are mediated by specific signaling pathways, highly complex intracellular pathways triggered by the attachment of this peptide to its specific cell surface receptors (AT_1 and AT_2), coupled through effector G proteins, including phospholipase C and adenylate cyclase [15,18,19].

The important pathophysiological implications of ANG II has stimulated the pharmacological research of drugs capable to inhibit its effects, such as the ACE inhibitors and AT_1 blockers (AT_{1B}) [20], that have many potential pharmacologic indications.

2. ANGIOTENSIN RECEPTORS

The effects of ANG II are a consequence of its interaction with specific cell surface receptors on the target cells, called AT_1 and AT_2; AT_3 receptors have also been described in neuroblastoma cells and in rat mesangial cell cultures, and AT_4 receptors have been described in bovine adrenal cortex and in human placenta [21, 22]. AT_1 and AT_2 receptors are functionally distinct polypeptides, with homologous sequence of only 30%. The AT_1 gene is in chromosome 3 and AT_2 gene is in the X chromosome [3].

AT1 Receptor

AT_1 receptors have 359 aminoacids, they are part of the superfamily of G protein coupled receptors [23]. They have 7 transmembrane domains, the C-terminus is located within the cytoplasm and the N-terminus is glycosylated at the surface of the cell [18, 20]. They bind ANG II with affinity that depends on serum concentrations and they have a highly specific structure and a limited binding capacity (saturability). The interaction of these receptors with ANG II elicits intracellular responses [3].

AT_1 receptors are present in all tissues. In humans there is only one subtype of this receptor, while in rodents two subtypes have been identified as AT_{1a} and AT_{1b} receptors [1]. In blood vessels AT_1 are mainly expressed in smooth muscle cells, and in the heart they are found in myocardium and fibroblasts [15]. ANG II decreases the expression of AT_1 receptors in smooth muscle cells, liver and mesangial cells; on the contrary, ANG II increases mRNA of the AT_1 receptor in kidney. The expression of AT_1 receptor increases after chronic treatment with ACE inhibitors, AT_{1B}, cyclosporine A, estrogens, progesterone, glucocorticoids and aldosterone, and in some pathologic situations such as myocardial hypertrophy and heart failure; whereas its expression decreases during exposure to growth factors such as PDGF, FGF and EGF [20].

AT_1 Receptor Stimulation Produces Several Effects

a) Vascular effects: it is a powerful arterial and venous vasoconstrictor that increases peripheral vascular resistance and the blood pressure due to direct effect on the cells that release aldosterone, endothelin-1 (ET-1) and vasopressin, an increase in sympathic tone, a central effect and stimulation of protein and DNA synthesis that finally, increases the volume and number of smooth muscle vessel cells, fibroblasts and the middle sheath of the arteries, decreasing external and internal vascular diameters. These changes decrease coronary and brain vascular reserve and they also decrease arterial distensibility [24-26]; *b) Actions on myocardium:* such as increased contractility, heart rate and oxygen requirements and intense coronary artery vasoconstriction [27, 28]; *c) Cell proliferation and apoptosis:* in heart cell cultures, vascular cell cultures and fibroblast cultures, AT_1 receptor stimulation increases phenylalanine-H^3 incorporation, a marker of increased protein synthesis and hypertrophy, and it also increases timidine-H^3 incorporation, a marker of DNA synthesis. It also induces activation of mitogen activated protein kinases, it increases the expression of several growth factors, it increases collagen and laminin synthesis during fibroblast proliferation, it increases the expression of several metalloproteinase inhibitors, it increases the plasminogen activator inhibitor type 1 (PAI-1) that inhibits extracellular matrix degradation and it increases the transforming growth factor β1 that increases the synthesis of collagen type I. The final results are increased interstitial and perivascular heart fibrosis, decreased ventricle distensibility and decreased intracardiac conduction speed, favoring diastolic heart failure and heart arrhythmias [20, 29, 30]. The activation of AT_1 receptor can control vascular smooth muscle cell growth through proliferation or inhibition of apoptosis [31]. In the classic view, AT_1 receptor has an antiapoptotic effect, contrary to AT_2 receptor, however in vascular smooth muscle epithelioid phenotype cells, it is able to induce apoptosis mediated by the entrance of calcium [32]; *d) Proinflammatory and proatherothrombotic actions:* the stimulation of AT_1 receptor stimulates the interaction of monocytes and endothelial cells through the expression of adhesion molecules (VCAM-1), proinflammatory cytokines release (TNFα, IL-6), monocyte migration to subendothelial tissue, expression and oxidation of receptors for low density lipoproteins (LDL*)* [9, 20]; *e) Renal effects:* vasoconstriction and decreased renal blood flow without changes in glomerular filtration, mesangial cells contraction with decreased surface for glomerular filtration, increased proximal tubular sodium reabsorption and potassium excretion, and inhibition of renin secretion. AT_1 receptor stimulation also increases aldosterone, catecholamines, vasopressin, adrenocorticotropic hormone (ACTH), prolactine and luteinizing hormone (LH) release, along with increased sympathic tone [20, 33, 34].

AT_2 Receptor

The second important form of the ANG II receptor is AT_2 receptor. It is constituted by 363 aminoacids with 7 transmembrane domains [35] but it is not coupled to G proteins [36]. This receptor is highly expressed in fetal tissues and it rapidly decreases after birth, playing an important role in growth and development. In the adult, it is located to adrenal cells, brain (areas controlling motor activity and sensitive information), myometrium, endothelial cells (mainly in adventitia and medial sheaths) and to a lesser extent, in pancreas, heart and kidney. It is overexpressed in pathologic conditions such as heart damage, myocardial hypertrophy, ovarian atrophy, ACE inhibitors and AT_{1B} treatment [4, 15, 37].

AT_2 Receptor Stimulation has Different Effects

a) Vascular effects such as brain arteriolar dilatation*; b) Proliferative, differentiation and apoptotic effects.* The antiproliferative effect has been demonstrated in coronary endothelial cells, mesangial cells and pheochromocytoma cell lines. The proapoptotic effects have been demonstrated in pheochromocytoma line cells, in mice fibroblasts and in rat ovarian granulose cells. Some studies have shown apoptosis induction through AT_1 receptor stimulation, however, these contradictory results are a consequence of differences in experimental conditions; c) Effects on diuresis and natriuresis; *d) Central actions* such as brain arteriolar dilatation, prostaglandin release, increased potassium conductance, LH and somatostatin secretion [20, 30, 31, 38].

This receptor is an antagonist of AT_1 receptor effects under physiologic conditions because it inhibits cell growth, it induces apoptosis and vasodilation [15, 39]. See Table **1**.

3. INTRACELLULAR PATHWAYS FOR AT_1 AND AT_2 RECEPTORS

Signaling Pathways for AT_1 Receptor

The AT_1 receptor is coupled to signal transmission mechanisms used by many other cell membrane receptors. Binding of ANG II to AT_1 receptor induces dissociation of its subunits [3], activating a Gqα protein and then phospholipase C-β1, that hydrolyses 4,5-phosphatidylinositol biphosphate into 1,4,5-phosphatydilinositol triphosphate (IP3) and diacylglycerol [26, 40]. IP3 acts on specific receptors located in the sarcoplasmic reticulum membrane and it facilitates the release of stored calcium and the entrance of extracellular calcium through calcium channels activated when intracellular calcium deposits are emptied [4, 15, 20].

ANG II increases calcium entrance through L and T channels of heart muscle cell membrane, facilitating the exit of negative charges from the cell, depolarizing the membrane and opening L calcium channels. The result of all these events is increased intracellular calcium concentrations producing powerful arterial and venous vasoconstriction, increased heart rate, contractility and cardiac output and it increases the release of neurotransmitters (catecholamines, aldosterone and vasopressin) [15, 41]. On the other hand, diacylglycerol activates and translocates protein kinase C (PKC) to the cell surface, where it stimulates the phosphorylation of several proteins that participate in the mitogenic effects of ANG II [4, 26, 40].

Mitogenic effects of ANG II mediated through AT_1 receptors imply the activation of several kinases, the first one is PKC and this one activates G Ras and the pathway of mitogen activated protein kinases (MAPK). MAPK phosphorylate several effector proteins that translocate towards the cell nucleus, where they increase the transcription of several genes that participate in cell hypertrophy, and hyperplasia in several tissues [15].

In some tissues, the stimulation of AT_1 receptor activates phospholipases A2 and D, increasing eicosanoid synthesis, particularly that of prostaglandin E2, that exhibits proinflammatory properties. In heart, the activation of this pathway also blocks several potassium channels, depolarizing the cell membrane and increasing the calcium entrance through L type channels, leading to increased

Table 1. Effects of AT_1 and AT_2 Receptors Stimulation

	AT_1	AT_2
BLOOD VESSELS	Arteries and veins vasoconstriction	Brain artery vasodilation
HEART	Increased contractility and heart rate Increased oxygen requirements Coronary vasoconstriction	
TROPHIC ACTIONS	Increased DNA and protein synthesis. Heart hypertrophy, hyperplasia and remodeling. Stimulation of angiogenesis	Cell growth and differentiation Inhibition of angiogenesis Activation of heart collagenases Antiproliferation and Apoptosis
CENTRAL ACTIONS	Increased sympathic tone Vasopressin, ACTH, LH and prolactin release.	Brain artery vasodilation Prostaglandin release Increased potassium conductance LH and somatostatin secretion Motor activity and sensitive information.
RENAL ACTIONS	Vasoconstriction Mesangial contraction and proliferation Increased proximal tubular sodium reabsorption Increased renal potassium excretion Increased prostaglandin synthesis Inhibition of renin secretion	Increased proximal tubular sodium reabsorption

Table 2. Factors Angiogenic and Antiangiogenic

	Stimulators	Inhibitors
Growth Factors	Epidermal growth factor Granulocyte Colony Stimulation Factor Fibroblast growth factor (acid, base) Hepatocyte growth factor Platelet derived growth factor Tumor necrosis factor α Vascular endothelium growth factor	
Proteases and protease inhibitors	Cathepsine Gelatinase A, B Estromelicine Urokinase type plasminogen activator	Inhibitor of fine tissue metalloproteinase Plasminogen-1 activator and inhibitor
Scan elements	Copper	Zinc
Oncogenes	c-myc ras c-src v-Royal Air Force c-jun	rb, p53
Signal transduction enzymes	Thymidine phosphorylase Farnesyl transferase Geranylgeranyl transferase	
Cytokines	Interleukin 1 Interleukin 6 Interleukin 8	Interleukin 10 Interleukin 12
Endogenous modulators	Angiopoietin 1 Endothelin Erytropoietin Hypoxia Nitric oxide Synthase Platelet activator factor Prostaglandin E Thrombopoitein	Angiotensin Caveolin 1, caveolin 2 Endostatin Interferon alfa Isoflavones Platelet factor 4 Prolactin Troponin 1

cardiac contractility and vascular tone; it also activates a Gi protein and inhibits adenylate cyclase, decreasing intracellular cAMP. As cAMP has vasodilator actions, its reduction would contribute to the vasoconstrictor effect of ANG II [4, 20, 23, 26, 40].

Several proteins such as c-Src, Jak-2 and FAK are phosphorylated at tyrosines in response to ANG II, favoring the generation of IP3 and the entrance of calcium to the cell, that in turn activate intracellular MAPK pathways, phosphorylation of signal transducers and activators of transcription (STATs), that stimulate the transcription of early growth response genes as *c-fos, c-jun, y c-myc* [23].

Signaling Pathways for AT_2 Receptors

The stimulation of AT_2 receptors can inhibit AT_1 receptor transduction pathways, leading to a growth inhibition effect through the activation of tyrosine phospholipases of several proteins. Some of these phospholipases are SHP-1, MKP-1 and PP2A, and they are able to inhibit STAT-1 phosphorylation, generating an antimitogenic and/or proapoptotic effect. AT_2 receptor stimulation also increases the *de novo* production of ceramide in pheochromocytoma cells through the activation of phospholipases.

AT_2 stimulation induces nitric oxide production, leading to cGMP stimulation in vascular cells of coronary arteries and aorta. AT_2 receptors are also able to stimulate phospholipase A2 through Gi proteins, with the corresponding release of arachidonic acid and of several serine/threonine phosphatase proteins that dephosphorylate several regulatory proteins and decrease the cellular concentrations of cGMP. [30, 42] See Fig. (**1**).

The effect of AT_1 receptor antagonists may be the result of the blockade of AT_1 receptor allowing ANG II to act mainly on AT_2 receptor.

4. ANGIOGENESIS

Angiogenesis is new blood vessel formation from pre-existing endothelial cells that cover capillaries and the other

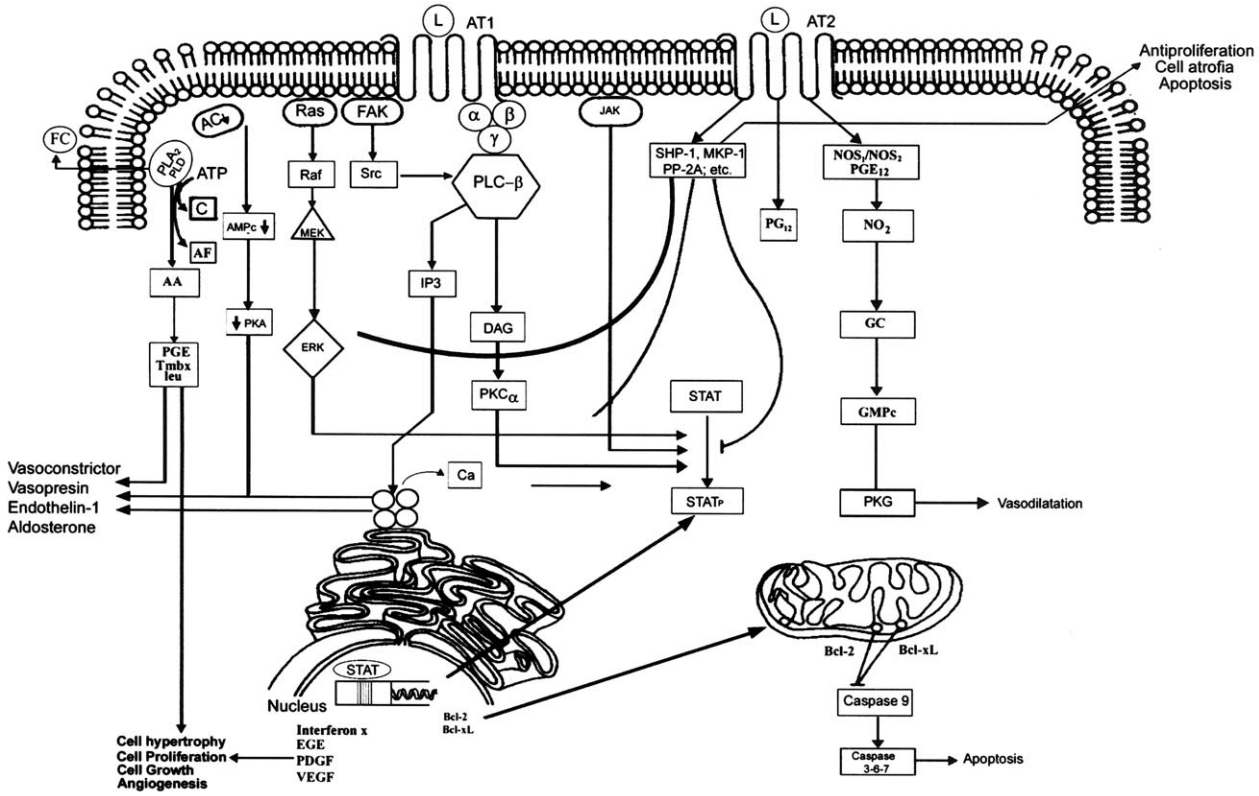

Fig. (1). Signaling pathways of the effects mediated through the stimulation of AT_1 and AT_2 receptors. AT_1 receptor stimulation results in Gqx protein activation (with three subunits α, β y γ and then phospholipase C activation (**PLC**β) that hydrolyses phosphatidylinositol (4,5) biphosphate in inositol-1,4,5 triphosphate (**IP3**) and diacylglycerol (**DAG**). IP3 acts on specific receptors from sarcoplasmic reticulum and enhances **Ca** release. DAG activates protein kinase C (**PKC**) and these enzymes stimulate the phosphorylation of several signal transducers and activators of transcription (**STAT**). AT_1 also activates focal adhesión kinase (**FAK**) and this enzyme activates **Src**, **Ras**, and **Raf** that in turn activate a mitogen-activated protein kinase (MAPK) called **MEK** resulting in the final activation of extracellular signal-regulated kinase (**ERK**) that also participates in STATs phosphorylation. AT_1 also activates a Janus Kinase (**JAK**) that participates in STAT phosphorylation that is translocated to the nucleus, where it promotes the transcription of genes that participate in cell hypertrophy and hyperplasia, as well as **Interferon-**α, vascular endothelial growth factor (**VEGF**), fibroblast growth factor (**FGFb**), platelet derived growth factor (**PDGF**), **BCL-2**, and **Bcl-xL**, that block the caspase cascade, inhibiting apoptosis. AT_1 activates phospholipases A and D (**PLA2** and **PLD**) that increase leukotriene **(Leu)**, thromboxane (**trmbx**) and prostaglandin E_2 (**PGE_2**) synthesis, all of which have proinflammatory properties. AT_1 inactivates adenylate cyclase (**AC**), decreasing **cAMP** along with its vasodilator action, favoring the vasoconstrictive effect of **ANG II**. AT_2 stimulation activates several phosphatases like serine/threonine phosphatase-2 (**PP2A)**, Mitogen activated protein Kinase phosphatase 1 (**MKP-1)** and Src homology (SH)2 protein containing tyrosine phosphatase (**SHP-1)**, which inactivate ERK and inhibit calcium release. ERK inactivation leads to serine dephosphorylation of STATs. AT_2 also induces the production of inducible nitric oxide synthase (**NOS**) generating nitric oxide (**NO)** that activates guanylcyclase (**GC**), increasing **cGMP** production, stimulating protein kinase C and favoring vasodilation.

blood vessel walls with smooth muscle. This is a natural and important process in normal and pathologic conditions, including wound healing, yellow body formation in ovaries and blood flow restoration in injured tissues. This process may be the primary event in pathologic conditions like diabetic retinopathy, ischemia and tumor vascularization [9, 43].

Vessel neoformation is finally regulated by the balance of angiogenic stimulating and inhibiting factors. Among the endogenous factors identified as angiogenic are TGF β, granulocytic colony stimulating factor (GSF), FGFb, hepatocytic growth factor (HGF), tumor necrosis factor α (TNFα), PDGF, VEGF, IL-8 and angiogenin. VEGF is currently considered the most important factor in the control of normal and pathologic angiogenesis because it is able to induce a cascade of responses in endothelial cells, such as proliferation, migration, increased vascular permeability and expression of pro-inflammatory genes. To induce such effects VEGF should be coupled to a cell surface receptor.

When there is an excess of angiogenic growth factors compared to angiogenesis inhibitors, the balance favors the growth of a new blood vessel. When there is an excess of inhibitors, angiogenesis stops.

A healthy individual maintains a perfect equilibrium between angiogenic and antiangiogenic factors. However, during many serious diseases, the individual looses control

over angiogenesis. Diseases that develop as a result of excessive angiogenesis include cancer, diabetic retinopathy, age related macular degeneration, rheumatoid arthritis, psoriasis and other conditions [43].

Angiogenesis has an important role in growth and spread of neoplastic cells that are able to express one or many angiogenic promoters that act synergistically. Neovascularization favors more efficient tumor perfusion. Neoplasms may exhibit a low angiogenic ability during a pre-malignant phase and the change of phenotype towards a cell type able to produce more angiogenic factors may generate a malignant invasive cell with ability to spread and generate metastases. Angiogenesis inhibition is an attractive therapeutic target for several malignant neoplasms; in fact there are several studies that have explored this therapeutic option [44-48].

5. ANGIOTENSIN II AND ANGIOGENESIS

Several studies led to the hypothesis that ANG II was involved in the regulation of blood vessel neoformation. In experiments of aorta ligation, it was demonstrated that the administration of exogenous ANG II to nephrectomized rats increased the blood flow recovery in the muscles of lower extremities. Other experiments have also shown that ANG II favors the development of collateral blood flow in peri-renal blood vessels after renal ischemia produced by renal artery ligation [49]. However, until 1985, it was demonstrated that ANG II is able to stimulate angiogenesis in rabbit cornea from collateral circulation [50]. Besides, ANG II is able to decrease mortality in gerbils after unilateral carotid artery ligation, possibly due to increased development of collateral circulation and decreased cerebral ischemia [51, 52]. In the same line, when ACE inhibitors were administered to gerbils with progressive occlusion of the carotid artery, mortality increased, suggesting that ANG II has a protective effect against chronic ischemia, however, the administration of PD123319 an AT_2 antagonist, or losartan, an AT_1 antagonist, increased survival in this stroke model, suggesting that AT_2 stimulation is involved with the protective effect of ANG II against ischemia [53].

Angiogenic effect of ANG II was also demonstrated in other models. One of them is the chicken embryo chorioalantoid membrane, where ANG II caused precapillary and postcapillary vessel neoformation in 30-40% of cases in a dose-dependent manner [54]. Interestingly, angiogenesis stimulation was neither blocked by losartan (AT_1 receptor antagonist) nor by PD123319 (non peptidic antagonist of AT_2 receptor) but it was blocked by CGP42112A (an AT_2 ligand) [55]; however, the inhibition of angiogenesis by this last compound was not observed without the presence of ANG II. These findings suggest that the angiogenic effect of ANG II is mediated by different receptor subtypes.

Another model in which ANG II has demonstrated pro-angiogenic effect is the sponge implant model in mice, where extracellular matrix protein synthesis is also increased in a dose dependent manner [56].

In a rat cremasteric muscle model, ANG II infusion significantly increased microvascular density measurement, an effect that was potentiated by co-infusion of high doses of PD123319, in contrast to the effect observed with co-infusion of losartan that generated an important decrease of microvessel density. This is just like in the model of chicken chorioalantoid membrane in which the separated administration of both antagonists did not modify microvascular density, suggesting that AT_1 receptor favors ANG II induced angiogenesis and that AT_2 receptor mediates an inhibition of angiogenesis [57]. Using the ^{133}Xe clearance technique to determine subcutaneous sponge granuloma blood flow, infusion of ANG II caused an intense neovascularization and increased the fibrovascular growth area, while co-administration of losartan inhibited them in 50% and PD123319 induced no change [58, 59]; however, the isolated administration of both antagonists did not modify angiogenesis. In this same model, the experimental administration of ANG II induces the presence of AT_1, AT_2 and ACE during microvascular maturation, suggesting that ANG II induced angiogenesis depends on the balance of the ACE, AT_1 and AT_2 expression [59]. The administration of ANG II to mice with the angiogenesis *in vivo* model of Matrigel, significatively increased angiogenesis, an effect that was inhibited by candesartan (AT_1 blocker) but not by PD123319 [9].

Ischemia is able to stimulate angiogenesis, a process that is experimentally inhibited in mice with limb ischemia with the pharmacological blockade of AT_1 or ACE inhibitors [60]. Seemingly, this process is affected in AT_1 receptor knockout mice ($AT_{1a-/-}$), suggesting that AT_1 receptor participates in angiogenesis induced by ischemia *in vivo* [61]. Besides ischemia, regular aerobic exercise training and electric stimulation induces physiologic angiogenesis in striated muscle as an adaptative response; in these cases ACE inhibitors and losartan have demonstrated blockade of VEGF expression and angiogenesis inhibition. It seems that RAAS is needed for VEGF expression during angiogenesis because Dahl S rats with low levels of renin do not induce angiogenesis as a response to electric stimulation compared to Dahl S rats transferred with renin gene, that show VEGF expression and vascular neoformation restoration [62].

AT_1 receptor and ACE expression can be modulated by ischemia. Initially (1 to 3 days) limb ischemia in mice decreases ACE activity and it is restored afterwards (day 7 to 14); besides, ischemia decreases AT_1a expression, and initially increases AT_1b expression [60]. However, it seems that AT_1 is not the only receptor involved in angiogenesis induced by ischemia, because femoral artery ligation in mice increases AT_2 expression; besides, in AT_2-deleted mice there is increased vascular density, capillaries and blood flow during ischemia compared to controls, indicating that AT_2 could modulate in a negative way angiogenesis induced by ischemia. VEGF expression is increased during ischemia with the administration of ANG II but in AT_2-deleted mice, VEGF remains similar to control mice, indicating that changes in VEGF are not related to AT_2 receptor. Anti-angiogenic effect of AT_2 could be associated to apoptosis activation [63].

Many components of the RAAS may have different and even opposing properties and functions. They are ANG II peptides generated by aminopeptidases, endopeptidases and carboxipeptidases. One of them is ANG-(1-7) generated

from ANG I and to a lesser extent, by ANG II. In the mice sponge implants model, ANG II has also shown an increase in fibrovascular tissue growth, in contrast to ANG-(1-7) that significantly inhibited angiogenesis, suggesting that some bio-active fragments from RAAS are endogenous angiogenesis regulators [64].

The concept that ANG II has pro-angiogenic properties is not universally accepted because there are some reports in which ACE inhibitors increase vascular density in several models such as the rat limb muscle model [65, 66] and the coronary microvessel model [67]. VEGF administration and ACE inhibition with quinalapril but not with captopril, increase capillary density and collateral circulation determined by angiography in relation to controls in rabbit models of ischemia induced by femoral artery resection, suggesting that ACE inhibition promotes angiogenesis and that the possible benefit of this drugs in patients with coronary artery disease may be partly explained by increased collateral coronary circulation [66]. These are controversial results, possibly because the regulation of angiogenesis by RAAS can be modified by many factors in different experimental or clinical situations, generating paradoxical results.

6. MECHANISMS BY WHICH ANG II INDUCES ANGIOGENESIS

The main mechanisms involved in angiogenesis induced by ANG II is the regulation of the expression of growth factors such as VEGF, angiopoietin, IGF, PDGF, TGF-β, FGF and HGF, as well as its receptors, proliferation stimulation and apoptosis inhibition in endothelial cells, proinflammatory effects, generation and induction of matrix metalloprotinase (MMP). See Fig. (**2**).

6.1 Growth Factors

VEGF is a homodimeric glycoprotein that stimulates proliferation of endothelial cells as well as several cell lines and it promotes angiogenesis by chemotaxis stimulation and the expression of plasminogen activators and collagenases through its KDR and Flk-1 receptors. It is synthesized by several tumors, being an attractive therapeutic target [67]. ANG II is a powerful stimulator for VEGF expression in several models. For instance, in endothelial cells of rat hearts, losartan but not PD123319 blocked ANG II induced VEGF synthesis (8). In the Matrigel angiogenesis model, the administration of ANG II increases VEGF in 144% compared to controls; that effect is blocked by AT_1 antagonists but not by AT_2 antagonists. In the same model, ANG II induced angiogenesis is blocked by neutralizing antibodies against VEGF. In bovine microcapillary endothelial cells, ANG II stimulation induces expression of KDR in a dose-dependent manner; this is blocked almost completely (90%) using the non selective ANG II antagonist saralasin and the AT_1 antagonist DuP753, and there is almost no change (20%) using AT_2 receptor antagonist PD123319 [68]. At the same time ANG II potentiates angiogenesis induced by VEGF in a dose dependent manner and such effect is blocked by AT_1 antagonists but not by AT_2 antagonists [9]. In conclusion, angiogenesis induced by ANG II is VEGF-dependent, increasing the expression of that factor and of its receptor through AT_1 receptor.

Tie2 ligands angiopoietin 1 and 2 are multifunctional proteins involved in embryogenesis, reproductive system and vascular neoformation. The main regulators of its expression are hypoxia, VEGF, FGFb, TNF and ANG II [69]. Recent studies have demonstrated that ANG II is able to stimulate angiopoietin 2 but not angiopoietin 1 synthesis in bovine

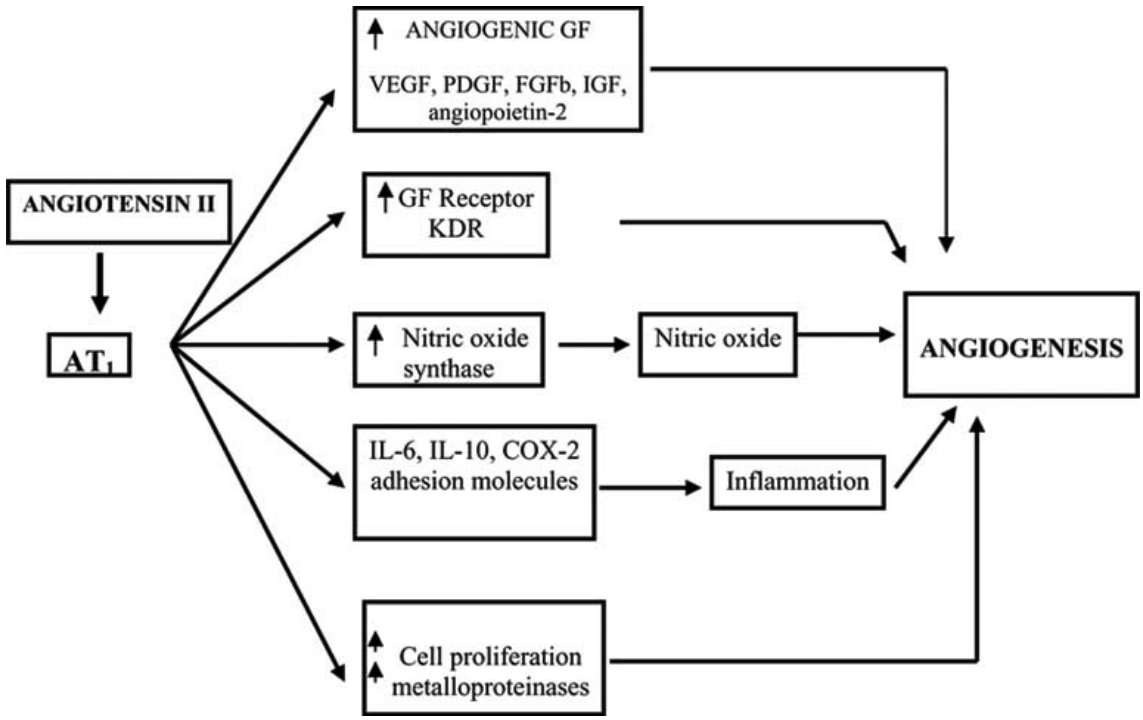

Fig. (2). Mechanisms involved in angiogenesis.

retinal endothelial cells in a dose dependent manner, due to increased expression rather than angiopoietin 2 mRNA stabilization. This response was inhibited by losartan but not by PD123319; angiopoietin 2 expression in response to ANG II is blocked by the inhibition of protein kinase C (PKC) and mitogen-activated protein kinases (MAPK) [70]. In fact, in endothelial heart cells, ANG II induced expression of mRNA of angiopoietin 2 in a dose dependent manner and it did not produce changes on angiopoietin 1 or Tie2. On the other hand, AT_1 blockade decreased in 76% the accumulation of angiopoietin 2 mRNA induced by ANG II while AT_2 blockade with PD123319 induced no changes [14]; in the same line, angiopoietin 2 expression through AT_1 stimulation was also blocked with EGF receptor antagonist AG1478 and with the MEK inhibitor PD98059 [63]. This fact suggests that ANG II increases angiopoietin 2 expression through EGF receptor pathway that involves ERK, indicating that ANG II has an important role on pathologic angiogenesis in diabetic retinopathy, ischemic diseases and maybe tumoral angiogenesis.

Insulin like growth factor I (IGF-I) and its receptor regulate multiple cell functions; it may favor neoplasm formation increasing cell survival, motility and invasion and inducing angiogenesis; it is expressed in several tissues and cell types with autocrine and paracrine effects. For these reasons, it is a potential therapeutic target against cancer [71]. ANG II stimulates IGF-1 expression, IGF-I receptor expression associated to DNA synthesis in vascular smooth muscle, effects blocked by antibodies against IGF-I [72, 73]. The stimulation of ANG II on IGF-I has also been observed in heart muscle, inducing heart structure changes during hypertension and inhibiting these changes after the administration of losartan and hydralazine, indicating that these effects may be explained by hemodynamic and non hemodynamic changes [74, 75].

In fact, it seems that the effect of ANG II on IGF-1 is not exclusive for vascular muscle because in heart fibroblast cultures, stimulation with ANG II induces IGF-I receptor over-expression, and AT_1 inhibition decreases the expression of genes such as c-Fos, Egr-1 and Sp1 as well as IGF-I induced fibroblast [76]. The expression of IGF-I receptor induced by ANG II in vascular smooth muscle cell culture (VSMCs) is inhibited by antioxidants, tyrosine kinase protein inhibitors (genistein and tyrphostin A25) and the intracellular calcium chelator BAPTA-AM, indicating that the possible intracellular IGF-I stimulating pathways that involve ANG II are calcium dependent, mediated by redox sensitive tyrosine kinase pathways [77]. Other experiments have demonstrated that the pathways by which ANG II induces IGF-I expression involve nuclear transcription factor κB (NF-κB) [13]. Finally, we know that AT_1 regulates IGF-I and also that IGF-I stimulates AT_1 receptor expression in vascular smooth muscle, an interaction that can modulate heart cell and vascular smooth muscle cell growth [73, 78].

Platelet-derived growth factor (PDGF) is constituted by an A chain and/or a B chain, forming a homodimer or a heterodimer with sulphate bounds. It is an important mitogen and chemotactic for mesenchymal cells, with vasoconstrictive properties; it also stimulates several cell functions such as growth, proliferation and differentiation. PDGF is expressed by different tissues and different solid tumors [79]. ANG II induces expression of PDGF-A chain in rat muscle cells and this effect is blocked by saralasin (AT_1 blocker) [10]. ANG II can activate a transcription factor called early growth response factor 1 (Erg-1) and it can induce PDGF expression [80].

MEK pathway specific inhibitor PD98059 and AT_1 antagonist losartan block Erg-1 activation and PDGF-A expression, indicating that MEK-Erk is an important component in ANG II transduction pathways to increase PDGF expression through AT_1 receptor [81, 82]. Interestingly, the AT_2 antagonist PD123319 has no effect on the PDGF-A production. On the other hand, PDGF-B chain expression is stimulated via AT_1 receptor in smooth muscle cells of newborn rats but not in adult rats, through two members of the MAPK cascade: ERK (extracellular signal regulated protein kinase) and JNK (c-jun N-terminal protein kinase) [83]. ANG II also plays a direct role in the activation of both PDFG chains in response to mechanic damage of vessel walls, an effect that is blocked by ACE inhibitors [84]. Besides increasing PDGF expression through AT_1 receptor, ANG II also converges with transduction pathways of several mitogenic growth factors such as PDGF receptor, stimulating the phosphorylation of some proteins that are members of the Ras activator family such as Shc [85]. It has also been observed that ANG II stimulates tyrosine phosphorylation of PDGF-B receptor *in vitro* and *in vivo* [7, 86]. PDGF expression stimulated by ANG II may modulate vascular smooth muscle cell proliferation (hypertrophy and hyperplasia) and vasoconstriction.

Transforming Growth Factor β (TGFβ) is a multifunctional cytokine with several homodimeric isomorphs (TGFβ 1, 2 and 3) that modulate cell proliferation, differentiation, apoptosis and embryogenesis. This molecule induces the expression of several extracellular matrix proteins such as collagen, it has a powerful chemotactic effect for fibroblasts, it induces the release of other cytokines such as PDGF that plays an important role in normal cicatrization process and in pathologic fibrosis. Several neoplasms express these cytokines, favoring tumor progression caused by angiogenesis stimulation and immune cell suppression [87]. In hypertensive rats there is increased expression of TGF-β1 associated to extracellular matrix proliferation in heart muscle and in vascular smooth muscle, and AT_1 inhibitors as well as ACE inhibitors can reverse this process [88-90]. ANG II induces phosphorylation and activation of extracellular signal-regulated kinases (ERK 1 and 2) followed by an increase in activator protein 1 (AP-1) and in TGF-β mRNA. It is now known that PD98059, an ERK specific inhibitor can decrease TGF-β1 expression and AP-1 activation, suggesting that ERK is involved in TGF-β1 induction by ANG II and that it participates in vascular remodeling in hypertension [91]. These phenomena are not exclusive for the cardiovascular system; in glomerular and mesangial rat cells, ANG II increases the expression of TGF-β and of some extracellular matrix components such as fibronectin and type I collagen, in a dose-dependent manner, *in vitro* and *in vivo*. Interestingly, ANG II promotes conversion of TGF-β from an inactive to an active form and AT_1 receptor inhibitors prevent this phenomenon [92].

It is known that TGF-β promoter activation by ANG II is blocked by the inhibition of PKC and TGF-β promoter activation by MAPK is blocked by BIS-1 and SB 203580, therefore TGF-β1 increase mediated by ANG II depends on PKC and p38 MAPK [93]. Interestingly, it is known that AT_2 receptor over-expression on vascular smooth muscle cells (VSMCs) suppresses the expression of TGF-β1 receptor [94] a fact that explains why the treatment with an AT_1 receptor inhibitor decreases TGF-β1 production and may indirectly generate greater stimulation of AT_2 and greater inhibition of TGF-β1 receptor.

Fibroblast growth factors (FGFs) are members of a polypeptide family synthesized by several cell types. They have an important role in cell differentiation and development; they are expressed in embrions as well as in adult tissues and they stimulate several biologic processes as mitogenesis and angiogenesis [95]. In bovine adrenal cells ANG II stimulation increases FGF-2 concentrations [11]. In these cells, AT_1 and AT_2 stimulation increase FGF-2 gene transcription and genistein (tyrosine kinase inhibitor) decrease it, indicating that tyrosine kinase activity may be essential for FGF induction by angiotensin [12, 13]. Paradoxically, ANG II inhibits FGF-2 induced proliferation in adrenal cells, maybe inducing PGE2 synthesis in the case of AT_1, and inducing the activation of pathways that require tyrosine kinase phosphorylation in the case of AT_2 [96]. FGF-2 can also regulate AT_1 expression in vascular smooth muscle; however, this is a negative regulation [97].

Hepatocyte Growth Factor (HGF) is a multifunctional protein that regulates cell growth and motility and it has a powerful angiogenic effect mediated by its c-met receptor [98]. Its overexpression has been found in several neoplasms and it is related to malignancy degree, recurrence and prognosis [99]. HGF and its receptor have been identified *in vivo* and *in vitro* in vascular cells. There is evidence that indicates that ANG II can regulate HGF synthesis, for instance ANG II decreases local production of HGF in a dose-dependent manner in vascular smooth muscle cells. In other studies, endothelial damage induced by intravascular balloon decreased HGF production, while the ACEI cilazapril and E-4177 increased its concentrations and inhibited the formation of a neointimal sheath [100]. Hypertense rats have decreased HGF tissue concentrations (kidney, heart, vessels) and AT_1 blockade or ACE inhibition can increase HGF tissue levels [101], indicating that local HGF production could have a protective effect on endothelium and that the decrease of HGF mediated by ANG II could accelerate endothelial damage. In fact, the benefit of RAAS blockade may be due to increased HGF tissue levels. Other studies have demonstrated that pre-treatment with candesartan (AT_1 blocker) in ischemic liver damage with reperfusion, increase tissular HGF levels and prevents post reperfusion damage [102].

6.2 Inflammation

Recent studies have shown that ANG II has proinflammatory actions including free oxygen radical production, release of proinflammatory cytokines like interleukin-6 (IL-6) and cell adhesion molecules, mediated by the activation of nuclear factor-κB (NF-κB) and protein kinase C [103], inducing endothelial dysfunction and increased atherosclerosis. Cytokine synthesis in vascular smooth muscle apparently is AT_1 dependent because its antagonist losartan inhibits IL-6 production. Besides, this cytokine induces angiotensinogen synthesis in the liver, increasing this important substrate for RAAS [104, 105]. These facts suggest that ANG II may contribute to the pathogenesis of atherosclerosis.

While inflammatory processes and cyclooxygenase-2 (COX-2) can regulate angiogenesis [106, 107], it is also known that ANG II stimulates monocyte recruitment, macrophage activation and increase COX-2 expression. For instance, in angiogenesis models with Matrigel, ANG II induced macrophage recruitment and COX-2 expression in 42% and these effects were blocked by AT_1 receptor antagonists. Besides, nimesulide, a COX-2 inhibitor, decreased angiogenesis induced by ANG II [9], indicating that ANG II-induced angiogenesis also depends on inflammatory processes.

6.3 Metaloproteinases

The presence of enzymes that degrade extracellular matrix is necessary during angiogenesis [108]. ANG II regulates the production and activation of several matrix metalloproteinases (MMP), important enzymes for collagen degradation. In heart muscle cells, stimulation with ANG II induces the expression of MMP-9, an effect that is inhibited by calfostin C a specific inhibitor of Protein Kinase C [103]. ANG II can also increase the expression of MMP-1 and 2 [109].

6.4 Nitric Oxide

Another mechanism by which ANG II could favor angiogenesis is through the regulation of the expression of nitric oxide synthetase (NOS) mediated by VEGF. Nitric oxide is a vascular tone modulator that has been implicated in neovascularization modulation in response to ischemia [110]. In a murine model of ischemic tissue, angiotensin administration increases the tissue expression of NOS, interestingly, AT_2 receptor knock-out mice have also increased basal NOS expression compared to control mice [63]. Other studies have shown that angiogenesis induced by angiotensin in the Matrigel model is blocked by the NOS inhibitor L-NAME; besides, the increase in tissue NOS concentrations can be prevented using AT_1 blocker candesartan and stimulated using PD123319, in conclusion, it seems that angiogenesis induced by ANG II depends on nitric oxide, because AT_1 stimulation increases nitric oxide synthesis [9].

7. ANGIOTENSIN AND CANCER

Angiogenesis is a mandatory process in neoplasia growth and development [111]. The angiogenic effect of ANG II can influence on carcinogenesis or in the growth of several neoplasms. There are two epidemiologic studies involving hypertensive patients that suggest that the treatment with ACE inhibitors could decrease cancer risk (relative risk = 0.73 and 0.79) [112, 113] although the results had no statistical significance. In the same line, a retrospective study that included more than 4,900 hypertensive patients has

shown that patients treated with ACE inhibitors had significantly reduced cancer risk compared to patients without this treatment, with a relative risk of 0.72 (95% CI: 0.55-0.92) for any neoplasm and 0.48 (95% CI 0.23-0.88) for fatal neoplasms; there was no relation when comparing patients treated with other antihypertensive drugs. Those neoplasms that had the lower incidences in ACE inhibitors users were lung and colon, as well as female sex specific neoplasms [114]. However, these results have not been confirmed by randomized studies [115- 117]. The lack of association of the use of ACE inhibitors and the reduction in cancer risk in most of the retrospective studies does not indicate necessarily that ANG II does not participate in neoplasm development, neither that the inhibition of RAAS blockade has no antineoplastic activity. An example of a similar situation is the indication of tamoxifen use. It is well known that tamoxifen is an anti-hormonal treatment with anti-neoplastic activity in breast cancer bearing estrogen and progestagen receptors, however, its long term use prevents breast cancer only in high risk patients, not in general population [118].

The RAAS has been associated with growth stimulation for several neoplasms at experimental level. In fact angiotensin receptors have been found in cell surface and in cytoplasm of human tumors such as breast cancer, malignant gliomas, hepatic carcinoma, renal carcinoma, melanoma, pancreas cancer and sarcomas.

Binding assay studies for ANG II receptor subtypes in medroxyprogesterone induced breast adenocarcinoma in mice have shown high expression of AT_1 receptor in the ductal tumors in contrast to lobular adenocarcinomas, while AT_2 receptor was found in peritumoral connective tissue and the control tissue did not express any of these receptors. In addition, this study has shown that ACE was expressed in all adenocarcinomas, mainly in ductal adenocarcinomas [119].

Tahmasebi has also shown the presence of AT_1 receptor in 28 of 30 samples of in situ ductal carcinoma and infiltrating ductal carcinoma, detected by immunohistochemistry [120]. Another study confirmed the presence of AT_1 receptor by the same method as well as by immunoblotting and ligand binding analysis in benign and malignant mamarian tissue in 22 patients, showing that AT_1 receptor is distributed in epithelial cell cytoplasm of normal and non-malignant tumor tissue. For malignant tumors there was a graded distribution of staining intensity, each varied from cell to cell and from one part of the tumor to another [121]. However in another study, AT_1 histochemistry was negative in 25 breast invasive human carcinoma samples and positive in 31 of 33 hyperplastic tissue samples and also positive in 18 of 23 in situ carcinoma tissue samples, suggesting that AT_1 expression may contribute to premalignant tissue development [122]. On the other hand, AT_2 receptor has been found in hyperplastic lesions, in situ and invasive carcinoma tissue, using immunocytochemistry and in situ hybridization, along with increased NOS expression [123].

In MCF-7 cell line of human breast cancer, angiotensin increases β_1 integrin expression, a cell adhesion molecule that has been found in breast cancer and that plays an important role in cell growth and differentiation as well as in [124]. Additionally, in MCF-7 cell line there is AT_1 and AT_2 receptor expression, and ANG II had a dose dependent proliferative effect, associated to AT_1 receptor mediated Na+/K+ ATPase activation [125]. In other study using normal breast cell and invasive intraductal carcinoma cell cultures from 5 mastectomies, AT_1 mRNA expression was measured and its levels were higher in carcinoma cells. In these same cultures, ANG II increased calcium intracellular concentrations in a dose dependent manner through AT_1 receptor, mainly in malignant cells, maybe due to a higher AT_1 receptor expression; additionally, angiotensin stimulates cell proliferation through PKC α and $\beta 1/2$ and phosphorylated extracellular-regulated kinases 1 and 2 (ERK1 and ERK2), a mandatory step for proliferation of these cells induced by EGF, a cytokine involved in neoplasm invasiveness. In this experiment cell proliferation induced by ANG II is inhibited by losartan, Gö6976 (PKC inhibitor) and AG1478 (EGF receptor tyrosine kinase inhibitor). Therefore, AT_1 regulates mitogenic signaling pathways by two simultaneous mechanisms: PKC activation and EGF receptor activation [126, 127].

In the ductal breast carcinoma human cell line, captopril significantly decreased the expression of estrogen receptors and increased the expression of progesterone receptors, inhibiting cell proliferation in a dose dependent manner; these measurements were made by [^{3}H] thymidine incorporation, and the results suggest that captopril has a cytostatic activity not mediated by estrogen receptor [128]. All these facts suggest that tissue RAAS contributes to breast cancer development and progression.

It is well known that there is a local brain RAAS [129], where neurons and glial cells can express ANG II. Astrocytomas are brain tumors that originate from glial cells and are the most common primary brain tumors. Anaplastic astrocytoma and glioblastoma multiform are aggressive tumors that show extense endothelial cell proliferation and angiogenesis, as well as high production of growth factors as PDGF, VEGF and HGF. Some studies have shown that glioblastoma multiform can synthesize renin, unlike low grade reactive gliosis tumors, indicating a possible relationship between endogenous intratumoral renin and angiogenesis [130]. Additionally, Fogarty has shown the presence of different AT_1 and AT_2 receptor subtypes (AT_{1a} and AT_{1b}) by RT-PCR in rat astrocytoma cells, and the incubation of these cells with different types of angiotensins induced cell proliferation, regardless AT_1 and AT_2 receptor expression, suggesting that astrocytomas can express several angiotensin receptor subtypes [131]. In concordance, an *in vivo* study with C6 rat glioma shown that the administration of losartan inhibited tumor growth in a dose dependent manner, as well as cell proliferation and vascular density, suggesting that AT_1 receptor blockade may lead to decreased synthesis of growth factors that depend on angiotensin, representing an attractive therapeutic target [132].

Several experimental studies have shown that ACE inhibitors decrease preneoplastic lesions and hepatic carcinoma cell growth [133-135]. In mice BNL-hepatic carcinoma, treatment with perindopril, an ACE inhibitor decreased tumor growth, angiogenesis and VEGF, unlike treatment with losartan or candesartan, suggesting that the

antineoplastic effect could be independent from AT_1 receptor blockade. [136-138].

There is also evidence that suggests the participation of RAAS in pancreatic cancer. For example, it is known that pancreatic cancer tissue expresses high levels of ANG II compared to normal pancreatic tissue; interestingly ACE activity is the same in both tissues, indicating that ANG II production in pancreatic cancer is ACE-independent [139]. Experimental studies using hamster pancreatic cancer cultured cells have shown that captopril decreased cell proliferation and the cell proliferation antigen (PCNA), a DNA polymerase cofactor that is used as a marker for synthesis phase [140]. Other studies using pancreatic cancer cell lines and tissues from patients with pancreatic cancer that underwent surgery, have shown AT_1 receptor expression using RT-PCR in 6 of 8 malignant neoplasms, 1 of 4 normal pancreatic tissue and 3 of 3 cell lines, and immunohistochemistry revealed that AT_1 receptor was present in 27 of 50 cases of pancreatic cancer. AT_1 receptor blockade decreased cell line proliferation without toxic effects [141].

It is well known that AT_1 and AT_2 receptors are present in normal kidney, however, AT_1 receptor has also been detected with immunohistochemistry at different intensities, in 9 of 10 metastatic renal cancers as well as in mice lung metastases from renal cancer [142]. Besides, in a xenograft model of human renal cell carcinoma, captopril inhibited *in vivo* tumor growth without *in vitro* cell proliferation inhibition [143]. In another study, AT_1 blocker candesartan administration in mice with renal cancer, decreased the incidence of lung metastases, tumoral contents of TGFβ and expression of VEGF, suggesting that AT_1 antagonists could be used as antineoplastic treatment for these tumors [142].

The treatment of soft tissue malignancies is a challenge for the oncologist. Animal models have contributed in our understanding about these neoplasms. For example, treatment with captopril inhibited fibrosarcoma growth in a rat model [134]; in addition, there are reports which indicate that TCV-116 (AT1 receptor antagonist) and lisinopril significantly inhibit sarcome-180 and NFSA fibrosarcoma growth *in vivo*, decreasing also microvessel density and pulmonary metastases number. Both tumors expressed AT_{1a} receptor, and the administration of TCV-116 and lisinopril decreased AT_{1a} receptor mRNA levels [144]. These facts also indicate a potential therapeutic role for AT_1 receptor blockers.

There are some interesting results involving cancer colon. At least three colon cancer cell lines express AT_1 and AT_2 receptors [145]. In AT_2 receptor deficient mice, azoxymethane-induced colon tumor prevalence was decreased to 11% compared to 100% in control animals, besides the size of the tumors was significatively higher along with multiplicity frequency, suggesting that AT_2 receptor could be crucial during tumor genesis and growth, representing a potential mechanism of chemoprevention for human colorectal cancer [146].

In human squamos cell carcinoma AT_1 receptor expression has been detected in 46 of 50 cases, with high immunohistochemistry intensity in 74%, compared with benign keratoacanthoma lesions that shown 23.7% of positive immunohistochemistries, suggesting a pathophysiologic role of AT_1 in skin cancer [147, 148]. Interestingly, treatment with captopril in radiated rats decreased the incidence of fibrosarcoma and squamous cell carcinoma in 80% compared to controls [133]. Recently, some reports indicate that in AT_1 receptor deficient mice implanted with B16 F1 melanoma and QRsP-11 fibrosarcoma cells, capilar density and tumor size significatively decreased and overall survival increased in comparison to control mice. Macrophage infiltration and VEGF expression by these cells was poorer in AT_1 receptor deficient mice than in controls, suggesting that ANG II plays an important poinflammatory role that favors tumor angiogenesis. Additionally, the administration of TCV-116 (AT_1 receptor antagonist), significatively decreased melanoma tumor volume in mice [149].

CONCLUSION

It is well known that angiogenesis plays an indisputable role in the development of solid tumors to provide them with oxygen and essential nutrients, constituting an attractive therapeutic target. To date, there are several potentially useful drugs to inhibit angiogenesis and they could be associated to cytotoxic agents to improve antitumoral response. It is of great relevance that multi-functional hormonal systems such as renin angiotensin aldosterone system could influence in neoplasms development, angiogenesis and invasiveness. The discovery of the influence of hormones on neoplasms, such as estrogens and progesterone, has represented a great advance for prevention and treatment of neoplasms such as breast and endometrium cancer, where the presence of such receptors define which patients may benefit from hormonal treatment. It is clear that treatment with ACE inhibitors and ANG II receptor blockers may decrease the production of growth factors in neoplasms with positive receptors, representing an adjuvant therapy with synergistic effects to chemotherapy. The antineoplastic effect of ANG II inhibition is not clear. Whereas in some tumors it seems to be mediated by AT_1 receptor blockade, it is possible that ANG II inhibition produced AT_1/AT_2 stimulation disequilibrium that favored AT_2 stimulation and would lead cells to apoptosis.

In the following years, we will witness the increase in all the information concerning angiotensin receptors in several tumors, along with clinical trials that may correlate the presence of such receptors with treatment and prognosis of human neoplasms, opening the doors to a new research area in oncology.

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REFERENCES

[1] Matsusaka T, Ichikawa. Biological functions of angiotensin and its receptors. Annual Reviews of Physiology1997; 59:395-412.

[2] Johnston CI. Renin-angiotensin system: a dual tissue and hormonal system for cardiovascular control. J Hypertens Suppl 1992; 10: S13-S26.

[3] Goodfriend TL, Elliot ME, Catt KJ. Angiotensin receptors and their antagonists. Drug Therapy 1996; 334: 1649-54.

[4] Dzau V. A symposium: the relevance of tissue angiotensin-converting-enzyme: manifestations in mechanistic and endopoint data. Am J Cardiol 2001b; 88:1-20.

[5] Gavras I, Gavras H. Angiotensin II possible adverse effects on arteries. Heart, brain, and kidney; experimental clinical, and epidemiological evidence. In: Robertson JIS, Nicholls MG, eds. The rennin-angiotensin system Vol. 1. Biochemistry/physiology. London: Gower Medical Publishing. 1993; 40.1-40.11.

[6] Ferrario C. Importance of the rennin-angiotensin-aldosterone systems (RAS) in the physiology and pathology of hypertension. Drugs 1990; 39: 1-8

[7] Kim S, Zhan Y, Izumi Y, Yasumoto H, Yano M, Iwao H. *In vivo* activation of rat aortic platelet-derived growth factor and epidermal growth factor receptors by angiotensin II and hypertension. Arterioscler Thromb Vasc Biol 2000; 20: 2539-45.

[8] Chua CC, Hamdy RC, Chua BHL. Upregulation of vascular endothelial growth factor by angiotensin II in rat heart ECs. Biochem Biophys Acta 1998; 1401:187-94.

[9] Tamarat R, Silvestre JS, Durie M, Levy BI. Angiotensin II Angiogenic Effect *in Vivo* Involves Vascular Endothelial Growth Factor- and Inflammation- Related Pathways. Lab Invest 2002; 82: 747-56.

[10] Naftilan AJ, Pratt RE, Dzau VJ. Induction of platelet derived growth factor A-chain and c-myc gene expressions by angiotensin II in cultured rat vascular smooth muscle cells. J Clin Invest 1989; 83: 1419-24.

[11] Stachowiak MK, Moffet J, Joy A, Puchacz E, Florkiewicz R, Stachowiak EK. Regulation of bFGF gene expression and subcellular distribution of bFGF protein in adrenal medullary cells. J Cell Biol 1994; 127: 203-23.

[12] Peng H, Moffett J, Myers J, Fang, Stachowiak EK, Maher P, et al. Novel Nuclear signaling pathway mediates activation of fibroblast growth factor-2 gene by type 1 and type 2 angiotensin II **r**eceptors. Mol Bio Cell 2001; 12: 449-62.

[13] Scheidegger KJ, Du J, Delafontaine P. Distinct and common pathways in the regulation of insulin-like growth factor-1 receptor gene expression by angiotensin II and basic fibroblast growth factor. J Biol Chem. 1999; 274: 3522-30.

[14] Fujiyama S, Matsubara H, Nozawa Y, Maruyama K, Mori Y, Tsutsumi Y, et al. Angiotensin AT1 and AT2 receptors differentialy regulate angiopoietin-2 and Vascular endothelial Growth factor Expression and angiogenesis by modulating Heparin Binding-epidermal Growth factor (EGF) Mediated EDF receptor transactivation. Circ Res 2001; 88: 22-9.

[15] Touyz RM, Berry C. Recent advances in angiotensin II signaling. Braz J Med Biol Res 2002; 35: 1001-15

[16] Hsueh WA, Do YS, Anderson PW, Law RE. Angiotensin II in cell growth and matrix production. Advances in Experimental Medicine and Biology. 1995; 377: 217-23.

[17] Stoll M, Steckelings UM, Paul M, Bottari SP, Metzger R, Unger T. The angiotensin AT2-receptor mediates inhibition of cell proliferation in corona endothelial cells. J Clin Invest 1995; 95: 651-7.

[18] Bergsma DJ, Ellis C, Kumar C, Nuthulaganti P, Kersten H, Eishourbagy N, et al. Cloning and characterization of a human angiotensin II type 1 receptor. Biochem Bioph Res Co 1992; 3: 989-95.

[19] Stroth U, Unger T. The renin-angiotensin system and its receptors.J Cardiovasc Pharmacol. 1999; 33 Suppl 1:21-8; discussion 41-3. Review

[20] Tamargo J, Caballero R, Moreno I, Cogolludo A. Los anatagonistas de los receptores de la angiotensina II han cumplido las expectativas. Monocardio 2002; 4: 124-38.

[21] De Gasparo M, Husain A. Alexander W ycols. Proposed update of angiotensin receptor nomenclature. Hypertension. 1995; 25: 924-7.

[22] Timmermans PBMWM, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ, et al. Angiotensin II receptors and angiotensin II receptor antagonist. Pharmacol Rev 1993; 45: 206-51.

[23] Sayeski PP, Ali MS, Sameniuk DJ, Doan TN, Bernstein KE. Angiotensin II signal transduction pathways. Regul Peptides 1998; 78: 19-29.

[24] Griffin SA, Brown WC, McPherson et al. An giotecnsin II causes vascular hypertrophy in part by a non-pressor mechanism. Hipertension 1991; 17: 626-35.

[25] Laragh JH, Angers M, Kelly WG. Lieberman S. Hypotensive agents and pressor substances: the effect of epinephrine, norepinephrine, angiotensin II, and others on the secretory rate of aldosterone in man. JAMA 1960; 174:234-40.

[26] Van Bilsen M. Signal transduction revisited: recent developments in angiotensin II signaling in the cardiovascular system. Cardiovasc Res 1997; 36: 310-22.

[27] Moravec CS, Schluchter MD, Paranandi L, et al. Inotropic effects of angiotensin II on human cardiac muscle *in vitro.* Circulation 1990; 82: 1973-84.

[28] Sudoshima J, Izumo S. Molecular Characterization of angiotensin II induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts: critical role of the AT1 receptor subtype. Circ Res 1993; 73: 413-23.

[29] Cook JL, Zhang Z, Re RN. *In vitro* evidence for intracellular site of angiotensin action. Cir Res 2001; 89: 1138-46.

[30] Horiuchi M, Akishiita M, Dzau VJ. Recent progress in angiotensin II type 2 receotor research in the cardiovascular system. Hypertension 1999; 33: 613-21.

[31] Silvestre JS, Levy BI. Angiogenesis therapy in ischemic disease. Arch Mal Coeur Vaiss 2002; 95: 189-96.

[32] Bascands JL, Girolami JP, Troly M, Escargueil-Blanc I, Nazzal D, Salvayre R, et al. Angiotensin II induces Phenotype dependent apoptosis in vascular smooth muscle cells. Hypertension 2001; 38:1294-9.

[33] Mitchell KD, Braam B, Navar LG. Hypertendinogenic mechanisms mediated by renal actions of renina-angiotensin system. Hypertension 1992; 19 Suppl: 118-27.

[34] Schubert B, Fassnacht M, Beuschlein F, Zenkert S, Allolio B, Reincke M. Angiotensin II type 1 receptor and ACTH receptor expression in human adrenocortical neoplasms. Clin Endocrinol 2001; 54: 627-32.

[35] Tsuzuki S, Ichiki T, Nakakubo H, Kitami Y, Guo D-F, Shirai H, et al. Molecular dloning and expression of the gene encoding human angiotensin II type 2 receptor. Biochem Bioph Res Co 1994; 3: 1449-54.

[36] Mukoyama M, Nakajima M, Horiuchi M, Sasamura H, Pratt RE, Dzau VJ. Expression cloning of type 2 angiotensin II receptor reveals a unique class of seven-transmembrane receptors. J Biol Chem 1993; 268: 24539-42.

[37] Nahmias C, Strosberg D. The angiotensin AT receptor: searching for signal-transduction pathways and physiological function. Trehds in Pharmacological Sciences. 1995; 16: 223-5.

[38] Wolf G, Harendza S, Schroeder R, Wenzel U, Zahner G, Butzmann U, et al. Angiotensin II´s Antiproliferative Effects Mediated Through AT2 Receptors Depend On Down Regulation of SM-20. Lab Invest 2002; 82: 1305-17.

[39] Horiuchi M, Hayashida W, Kambe T, Yamada T, Dzau VJ. Angiotensin type 2 receptor dephosphorylates Bcl-2 by activating mitogen-activated proein kinase phosphatase 1 and induces apoptosis. J Biol Chem 1997; 272: 19022-26.

[40] Berk B, Corson M. Angiotensin II signal transduction in vascular smooth muscle. Role of tyrosine kinases. Circ Res 1997; 80: 607-16.

[41] Touyz Rm, Schiffrin EL. Role of calcium influx and intracellular calcium stores in angiotensin II mediated calcium hyperresponsiveness in smooth muscle from spontaneously hypertensive rats. J Hypertension 1997; 15: 1431-9.

[42] Stroth U, Blume A, Mielke K, Unger T. Angiotensin AT2 receptor stimulates ERK1 and ERK2 in quiescent but inhibits ERK in NGF-stimulated PC12W cells. Mol Brain Res 2000; 78: 175-80.

[43] Folkman J. Clinical applications of research on angiogenesis. NEJM 1995; 333:1757-63.

[44] D´Amaro RJ, Loughnan MS, Flynn E, Folkman J. Thalidomide is an inhibitor of angiogenesis. Proc Natl Acad Sci USA. 1994; 91: 4082-5.

[45] Arrieta O, Guevara P, Reyes S, Ortiz A, Rembao D, Sotelo J. Protamine Inhibits Angiogénesis and Growth of C6 Rat Glioma; a Synergistic Effect when Combined with Carmustine. Eur J Cancer 1998; 34: 2101-6.

[46] Kisker O, Becker CM, Prox D, Fannon M, D´Amato R, Flynn E, et al. Continuous administration of endostatin by intraperitoneally implanted osmotic pump improves the efficacy and potency of therapy in a mouse xenograft tumor model.Cancer Res 2001; 61: 7669-74.

[47] Arrieta O, Guevara P, Tamariz J, Rembao D, Rivera E, Sotelo J. Antiproliferative effect of thalidomide alone and combined with carmustine against C6 rat glioma. Int J Exp Pathol 2002; 83: 99-104.

[48] Folkman J. Role of angiogenesis in tumor growth and metastasis. Semin Oncol 2002; 29:15-8.

[49] Fernandez LA, Caride VJ, Twickler J, Galardy RE. Renin-angiotensin and development of collateral circulation after renal ischemia. Am J Physiol 1982; 243: 869-75.

[50] Fernandez LA, Twickler J, Mead A. Naovascularization produced by angiotensin II. J Lab Clin Med 1985; 105: 141-5.

[51] Kaliszewski C, Fernadez LA, Wicke JD. Differences in mortality rate between abrupt and progressive carotid ligation in the gerbil: role of endogenous angiotensin II. J Cereb Blood Flow Metab 1998; 8: 149-54.

[52] Fernandez LA, Spencer DD, Kaczmar T Jr. Angiotensin II decrease mortality rate in gerbil with unilateral carotid ligation. Stroke 1986; 17: 82-5

[53] Fernandez LA, Caride VJ, Stromberg C, Naveri L, Wicke JD. Angiotensin AT2 receptor stimulation increases survival in gerbils with abrupt unilateral carotid ligation. J Cardiovasc Pharmacol 1994; 24: 937-40.

[54] Le Noble FA, Schreurs NH, Van Straaten HW, Slaaf DW, Struyker-Boudier HAJ. Angiotensin II stimulates angiogenesis in the chorioallantoic membrane of the chick embryo. Eur J Pharmacol 1991; 195: 305-6.

[55] Le Noble FA, Schreurs NH, van Straaten HW, Slaaf DW, Smits JF, Rogg H, et al. Evidence for a novel angiotensin II receptor involved in angiogenesis in chick embryo choriallantoic membrane. Am J Physiol 1993; 264: R460-5.

[56] Andrade Sp, Cardoso CC, Machado RD, Beraldo WT. Angiotensin II induced angiogenesis in sponge implants in mice. Int J Microcirc Clin Exp 1996; 16: 302-7.

[57] Munzenmaier DH, Greene AS. Apposing Actions of angiotensin II on Microvascular Growth and Arterial Blood Pressure. Hypertension 1996; 27: 760-5.

[58] Hu DE, Hiley CR, Fan TP. Comparative studies of the angiogenic activity of vasoactive intestinal peptide, endothelins-1 and -3 and angiotensin II in a rat sponge model. Br J Pharmacol 1996; 117: 545-51.

[59] Walsh DA, Hu DE, Wharton J, Catravas JD, Blake DR, Ping DT. Sequential development of angiotensin receptors and angiotensin I converting enzyme during angiogenesis in the rat subcutaneous sponge granuloma. Brit J Pharmacol 1997; 120: 1302-11.

[60] Emmanueli C, Bonaria SM, Stacca T, Pinna A, Gaspa L, Meddeddu P. Angiotensin AT1 receptor signaling modulates reparative angiogenesis induced by limb ischaemia. Brit J Pharmacol 2002; 135: 87-92.

[61] Sasaki K, Murohara T, Ikeda H, Sugaya T, Shimada T, Shintani S, et al. Evidence for the importance of angiotensin II type 1 receptor in ischemia- induced angiogenesis. J Clin Invest 2002; 109: 603-11.

[62] Amaral SL, Linderman JR, Morse MM, Greene AS. Angiogenesis induced by electrical stimulation is mediated by angiotensin II and VEGF. Microcirculation 2001; 8:57-67.

[63] Silvestre JS, Tamarat R, Senbonmatsu T, Icchiki T, Ebrahimian T, Iglarz M, et al. Antiangiogenic effect of angiotensin II type 2 receptor in ischemia induced angiogenesis in mice hindlimb. Circ Res 2002; 90: 1072-9.

[64] Machado RD, Santos RA, Andrade SP. Opposing actions of angiotensins on angiogenesis. Life Sci 2000; 66: 67-76.

[65] Maxfield EK, Cameron NE, Cotter MA, Dines KC. Angiotensin II receptor blockade improves nerve function, modulates nerve blood flow ans stimulates endoneural angiogenesis in streptozocin-diabetic rats. Diabetologia. 1993; 36: 1230-7.

[66] Fabre JE, Rivard A, Magner M, Silver M, Isner JM. Tissue Inhibition of Angiotensin-Converting Enzyme Activity stiumulates Angiogenesis *in vivo*. Circulation 1999; 99: 3043-9.

[67] Thomas Kenneth A. Vascular Endothelial Growth Factor, a Potent and Selective Angiogenic Agent. J Biol Chem 1996; 271: 603-6.

[68] Otani A, Takagi H, Suzuma K, Honda Y. Angiotensin II potentiates vascular endotelial growth factor-induced angiogenesis activity in retinal microcapillary endotelial cells. Circ Res 1998; 82: 619-28.b

[69] Young Koh G, Kim I, Jin KH, Yun MJ, Chan LJ. Biomedical significance of endothelial cell specific growth factor, angiopoietin. Exp Mol Med 2002; 34:1-11.

[70] Atsushi O, Hitoshi T, Hideyasu O, Shinji K, Yoshihito H. Angiotensin II Induces Expression of the Tie2 Receptor Ligand, Angiopoietin 2, in Bovine Retinal Endotelial Cells. Diabetes 2001; 50: 867-75.

[71] Samani AA, Brodt P. The receptor for the type I insulin-like growth factor and its ligands regulate multiple cellular functions that impact on metastasis. Surg Oncol Clin N Am 2001; 10: 289-312,

[72] Delafontaine P, Lou H. Angiotensin II regulates Insulin-like Growth Factor I Gene Expression in Vascular Smooth Muscle Cells. J Biol Chem 1993; 268: 16866-70.

[73] Ververis JJ, Ku L, Delafontaine P. Regulation of insulin-like growth factor I receptors on vascular smooth muscle cells by growth factors and phorbol esters. Circ Res 1993; 72: 1285-92.

[74] Brink M, Chrast J, Russ PS, Mitch WE, Delafontaine P. Angiotensin II stimulates gene expression of cardiac Insulin-like growth factor I and its receptor through effects on blood pressure and food intake. Hypertension 1999; 34: 1053-9.

[75] Haddad GE, Blackwell K, Bikhazi A. Regulation of insulin like growth factor-1 by the rennin-angiotensin system durir regression of cardiac eccentric hypertrophy through angiotensin-converting enzyme inhibitor and AT1 antagonist. Can J Physiol Pharmacol 2003; 81: 142-9.

[76] Van Eickels M, Vetter H, Grohé C. Angiotensin converting enzyme (ACE) inhibition attenuates insulin like factor I (IGF-I) induced cardiac fibroblast proliferation. Brit J Pharmacol. 2000; 131: 1592-6.

[77] Du J, Peng T, Scheidegger KJ, Delafontaine P. Angiotensin II activation of insulin-like growth factor 1 receptor transcription is mediated by tyrosine kinase-dependent redox-sensitive mechanism. Arterioscler Thromb Vasc Biol 1999; 19: 2119-26.

[78] Muller C, Reddert A, Wassman S, Strehlow K, Bohm M, Nickenig G. Insulin like growth factor induces up-regulation of AT(1) receptor gene expression in vascular smooth muscle cells. J Renin Angiotensin Aldosterone Syst. 2000; 1: 273-7.

[79] Yu J, Ustach C, Kim HR. Platelet-derived growth factor signaling and human cancer. Biochem Mol Biol 2003; 36: 49-59.

[80] Cook JL, Giardina JF, Zhang Z, Re RN. Intracellular angiotensin II increases the long isoform of PDGF mRNA in rat hepatoma cells. J Mol Cell Cardiol 2002; 34: 1525-37.

[81] Day FL, Rafty LA, Chesterman CN, Khachigian LM. Angiotensin II (ATII) inducible Platelet-derived growth factor A-chain gene expression is p42/44 extracellular signal-regulated kinase $^{1}/_{2}$ and Egr-1 dependent and mediated *via* the ATII type 1 but not type 2 receptor. J Biol Chem 1999; 274: 23726-33.

[82] Khachigian LM, Takuwa Y, Collins T. Mechanisms of angiotensin II -induced platelet-derived growth factor gene expression. Mol Cell Biochem 2000; 212: 183-6.

[83] Deguchi J, Makuuchi M, Nakaoka T, Collins T, Takuwa Y. Angiotensin II stimulates platelet-derived growth factor-B chain expression in newborn rat vascular smooth muscle cells and neointimal cells through Ras, extracellular signal-regulated protein kinase, and c-Jun N-terminal protein kinase mechanisms. Circ Res 1999; 85: 565-74.

[84] Wong J, Rauhoft C, Dilley RJ, Agrotis A, Jennings GL, Bobik, A. Angiotensin-converting enzyme inhibition abolishes medial smooth muscle PDGF-AB biosynthesis and attenuates cell proliferation in injured carotid arteries: relationships to neointima formation. Circulation 1997; 96:1631-40.

[85] Linseman DA, Benjamin CW, Jones DA. Convergence of angiotensin II and platelet-derived growth factor receptor signaling cascades in vascular smooth muscle cells. 1995; 270: 12563-8.

[86] Heeneman S, Haendeler J, Saito Y, Ishida M, Berk BC. Angiotensin II induces transactivation of two different populations of the platelet derived growth factor-β receptor. J Biol Chem 2000; 275: 15926-32

[87] Huang X, Lee C. From TGF-beta to cancer therapy. Curr Drug Targets. 2003; 4:243-50.

[88] Ohta K, Kim S, Hamaguchi A, Yukimara T, Miura K, Takaori K, et al. Role of angiotensin II in extracelluar matrix and transforming growth factor-beta 1 expression in hypertensive rats. Eur J Pharmacol 1994; 269: 115-9.

[89] Kim JA, Berliner JA, Nadler JL. Angiotensin II Increases Monocyte Binding to Endothelial Cells. Biochem Bioph Res Co 1996; 226: 862-8.

[90] Gibbons GH, Pratt RE, Dzau VJ. Vascular smooth muscle cell hypertrophy vs. hyperplasia. Autocrine transforming growth factor-

beta 1 expression determines growth response to angiotensin II. J Clin Invest 1992; 90: 456-61.
[91] Hamaguchi A, Kim S, Izumi Y, Zhan Y, Yamanaka S, Iwao H. Contribution of extracellular signal-regulated kinase to angiotensin II-induced transforming growth factor-b1 expression in vascular smooth muscle cells. Hypertension 1999; 34: 126-31.
[92] Kagami S, Border WA, Miller DE, Noble NA. Angiotensin II stimulates extracellular matrix protein synthesis through induction of transforming growth factor-beta expression in rat glomerular mesangial cells. J Clin Invest 1994; 93: 2431-7.
[93] Weigert C, Brodbeck K, Klopfer K, Häring U, Schleicher ED. Angiotensin II induces human TGF-B1 promoter activation: similarity to hyperglycaemia. Diabetologia 2002; 45: 890-8.
[94] Su JZ, Fukuda N, Jin XQ, Lai YM, Suzuki R, Tahira Y, et al. Effect of AT2 receptor on expression of AT1 and TGF-beta receptors in VSMCs from SHR. Hypertension 2002; 40: 853-8.
[95] Botta M, Manetti F, Corelli F. Fibroblast growth factors and their inhibitors. Curr Pharm Des 2000; 6: 1897-924.
[96] Liakos P, Bourmeyster N, Defaye G, Chambaz EM, Bottari SP. Angiotensin II AT1 and AT2 receptors both inhibit bFGF induced proliferation of bovine adrenocortical cells. Am J Physiol 1997; 273(Cell Physiol. 42):C1324-34.
[97] Nickenig G, Murphy TJ. Down-regulation by growth factors of vascular smooth muscle angiotensin receptor gene expression. Mol Pharmacol 1994; 46: 653-9.
[98] Boros P, Miller CM. Hepatocyte growth factor: a multifunctional cytokine. Lancet 1995; 345: 293-5.
[99] Arrieta O, Garcia E, Guevara P, Garcia NR, Ondarza R, Rembao D, et al. Hepatocyte Growth Factor is associated with poor prognosis of malignant gliomas and is a predictor for recurrence of meningioma. Cancer 2002; 94: 3210-8.
[100] Nakano N, Morishita R, Moriguchi A, Nakamura Y, Hayashi S, Aoki M, et al. Negative regulation of local hepatocyte growth factor expression by angiotensin II and transforming growth factor-β in Blood Vessels. Hypertension 1998; 32: 444-51.
[101] Nakano N, Atsushi M, Ryuichi M, Iwao K, Naruya T, Kunio M, et al. Role of angiotensin II in the Regulation of a Novel Vascular Modulator, Hepatocyte Growth Factor (HGF), in Experimental Hypertensive Rats. Hypertension 1997; 30: 1448-54.
[102] Araya J, Tsuruma T, Hirata K, Yagihashi A, Meguro M, Kawakami M, et al. The regulation of HGF and TGF-β by angiotensin II a Type 1 Receptor Antagonist in Hepatic Ischemia- Reperfusion Injury. Transplant P 2003; 35: 107-10.
[103] Rouet-Benzineb P, Gontero M, Dreyfus P, Lafuma C. Angiotensin II Induces Nuclear Factor-κB Activation in Cultured Neonatal Rat Cardiomyocytes Through Protein Kinase C Signaling Pathway. J Mol Cell Cardiol 2000; 32: 1767-78.
[104] Brasier AR, Recinos A, Eledrisi MS. Vascular inflammation and the rennin-angiotensin system. Arterioscler Thromb Vasc Biol 2002; 22: 1257-66.
[105] Kranzhofer R, Schmidt J, Pfeiffer CAH, Hagl S, Libby P, Kubler W. Angiotensin induces inflammatory activation of human vascular smooth muscle cells. Aterioscler Thromb Vasc Biol 1999; 19: 1623-9.
[106] Arras M, Ito WD, Scholz D, Winkler B, Schaper J, Schaper W. Monocyte activation in angiogenesis and collateral growth in the rabbit hindlimb. J Clin Invest 1997; 101: 40-50.
[107] Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M, DuBois RN. Cyclooxygenase regulates angiogenesis induced by colon cancer cells.
Cell. 1998 May 29;93(5):705-16
[108] Stetler-Stevenson WG. Matrix metalloproteinases in angiogenesis: A moving target for therapeutic intervention. J Clin Invest 1999; 103: 1237-41.
[109] Coker ML, Jolly JR, Joffs C, Etoh T, Holder JR, Bond BR, et al. Matrix metalloprotease expression and activity in isolated myocytes after neurohormonal stimulation. Am J Physiol Heart Circ Physiol 2001; 281:H543-51.
[110] Murohara T, Asahara T, Silver M, Bauters C, Masuda H, Kalka C, et al. Nitric Oxide Synthase Modulates Angiogenesis in response to tissue ischemia. J Clin Invest 1998; 101: 2567-78.
[111] Folkman J, Watson K, Ingber D, Hanahan D. Induction of angopgenesis during the transition from hyperplasia to neoplasia. Nature 1989; 339: 58-61.
[112] Pahor Marco, Jack M. Guralnik, Marcel E. Salive, Maria-Chiara Corti, Pierugo Carbonin and Richard J. Havlik Do Calcium Channel Blockers Increase the Risk of Cancer? Am J Hypertens 1996; 9: 695-9.
[113] Jick H, Jick S, Derby EL, Vasilakis C, Wald MM, Meier RC. Calcium-channel blockers and risk of cancer. Lancet 1997; 349: 525-8
[114] Lever AF, Hole DJ, Gillis CR, McCallum IR, Mclnnes GT, Mackinnon PL, et al. Do inhibitors of angiotensin I converting enzyme protect against risk of cancer?. Lancet 1998; 352: 179-84
[115] Meier CR, Derby LE, Jick SS, Jick H. Angiotensin converting enzyme inhibitors, calcium chanel blockers and breast cancer. Arch Intern Med 2000; 160: 349-53
[116] Friis S, Sorensen HT, Mellemkjaer L, McLaughlin JK, Nielsen GL, Blot WJ, et al. Angiotensin- converting enzyme inhibitors and the risk of cancer: a population-based cohort study in Denmark. Cancer 2001; 92: 2462-70.
[117] Grossman E, Messerli FH, Goldbourt U. Carcinogenicity of antihypertensive therapy. Current Hypertension Reports 2002: 195-201.
[118] Veronesi U, Maisonneuve P, Rotmensz N, Costa A, Sacchini V, Travaglini R, et al. Early Breast Cancer Trialist´s Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomised trials. Lancet 1998; 351: 1451-7.
[119] Guerra FK, Ciiuffo GM, Elizalde PV, Charreau EH, Saavedra JM. Enhanced expression of angiotensin II receptor subtypes and angiotensin converting enzyme in medroxy progesterone-induced mouse mammary adenocarcinomas. Biochem Biophys Res Commun 1993; 193: 93-9.
[120] Tahmasebi M, Puddefoot JR, Inwang E.R, Goode AW, Carpenter R, Vinson GP. Transcription of the Prorenin Gene in Normal and Diseased Breast. Eur J Cancer 1998; 34: 1777-82.
[121] Inwang ER, Puddefoot JR, Brown CL, Goode AW, Marsigliante S, Ho MM, et al. Angiotensin II type 1 receptor expression in human breast tissues. Brit J Cancer 1997; 75: 1279-83.
[122] De Paepe B, Verstraeten VL, De Potter CR, Vakaet LA, Bullock GR. Growth stimulatory angiotensin II type 1 receptor is upregulated in breast hyperplasia and in situ carcinoma but not in invasive carcinoma. Histochem cell Biol 2001; 116: 247-54.
[123] De Paepe B, Verstraeten VM, De Potter CR, Bullock GR. Increased angiotensin II type 2 receptor density in hyperplasia, DCIS and invasive carcinoma of the breast is paralleled wirh increased iNOS expression. Histochem Cell Biol 2002; 117: 13-19.
[124] Berry MG, Goode AW, Puddefoot GP, Vinson and Carpenter R. Integrin β1 upregulation in MCF-7 breast cancer cells by angiotensin II. Eur J Surg Oncol 2000; 26: 25-9.
[125] Muscella A, Greco S, Elia MG, Storelli C, Marsigliante S. Angiotensin II stimulation of Na+/K+ATPase activity and cell growth by calcium-independent pathway in MCF-7 breast cancer cells. J Endocrinol 2002; 173: 315-23.
[126] Greco S, Elia MG, Muscella C, Sterolli C, Marsigliante S. AT1 angiotensin II receptor mediates intracellular calcium mobilization in normal and cancerous breast cells in primary culture. Cell calcium 2002; 32:1-10.
[127] Greco S, Muscella A, Elia MG, Salvatore P, Storelli C, Mazzotta A, et al. Angiotensin II activates extracellular signal regulated kinases *via* protein kinase C and epidermal growth factor receptor in breast cancer cells. J Cell Physiol 2003; 196: 370-7.
[128] Small Jr W, Molteni A, Kim YT, Taylor JM, Chen Z, Ward WF. Captopril modulates hormone receptor concentration and inhibits proliferation of human mammary ductal carcinoma cells in culture. Breast Cancer Res Tr 1997; 44: 217-24.
[129] Ganong WF: The brain rennin-angiotensin system. Ann Rev Physiol 1984; 46: 17-31.
[130] Ariza A, Fernandez LA, Inafami T, Kim JH, Manuelidis EE. Renin in glioblastoma multiforme and its role in neovascularization. Am J Clin Pathol 1988; 90: 437-41.
[131] Fogarty DJ, Sánchez GV, Matute C. Multiple Angiotensin Receptor subtypes in Normal and Tumor Astrocytes *In Vitro.* GLIA 2002; 39: 304-13.
[132] Rivera E, Arrieta O, Guevara P, Duarte-Rojo A, Sotelo J. AT1 receptor is present in glioma cells; its blockage reduces the growth of rat glioma. Brit J Cancer 2001; 85: 1396-9.
[133] Ward WF, Molteni A, Ts´ao C, Hinz JM. The effect of captopril on benign and malignant reactions in irradiet rat skin Br J Radiol 1990; 63: 349-54.

[134] Volpert OV, Ward WF, lingen MW, Chesler L, Solt DB, Johnson MD, et al. Captopril inhibits angiogenesis and Slows the Growth of Experimental Tumors in Rats. J. Clin. Invest 1996; 98: 671-9.

[135] Yoshiji H, Yoshiji J, Ikenavaba Y, Noguchi R, Tsujinoue H, Nakatani T, et al. Inhibition of renin-angiotensin system attenuates liver enzyme altered preneoplastic lesions and fibrosis development in rats. J Hepatol 2002; 37: 22-30.

[136] Yoshiji H, Kuriyama S, Kawata M, Yoshii J, Ikenaka Y, Noguchi R, et al. The Angiotensin- I- converting Enzyme Inhibitor Perindopril Supresses Tumor Growth and Angiogenesis: Possible role of the Vascular Endothelial Growth Factor. Clin Cancer Res 2001; 7: 1073-8.

[137] Yoshiji H, Yoshii J, Ikenaba Y, Noguchi R, Yanase K, Tsujinoue H, et al. Suppression of the renin-angiotensin sistems attenuates vascular endothelial growth factor- mediated tumor development and angiogenesis in murine hepatocellular carcinoma cells. Int J Oncol 2002; 20: 1227-31.

[138] Yoshiji H, Kuriyama S, Fukui H. Perindopril: possible use in cancer therapy. Anti-cancer drugs 2002; 13: 221-8.

[139] Ohta T, Amaya K, Yi S, Kitagawa H, Kayahara M, Ninomiya I, et al. Angiotensin converting enzyme-independent, local angiotensin II -generation in human pancreatic ductal cancer tissues. Int J Oncol 2003; 23: 593-8.

[140] Reddy MK, Baskaran K, Molteni A: Inhibitors of angiotensin-converting enzyme modulate mitosis and gene expression in pancreatic cancer cells. Proc Soc Exp Biol Med 1995; 210: 221-6.

[141] Fujimoto Y, Sasaki T, Tsuchida A, Chayama K. Angiotensin II type 1 receptor expresion in human pancreatic cancer and growth inhibition by angiotensin II type 1 receptor antagonist. FEBS. Letters 2001; 495: 197-200.

[142] Miyajima A, Kosaka T, Asano T, Seta K, Kawai T, Hayakawa M. Angiotensin II type I antagonist prevents pulmonary metastasis of murine renal cancer by inhibiting tumor angiogenesis. Cancer Res 2002; 62: 4176-9.

[143] Hii SI, Nicol DL, Gotley DC, Thompson LC, Green MK, Jonsson JR. Captopril inhibits tumour growth in a xenograft model of human renal cell carcinoma. Br J Cancer 1998; 77: 880-3.

[144] Fujita M, Hayashi I, Yamashina S, Itoman M, Majima M. Blockade of angiotensin AT1 a receptor signaling reduces tumor growth, angiogenesis, and metastasis. Biochem Biophys Res Commun 2002; 294: 441-7.

[145] Kucerova D, Zelezma B, Sloncova E, Sovova VV. Angiotensin II receptors on colorectal carcinoma cells. Int J mol Med 1998; 2: 593-5.

[146] Takagi T, Nakano Y, Takekoshi S, Inagami T, Tamura M. Hemizygous mice for the angiotensin II type 2 receptor gene have attenuated susceptibility to azoxymethane-induced colon tumorigenesis. Carcinogenesis 2002; 2: 1235-41.

[147] Takeda H, Kondo S. Differences between Squamous Cell Carcinoma and Keratoacanthoma in Angiotensin Type 1 Receptor Expression. Am J Pathol 2001; 158: 1633-7.

[148] Takeda H, Katagata Y, Kondo S. Inmunohistochemical study of angiotensin receptors in human anagen hair follicles and basal cell carcinoma. Br J Dermatol 2002; 147: 276-80.

[149] Egami K, Morohara T, Shimada T, Sasaki K, Shintani S, Sugaya T, et al. Role of host angiotensin II type 1 receptor in tumor angiogenesis and growth. J Clin Invest 2003; 112: 67-75.