

The Importance of the RAS Interacting with the HGF/c-Met Receptor System in Hypertensive Type 2 Diabetes Mellitus

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

ABSTRACT

The classic renin-angiotensin system (RAS) is described as a circulating hormone system with primary roles in the regulation of blood pressure, body water balance and thirst, and control over vasopressin and aldosterone release. Recently local tissue RASs have been identified with regulatory physiological functions and also with pathophysiological processes including fibrosis, inflammation, and dysfunctional cell proliferation. There is a strong correlation between organs vulnerable to diabetic-induced hyperglycemic injury (eg. kidney and retina) and the over activation of local RASs. Increased angiotensin II concentrations in these tissues promotes hypertension and end-organ damage in at least two ways: 1) By activating AT₁ receptor proteins thus inducing changes in local blood flow and tissue hydration; and 2) Exacerbating hyperglycemic-induced oxidative stress, elevated polyol and hexosamine pathway variability, and facilitating glycation end-products. Thus, inhibition of the RAS has become an important treatment approach to control diabetic related hypertension, nephropathy, and to a lesser extent retinopathy. The present review emphasizes the recently established importance of the hepatocyte growth factor (HGF)/c-Met receptor system interacting with the RAS in Type 2 diabetes, and their likely contribution to end-organ damage. A hypothesis is offered concerning how the pancreatic RAS may affect dimerization of HGF, and in turn activation of the c-Met receptor to promote β cell proliferation and insulin synthesis. We conclude with details concerning the development of an AngIV-based small molecule HGF mimetic designed to act as an insulinotropic factor.

Keywords: : Hypertension; Type 2 diabetes; Renin-angiotensin system; Angiotensin II; Angiotensin IV; Hepatocyte growth factor; AT₁ receptor subtype; AT₄ receptor subtype; c-Met receptor.

26 1. INTRODUCTION

27

28 1.1 Background Information

29 The worldwide incidence of diabetes mellitus presently numbers 250 to 350 million adults,
30 and is predicted to reach 380-430 million by 2025 if new treatment strategies are not
31 introduced [1,2]. Diabetes is accompanied by a significant increase in the risk of
32 hypertension, heart attack, stroke, atherosclerosis, renal failure, and end-organ damage [3-
33 5]. Thus, the control of blood pressure is an especially important goal in preventing
34 cardiovascular and renal dysfunctions in Type 2 diabetic patients with hypertension.
35 Although discussion continues over the optimal target, several reports recommend
36 maintaining blood pressure at, or below, 130/80 mm Hg (reviewed in [6,7]). In the overall
37 population the risk of cardiovascular events doubles with each 20 mm Hg elevation in
38 systolic blood pressure above 115 mm Hg, and each 10 mm Hg elevation above 75 mm Hg
39 diastolic pressure [8]. Diabetic patients with hypertension suffer twice the risk of
40 cardiovascular dysfunction as nondiabetic individuals with hypertension, accompanied by an
41 increased likelihood of diabetes specific complications such as retinopathy [9-11] and
42 nephropathy [12]. Patients with Type 2 diabetes and hypertension have a sevenfold
43 increased risk of progression to end-stage renal disease as compared with normotensive
44 Type 2 diabetic patients [13]. The association of Type 2 diabetes with hypertension,
45 cardiovascular disease, retinopathy, and nephropathy portends over activation of the renin-
46 angiotensin system (RAS) as an important predisposing factor [14-16]. The relationship
47 between diabetes and the RAS is further supported in that increased local tissue angiotensin
48 II (AngII) levels can result in insulin resistance by impacting insulin-stimulated elevations in
49 insulin receptor substrate1-associated P13K activity [17], thus promoting nephropathy [18].

50

51 1.2 The Renin-Angiotensin System

52

53 The classic RAS is known for its roles in the regulation of blood pressure, body water
54 balance and thirst, and influences on vasopressin and aldosterone release [19-26]. This
55 system has been implicated in diabetes [14,21, 27-29], particularly as evidenced by the
56 ameliorating effects of angiotensin converting enzyme (ACE) inhibitors and angiotensin
57 receptor blockers (ARBs, designed to block the angiotensin AT₁ receptor subtype) in treating
58 Type 2 diabetes (see below). This review focuses on the role of the RAS in the development
59 and sustaining of Type 2 diabetes. We discuss the recently discovered relationship among
60 the renin-angiotensin system (RAS), the hepatocyte growth factor (HGF)/c-Met receptor
61 system, and diabetes. Next, we discuss the negative impact of elevated prorenin levels on
62 the pancreatic RAS and how this may promote end-organ damage. A working hypothesis is
63 presented concerning how HGF and the angiotensin IV (AngIV) peptide is facilitated during
64 treatment with an ACE inhibitor or ARB, thus activating the c-Met receptor and promoting β
65 cell proliferation while attenuating cellular insulin resistance. We conclude with a
66 description of the development of a small molecule HGF mimetic that may act as an
67 insulinotropic factor.

68

69 2. Formation of Angiotensin Ligands

70 Components of the RAS have been localized in the brain and a number of peripheral tissues
71 including heart, kidney, skeletal muscle, adipose tissue and pancreas [25, 26, 30]. All
72 angiotensin ligands are derived from the precursor protein angiotensinogen which is
73 synthesized and secreted from the liver, as well as adipose tissue in obese individuals [31].
74 Angiotensin ligands are formed via several enzymatic conversion pathways (Fig. 1 [32-34]).
75 Briefly, the decapeptide angiotensin I (AngI) is derived by renin (EC 3.4.23.15) acting upon
76 the amino terminal of angiotensinogen [35]. AngI serves as a substrate for ACE (EC

3.4.15.1) to form the octapeptide AngII [32, 36]. This conversion can also be accomplished by the chymotrypsin-like serine protease, chymase [37]. AngII is converted to the heptapeptide angiotensin III (AngIII) by aminopeptidase A (APA: EC 3.4.11.7) cleavage of aspartate [38-40]. Aminopeptidase N (APN: EC 3.4.11.2) cleaves arginine at the N-terminal of AngIII to form the hexapeptide angiotensin IV (AngIV) [41]. AngIV can be further converted to Ang(3-7) by carboxypeptidase P (Carb-P) and propyl oligopeptidase (PO) cleavage of phenylalanine. Endopeptidases such as chymotrypsin, along with dipeptidyl carboxypeptidase, reduce AngIV and Ang(3-7) to inactive peptide fragments and amino acid constituents [32,42-46].

AngI is biologically inactive; while AngII and AngIII are full agonists at the AT₁ and AT₂ receptor subtypes (Table 1 [25, 47]). AngIV and Ang(3-7) bind with low affinity at the AT₁ receptor subtype, but with high affinity and specificity at the AT₄ receptor subtype [48-53]. AngII and AngIII mediate pressor and dipsogenic effects via the AT₁ and AT₂ receptor subtypes [19]. AngIV exerts a much reduced pressor response by acting with low affinity as an agonist at the AT₁ receptor subtype [54-58].

Table 1. Binding affinity values (M) for native angiotensins, Candisartan and Telmisartan at three angiotensin receptor subtypes.

Ligands	AT ₁	AT ₂	AT ₄
Angiotensin II	7.92 X 10 ⁻⁹ M	5.22 X 10 ⁻¹⁰ M	1.00 X 10 ⁻⁶ M
Angiotensin III	2.11 X 10 ⁻⁸	6.48 X 10 ⁻¹⁰	1.60 X 10 ⁻⁷
Angiotensin IV	1.00 X 10 ⁻⁵	4.86 X 10 ⁻⁸	1.29 X 10 ⁻⁹
Candisartan	1.56 X 10 ⁻⁹	1.00 X 10 ⁻⁵	NA
Telmisartan	10.90 X 10 ⁻⁹	10.00 X 10 ⁻⁶	NA

The ligand binding affinities for the AT₁ and AT₂ receptor subtypes are from Bosnyak et al. [59] and Kukuta et al., [60]. Binding affinities for the AT₄ receptor subtype are from Harding et al. [50]. NA=not available.

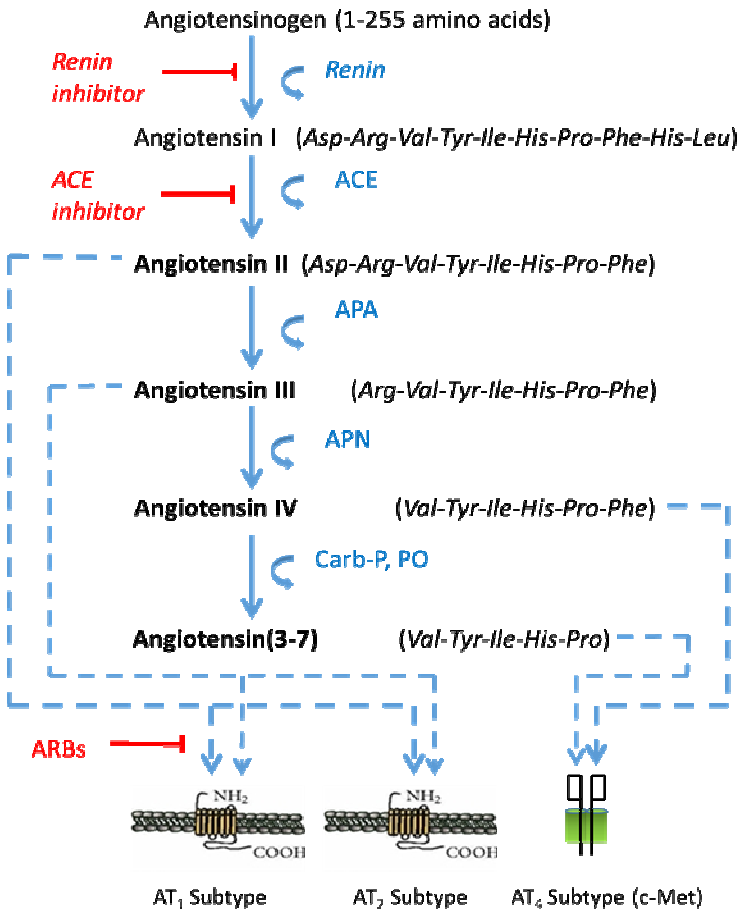


Fig. 1. RAS Synthetic Pathway

The renin-angiotensin pathway, including active ligands (bold), enzymes and receptors, is presented. The location of action for clinically available inhibitors designed to control angiotensin mediated hypertension are indicated in red. Abbreviations: ACE=angiotensin converting enzyme; APA=aminopeptidase A; APN=aminopeptidase N; ARBs=angiotensin receptor blockers. Carb-P=carboxypeptidases, PO=propyl oligopeptidase.

3. Characterization of the AT₁ and AT₂ Receptor Subtypes

The AT₁ receptor subtype is a G-protein coupled receptor with signaling via phospholipase-C and calcium. Thus, the angiotensin ligand binds to the AT₁ receptor and induces a conformational change in the receptor protein that activates G proteins, which in turn, mediates signal transduction. This transduction involves several plasma membrane mechanisms including phospholipase-C, -A₂, and -D-adenylate cyclase, plus L-type and T-type voltage sensitive calcium channels [19, 61, 62]. The AT₁ receptor (now designated AT_{1A}) is also coupled to intracellular signaling cascades that regulate gene transcription and the expression of proteins that mediate cellular proliferation and growth in many target tissues. Subsequently, a second AT₁ subtype was discovered and designated AT_{1B} that was also cloned in the rat [63, 64], mouse [65], and human [66]. This subtype is approximately 92-95% homologous with the amino acid sequence of the AT_{1A} subtype [67, 68]. Of these two isoforms the AT_{1A} subtype appears to be primarily responsible for the classic functions associated with the brain angiotensin system [69, 70].

The AT₂ receptor subtype has been cloned and sequenced using a rat fetus expression library [71, 72], and also evidences a 7-transmembrane domain characteristic of G-protein coupled receptors; however it shows only about 32-34% amino acid sequence identity with - the rat AT₁ receptor. Even though this AT₂ receptor possesses structural features in common with members of the 7-transmembrane family of receptors, it displays few if any functional similarities with this group, although it does appear to be G-protein coupled [19, 71, 72]. While the AT₁ receptor subtype is maximally sensitive to AngII, it is also responsive to AngIII. The AT₂ receptor subtype appears to be maximally sensitive to AngIII, but AngII also serves as a ligand at this receptor subtype. The functions associated with the activation of each of these receptors are presented in Table 2.

Table 2. Ligand activation of the AT₁, AT₂ and AT₄ receptor subtypes

AT ₁ receptor subtype	AT ₂ receptor subtype	AT ₄ receptor subtype
Vasoconstriction	Vasodilation	Dendritic arborization
Aldosterone release	Antifibrotic	Changes in blood flow
Vasopressin release	Antiproliferative	Memory facilitation
Cardiac hypertrophy	Antihypertrophic	Protection against seizures
Fibrosis	Antithrombotic	Facilitates wound healing
Proliferation		
Inflammation		
Platelet aggregation		
Oxidative stress		
Endothelial disruption		

4. Characterization of the AT₄ Receptor Subtype

During our attempt to purify and sequence the AT₁ receptor subtype we noticed that heat-denatured purified receptor from the bovine adrenal gland lost binding to [¹²⁵I]-Sar¹,Ile⁸-AngII, whereas [¹²⁵I]-AngIII binding persisted. It was initially suspected that an angiotensin

receptor specific to AngIII had been isolated. However, with sufficient peptidase inhibitors added to prevent the conversion of AngIII to shorter fragments, this binding activity was also lost. These results were puzzling given that the two known receptor types at that time, AT₁ and AT₂, each accepted AngII and AngIII as ligands, albeit with different affinities. A fragment of Ang III was suspected to be acting at this new site because Sar¹-AngII, Sar¹,Ile⁸-AngII (Sarile), Sar¹,Ala⁸-Ang II (Saralasin), DuP753 (Losartan), PD123177, CGP42112A, AngII(1-7) and AngIII did not act as ligands [50, 52]. In fact [¹²⁵I]-AngIV did bind at this site reversibly, saturably, and with high affinity (K_d = 1 nM) [73, 74]. Thus, we determined the binding profile of this protein to be distinct from the AT₁ and AT₂ receptor subtypes given that [¹²⁵I]-AngIV binding could not be displaced by AT₁ or AT₂ receptor antagonists.

We next set about determining the ligand requirements of this site. Using competitive assays against [¹²⁵I]-AngIV it was soon discovered that the three N-terminal amino acids of AngIV (valine-tyrosine-isoleucine) were necessary for binding [74-76]. Substituting a straight-chain aliphatic moiety with a carbon atom (norleucine) for valine produced the AT₄ receptor agonist, Nle¹-AngIV. This substitution resulted in an analogue with higher receptor affinity than native Ang IV, and greater resistance to enzymatic degradation. Further modification of Nle¹-AngIV by placing a reduced peptide bond (CH₂-NH₂) between norleucine and tyrosine yielded Norleucinal¹-AngIV and resulted in even greater resistance to degradation accompanied by nanomolar affinity at the receptor.

The AT₄ receptor is distributed within a number of brain structures [25] and in several peripheral tissues including heart, kidney, spleen, colon, prostate, bladder, adrenal gland, and pancreas [54, 77].

5. A Role for the RAS in Type 2 Diabetes Mellitus

There is no evidence of increased plasma AngII levels associated with diabetes. In fact, circulating AngII levels have been reported to be suppressed in diabetic patients [78]. However, increased plasma levels of the renin-precursor prorenin have been measured in diabetic patients and are suggested to serve as a predictor of the onset of retinopathy and nephropathy [79, 80]. Since these increases in prorenin do not appear to result in elevated plasma AngII levels, it is proposed that they act at the renin-prorenin receptor to induce tissue injury [81, 82]. Along these lines, the retina and kidney have been reported to have over-active local RASs during episodes of hyperglycemia [83, 84]. Elevated prorenin levels have been measured in the vitreous of the eye in diabetic patients with proliferative retinopathy [85]. Some older patients with this disorder evidence increases in vitreous AngII levels [86]. Further, there is evidence that vitreous AngII levels are positively correlated with degree of retinopathy [87].

Considerable experimental work has focused on understanding how hyperglycemia, and activation of local tissue RASs, lead to cellular damage. It has been known for some time that hyperglycemia induces oxidative stress; however elevated AngII tissue levels have also been shown to act as an oxidative stress inducer [88, 89]. In this way elevated AngII concentrations in diabetic tissues may exacerbate hyperglycemia-induced oxidative stress damage [90, 91]. As a result oxidative stress appears to both underlie, and be the result of, pathobiochemical mechanisms of diabetic-induced tissue damage [92]. The inhibition of the RAS with ACE inhibitors or ARBs in diabetic nephropathy rats reduced oxidative stress [93]. Recent clinical trials have been conducted with young Type 1 diabetic patients evidencing vascular superoxide overproduction (and early signs of angiopathy) due to hyperglycemia-related dysfunctional intracellular antioxidant enzyme production [94]. This dysfunction was reversed by treatment with the ARB Irbesartan. Further, the ARBs Candesartan and R-147176 (a sartan with low affinity for the AT₁ receptor subtype) appear to exert direct

antioxidant influences apparently independent of AT₁ receptor blockade [95]. Thus, these drugs show promise with regard to protection against diabetic-induced end-organ damage. Several clinical trials have focused on the efficacy of RAS blockade in diabetic patients. The Renin-Angiotensin System Study (RASS) monitored the efficacy of treatment with Losartan and Enalapril to Type 1 diabetic patients over a 5 year period [96]. The Diabetic REtinopathy Candesartan Trials (DIRECT) study tested the ARB Candesartan with Type 1 and 2 diabetic patients [97]. A report on An evaluation of telMisartan and losArtan in hypertensive Type 2 DiabEtic patients with Overt nephropathy (AMADEO) was recently published [98]. Specific details concerning the results of each of these trials follow. The RASS study monitored 2007 normotensive, normoalbuminuric Type 1 patients and focused on retinopathy. Both ACE inhibitors and ARB treatment attenuated the progression of retinopathy by 65 and 70%, respectively. However, since the majority of these patients (74%) had no evidence, or minimal evidence, of non-proliferative retinopathy at the outset of the study these results indicate the attenuation of new onset retinopathy. The DIRECT study consisted of 5000 Type 1 and 2 diabetic patients who for the most part were normotensive and normoalbuminuric. Three substudies were conducted. In one, DIRECT-Prevent, Type 1 patients showed no evidence of retinopathy. Candesartan reduced the incidence of retinopathy. In the second, DIRECT-Protect 1, Type 1 patients with existing retinopathy treated with Candesartan showed no effect on progression [97]. The third study, DIRECT-Protect 2, monitored normotensive and treated hypertensive Type 2 diabetic patients and found that Candesartan slowed the progression of retinopathy [99]. The AMADEO study randomly assigned Type 2 patients **with hypertension** to treatment with Telmisartan (final dose = 80 mg) or Losartan (final dose = 100 mg) **over the duration of** one year. Telmisartan was found to provide an effective anti-proteinuric effect in these hypertensive and overt nephropathy Type 2 patients. This effect was greater than that achieved with Losartan and blood pressure was maintained equivalently in the two groups [98]. Taken together these animal and clinical trials results indicate that inhibiting the action of the RAS with ACE inhibitors or ARBs offers significant clinical advantage in both Type 1 and 2 diabetic-induced retinopathy. These studies also illustrate the importance of gaining a better understanding of the underlying relationship between local RASs and diabetes.

6. The HGF/c-Met Receptor System

Several years ago our laboratory began searching for a molecular target with structural homology to AngIV and physiological functions in agreement with those identified for the AngIV/AT₄ receptor system. This yielded a partial match with the anti-angiogenic protein angiostatin, and the related plasminogen family member HGF. HGF is a mesenchyme-derived protein recognized as a potent mitogenic, morphogenic, and motogenic growth factor that acts via the Type 1 tyrosine kinase receptor c-Met [100]. HGF is intimately involved in cell survival, proliferation, migration, and differentiation [101-103]. c-Met has been shown to play a role in multiple types of cancer [104, 105], blunt neurodegenerative changes [106], facilitate long-term potentiation (LTP) [107], contribute to learning and memory consolidation [106, 108-111], and c-Met has been implicated in Alzheimer's disease [112, 113]. Also, inactivation of c-Met in the embryonic proliferative zones of mice results in an increase in parvalbumin-expressing cells in the dentate gyrus of the brain, accompanied by a loss of these cells in the CA3 field, with an overall loss of calretinin-expressing cells throughout the hippocampus [114]. These results suggest that c-Met is required for appropriate hippocampal development. The above functions associated with the HGF/c-Met system overlap with those mediated by the AngIV/AT₄ system including facilitated hippocampal LTP and memory consolidation, augmented neurite outgrowth, calcium signaling, dendritic arborization, facilitation of cerebral blood flow and cerebroprotection, seizure protection, and facilitated wound healing [25].

These observations prompted the hypothesis that AngIV analogues may exert their activity via the HGF/c-Met system. Investigations conducted in our laboratory demonstrated that the AT₄ receptor antagonist Norleual-AngIV inhibited HGF binding to c-Met and HGF-dependent cell signaling, proliferation, invasion, and scattering [115]. Additional studies indicated that Norleual-AngIV **bound** directly to HGF blocking the ability of HGF to dimerize, a process required for HGF activation and binding to the c-Met receptor [116]. Taken together these results suggest that the biological effects of AngIV, and AngIV analogues, are likely mediated through the HGF/c-Met system.

7. A Role for the HGF/c-Met System in Type 2 Diabetes Mellitus

The HGF/c-Met signaling pathway is involved in multiple functions including cellular proliferation, regeneration and branching morphogenesis [117]. As mentioned above, HGF must dimerize in order to bind and activate the transmembrane tyrosine kinase c-Met receptor [118]. The HGF/c-Met system is expressed in the pancreas where HGF is localized to endothelial islet and mesenchymal cells; while the c-Met receptor is present in islet, ductal and pancreatic progenitor cells [119-121]. Both HGF and c-Met are highly expressed during pancreatic development, and HGF functions as an insulinotropic factor promoting β cell proliferation and regeneration [122-124]. Pancreas specific c-Met knockout mice are susceptible to low dose streptozotocin-induced diabetes [125]. **These mice evidence elevated blood glucose levels accompanied by decreased glucose tolerance, hypoinsulinemia, and significantly decreased β cell mass when compared with wild type litter mates. Overall this HGF/c-Met knockout mouse evidences a pattern of β cell functioning and glucose metabolism very similar to what is seen in early phase β cell failure in Type 2 diabetic patients [120]. Thus, HGF/c-Met signaling appears to be essential for appropriate glucose-dependent insulin secretion and utilization.**

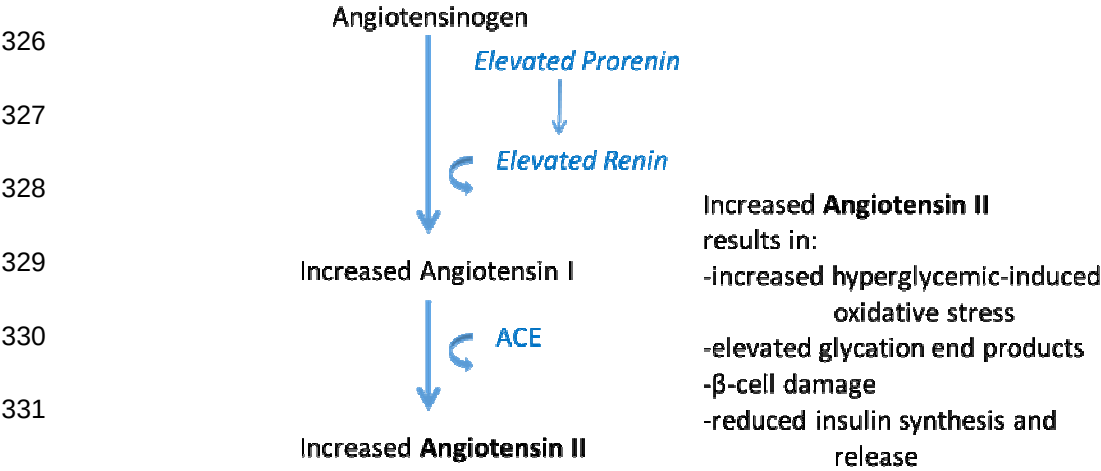
The HGF/c-Met system is also necessary for optimizing hepatic insulin responsiveness by interacting with the insulin receptor to form a hybrid complex of c-Met-insulin receptor [126]. These researchers reported that activation of the HGF/c-Met system facilitated insulin responsiveness in the ob/ob mouse model of Type 2 diabetes. The importance of this system is further emphasized in that HGF gene delivery slowed the progression of diabetic nephropathy in db/db mice by promoting antifibrotic and antiapoptotic actions [127]. In addition, HGF gene delivery in streptozotocin-induced diabetic mice triggered pro-survival Akt kinase activity as well as Bcl-xL expression in pancreatic islet cells thus preserving β cells [128, 129].

8. Hypothesis Concerning the RAS and HGF/c-Met Systems in Type 2

Diabetes

Several findings are relevant in designing a model to explain how small molecule HGF mimetics may be efficacious in the treatment of Type 2 diabetes. 1) It has been determined that prorenin levels associated with local RASs are elevated in the retina and kidney during hyperglycemia [79, 85, 130, 131]. 2) There is ample angiotensinogen available in local tissues to act as a substrate for elevated renin levels, along with the necessary ACE to convert AngI to AngII [132]. 3) Local tissue elevations in AngII have been linked with tissue injury [133, 134]. 4) Severity of retinopathy has been positively correlated with the level of vitreous AngII [135].

318 The above findings encourage the following hypothesis concerning the relationship between
319 the pancreatic RAS and cellular damage. Elevated prorenin levels in local tissues result in
320 renin acting on angiotensinogen to produce increases in AngI (Fig. 2). This elevated AngI is
321 converted to the biologically active peptide, AngII. Increased AngII levels facilitate
322 hyperglycemic-induced oxidative stress, and elevate glycation end-products resulting in β
323 cell damage. As β cells die off progressive reductions in insulin synthesis and release
324 follow.
325



332
333
334 **Fig. 2. Renin Angiotensin System and elevated Prorenin**
335 Changes in the pancreas renin-angiotensin system as a result of elevated prorenin levels.
336 Angiotensin II (bold) is biologically active.
337

338 From this prediction it is proposed that the treatment of Type 2 diabetic patients with an ACE
339 inhibitor or ARB reduces activation of the RAS with resulting reductions in hypertension and
340 oxidative stress (Fig. 3). Decreased RAS activity also impacts the local HGF/c-Met systems.
341 Specifically, treatment with an ACE inhibitor would be expected to reduce the formation of
342 AngII and result in compensatory increases in the nonapeptide, D-Asp¹,AngI. Cleavage of
343 aspartate by APA, histidine and leucine via carboxypeptidases, would convert this
344 nonapeptide to AngIII and then to AngIV via APN cleavage of arginine (Fig. 4; [47]). This
345 elevation in AngIV causes dimerization of HGF followed by increased activation of the c-Met
346 receptor thus optimizing hepatic insulin responsiveness accompanied by the facilitation of
347 cellular insulin receptivity. A similar outcome would be anticipated with ARB treatment of
348 Type 2 diabetic patients. Specifically, the positive response of Type 2 diabetic patients
349 treated with an ARB [136-138] is accompanied by an excess of AngII that is prevented from
350 binding at the AT₁ receptor subtype. This excess AngII may be converted to AngIII, and
351 then to AngIV and Ang(3-7) (see Figs. 1 and 3). These latter angiotensins are capable of
352 facilitating dimerization of HGF which then activates c-Met receptors in the pancreas and
353 elsewhere. Activation of c-Met receptors in turn increments insulin production and facilitate
354 cellular insulin responsiveness, with accompanying reductions in hyperglycemia-induced
355 oxidative stress and end-organ damage.
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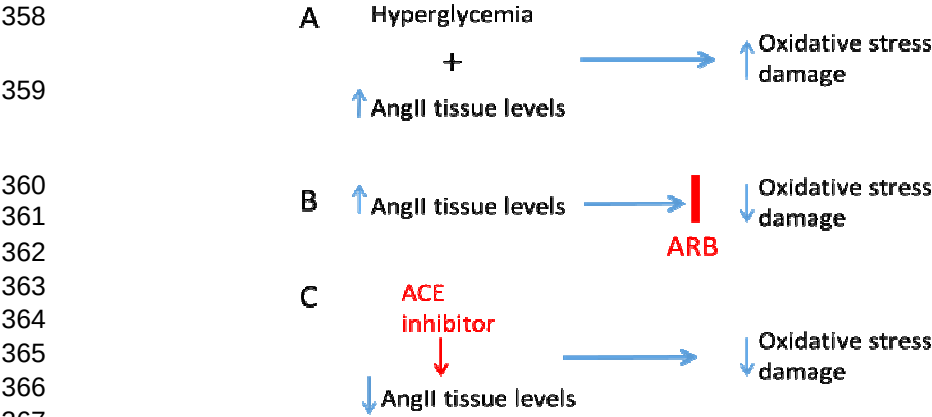


Fig. 3. Hyperglycemia and β Cell Damage: Treatment with and Angiotensin Receptor Blocker (ARB)

A) The contribution of hyperglycemia and increased tissue AngII levels results in facilitated hyperglycemic-induced oxidative stress leading to β cell damage. B) Treatment with an ACE inhibitor reduces the conversion of AngI to AngII resulting in decreased oxidative stress-induced β cell damage. C) Treatment with an ARB prevents AngII binding with the AT_1 receptor subtype resulting in decreased oxidative stress-induced β cell damage.

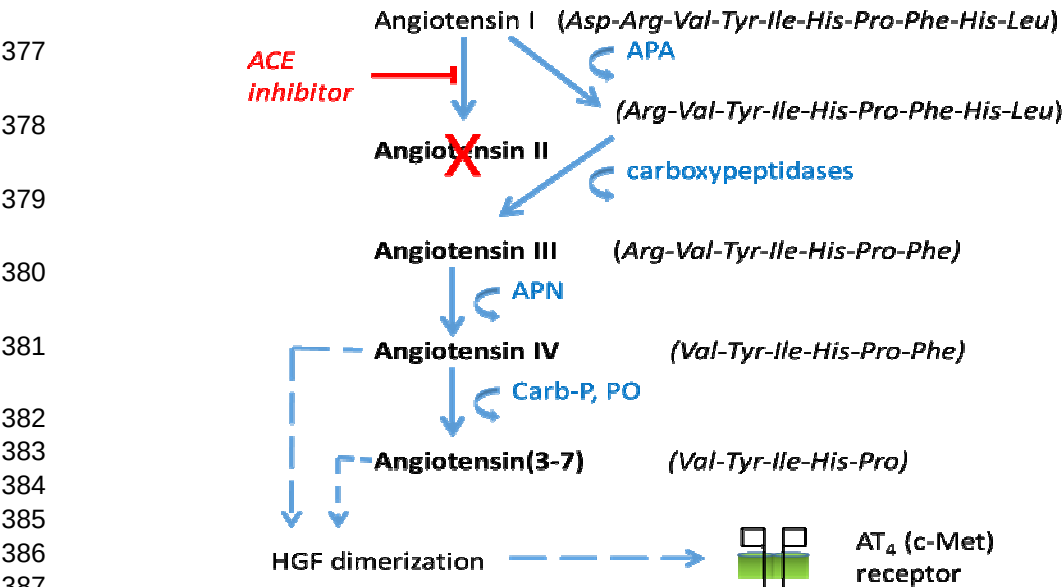


Fig. 4. Influence of ACE Inhibition on AngIV and Ang (3-7) Formation: Treatment with an Angiotensin Converting Enzyme (ACE) Inhibitor

Proposed changes in the synthesis pathway with ACE inhibitor treatment. This inhibitor reduces the formation of angiotensin II resulting in the increased formation of nonapeptide Ang(2-10). Carboxypeptidases then form angiotensin III which is converted to angiotensin IV by APN and angiotensin (3-7) by Carb-P and PO. Both AngIV and Ang(3-7) are capable of dimerizing HGF which then binds at the AT_4 (c-Met) receptor.

397 9. Development of a Small Molecule AngIV Analogue

398 During the development of AngIV analogues we determined the minimum structural features
399 of Nle¹-AngIV capable of promoting biological activity [139]. Previous studies indicated that
400 critical structural information resides at the N-terminal of AngIV [140, 141]. Thus, we
401 sequentially removed amino acids from the C-terminal of Nle¹-AngIV, while monitoring pro-
402 cognitive activity. This approach suggested that memory facilitation was achievable with
403 peptides as small as tetra- and tripeptides. Next, we modified these peptides in several
404 ways to enhance stability and hydrophobicity. Modifications included the addition of a D-
405 amino acid or non- α -amino acid in the #1 position, acylation of the N-terminal amino acid,
406 and conversion of the 1-2 peptide bond to a reduced-peptide bond. Several resulting
407 molecules offered the desired stability while maintaining the required biological activity [139,
408 142]. Included in this group were analogues protected at both the N- and C-terminals by
409 nonmetabolizable constituents, and in particular a tripeptide-sized small molecule with the
410 ability to reverse scopolamine-induced amnesia when delivered peripherally. We are
411 currently testing this compound for its ability to act as an insulinotropic factor in Type 2
412 diabetic animal models.

413 10. Conclusion

415 The number of individuals diagnosed with Type 2 diabetes is reaching epidemic proportions.
416 It is essential that new and novel treatments be considered [143-145]. The deleterious
417 contribution of local RASs has been documented in diabetic patients with hypertension. In
418 these patients AngII promotes end-organ damage by: 1) Promoting local vasoconstriction
419 accompanied by tissue ischemia and altered fluid electrolyte imbalance. 2) Exacerbating
420 hyperglycemic-induced oxidation stress and deleterious glycation end-products. Recently
421 the HGF/c-Met system has been implicated in diabetes [146, 147]. This growth factor
422 system appears to be essential for the appropriate development and maintenance of
423 pancreatic β cells [148, 149], and thus may hold the key to new treatment strategies. The
424 presently described approach concerns the use of a small molecule HGF mimetic to
425 increase HGF dimerization and binding to the c-Met receptor. Increased activation of c-Met
426 receptors has been shown to facilitate proliferation and optimization of pancreatic β cells. It
427 appears that appropriate insulin response is dependent upon engaging the insulin receptor
428 to form a hybrid complex with the c-Met receptor [148]. Insulin refractoriness is significantly
429 reduced when this complex is restored in Type 2 diabetic animal models. These research
430 findings, coupled with our discovery that a small molecule AngIV analogue induces HGF
431 dimerization (a prerequisite to c-Met binding), offer a unique potential treatment. The
432 availability of a small molecule may offer a significant advantage over the use of HGF, or
433 large HGF analogues, to accomplish this treatment goal. It remains to be seen whether
434 long-term treatment of Type 2 diabetes with small molecule HGF mimetics is efficacious.

435

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440

441 COMPETING INTERESTS

442 The authors indicate that they have no competing interests with regard to this publication.

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444 REFERENCES

- 445 1. Hossain P, Kavar G, El Nahas M. Obesity and diabetes in the developing world - a
446 growing challenge. *New Eng J Med*. 2007;356(3):213-215.
- 447 2. Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, Lin JK,
448 Farzadfar F, Khang YH, Stevens GA, Rao M, Ali MK, Riley LM, Robinson CA, Ezzati
449 M. National, regional, and global trends in fasting plasma glucose and diabetes
450 prevalence since 1980: Systematic analysis of health examination surveys and
451 epidemiological studies with 370 country-years and 2-7 million participants. *Lancet*.
452 2011;378(9785):31-40.
- 453 3. Haffner SM, Lehto S, Rönnekaa T, Pyörälä K, Laakso M. Mortality from coronary
454 heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and
455 without prior myocardial infarction. *New Eng J Med*. 1998;339(4):229-234.
- 456 4. Steckelings UM, Unger T. The renin-angiotensin-aldosterone system. In: Mancia G.
457 Grassi G. Kjeldsen S. eds. *Manual of Hypertension of the European Society of*
458 *Hypertension*. UK: Informa Healthcare London Publishing; 2008;14:110-116.
- 459 5. Tesfaye S, Chaturvedi N, Eaton SE, Ward JD, Manes C, Ionescu-Tirgoviste C, Witte
460 DR, Fuller JH. EURODIAB Prospective Complications Study Group: Vascular risk
461 factors and diabetic neuropathy. *New Eng J Med*; 2005;352(4):341-350.
- 462 6. Campbell NR, Gilbert RE, Leiter LA, Larochelle P, Tobe S, Chockalingam A, Ward R,
463 Morris D, Tsuyuki RT, Harris SB. Hypertension in people with type 2 diabetes;
464 Update on pharmacologic management. *Can Fam Physician*. 2011;57(9):997-1002.
- 465 7. Reboldi G, Gentile G, Angeli F, Ambrosio G, Mancia G, Verdecchia P. Blood
466 pressure lowering in diabetic patients. *J Hypertension*, 2012;30(2):438-439.
- 467 8. Lawes CM, Bennett DA, Lewington S, Rodgers A. Blood pressure and coronary
468 heart disease: A review of the evidence. *Seminars Vasc Med*. 2002;2(4):355-368.
- 469 9. Blumenthal JA, Babyak MA, Sherwood A, Craighead L, Lin PH, Johnson J, Watkins
470 LL, Wang JT, Kuhn C, Feinglos M, Hinderliter A. Effects of the dietary approaches to
471 stop hypertension diet alone and in combination with exercise and caloric restriction
472 on insulin sensitivity and lipids. *Hypertension*. 2010;55(5):199-205.
- 473 10. Blumenthal SS. Evolution of treatment for diabetic nephropathy: Historical
474 progression from RAAS inhibition and onward. *Postgrad Med*. 2011;123(6):166-179.
- 475 11. Shaw LC, Neu NB, Grant MB. Cell-based therapies for diabetic retinopathy. *Cur*
476 *Diabetic Rep*. 2011;11(4):265-274.
- 477 12. Miyata T, Dan T. Inhibition of advanced glycation end products (AGEs): An implicit
478 goal in clinical medicine for the treatment of diabetic nephropathy? *Diabetes Res*
479 *Clin Pract*. 2008;82(Suppl. 1):S25-29.
- 480 13. Ruggenenti P, Perna A, Ganeva, Ene-Iordache B, Remuzzi G: BENEDICT Group,
481 Impact of blood pressure control and angiotensin-converting enzyme inhibitor
482 therapy on new-onset microalbuminuria in type 2 diabetes: A post hoc analysis of
483 the BENEDICT trial. *J Amer Soc Nephrol*. 2006;17(12):3472-3481.
- 484 14. Luther JM, Brown NJ. The renin-angiotensin-aldosterone system and glucose
485 homeostasis. *Trends Pharmacol Sci*. 2011;32(12):734-739.
- 486 15. Roibeiro-Oliveira Jr. A, Nogueira AI, Pereira RM, Boas WW, Dos Santos RA,
487 Simoes e Silva AC. The renin-angiotensin system and diabetes: An update. *Vasc*
488 *Health Risk Man*. 2008;4(4):787-803.
- 489 16. Steckelings UM, Rompe F, Kaschina E, Unger T. The evolving story of the RAAS in
490 hypertension, diabetes and CV disease – moving from macrovascular to
491 microvascular targets. *Fund Clin Pharmacol*. 2009;23(6):693-703.
- 492 17. Foli T, Saad MJ, Velloso L, Hansen H, Carandente O, Feener EP, Kahn CR.
493 Crosstalk between insulin and angiotensin II signaling systems. *Exper Clin Endocr*
494 *Diabetes*. 1999;107(2):133-139.
- 495 18. Giacchetti G, Sechi LA, SRilli S, Carey RM. The renin-angiotensin-aldosterone
496 system, glucose metabolism and diabetes. *Trends Endocrinol Metab*.
497 2005;16(3):120-126.

- 498 19. de Gasparo M, Catt KJ, Inagami T, Wright JW, Unger T. International Union of
499 Pharmacology. XXIII. The angiotensin II receptors. *Pharmacol Rev.*
500 2000;52(3):415-472.
- 501 20. Gard PR. The role of angiotensin II in cognition and behavior. *Eur J Pharmacol.*
502 2002;438(1-2):1-14.
- 503 21. Schmieder RE, Delles C, Mimran A, Fauvel JP, Roulope LM. Impact of telmisartan
504 versus ramipril on renal endothelial function in patients with hypertension and type 2
505 diabetes. *Diabetes Care.* 2007;30(6):1351-1356.
- 506 22. Thomas WG, Mendelsohn FAO. Molecules in focus: Angiotensin receptors: form
507 and function and distribution. *Internat J Biochem Cell Biol.* 2003;35:774-779.
- 508 23. von Bohlen und Halbach O, Albrecht D. The CNS renin-angiotensin system. *Cell*
509 *Tissue Res.* 2006;326(2):599-616.
- 510 24. Wright JW, Harding JW. Regulatory role of brain angiotensins in the control of
511 physiological and behavioral responses. *Brain Res Rev.* 1991;17(3):227-262.
- 512 25. Wright JW, Harding JW. Brain renin-angiotensin-A new look at an old system. *Prog*
513 *Neurobiol.* 2011;95(1):49-67.
- 514 26. Wright JW, Yamamoto BJ, Harding JW. Angiotensin receptor subtype mediated
515 physiologies and behaviors: New discoveries and clinical targets. *Prog Neurobiol.*
516 2008;84(2):157-81.
- 517 27. Kalupahana NS, Moustaid-Moussa N. The renin-angiotensin system: A link between
518 obesity, inflammation and insulin resistance. *Obesity Rev.* 2012;13:136-149.
- 519 28. Singh VP, Le B, Khode R K, Baker KM, Kumar R. Intracellular angiotensin II
520 production in diabetic rats is correlated with cardiomyocyte apoptosis, oxidative
521 stress, and cardiac fibrosis. *Diabetes.* 2008;57(12):3297-3306.
- 522 29. Skipworth JR, Szabadkai, Olde Damink SW, Leung PS, Humphries SE, H
523 Montgomery HE. Review article: Pancreatic renin-angiotensin systems in health and
524 disease," *Alimentary Pharmacol Therap.* 2011;34(8):840-852.
- 525 30. Paul M, Poyan mehr A, Kreutz R. Physiology of local renin-angiotensin systems.
526 *Physiol Rev.* 2006;86(3):747-803.
- 527 31. Yasue S, Masuzaki H, Okada S, Ishii T, Kozuka C, Tanaka T, Fujikura J, Ebihara K,
528 Hosoda K, Katsurada A, Ohashi N, Urushihara M, Kobori H, Morimoto N, Kawazoe
529 T, Naitoh M, Okada M, Sakaue H, Suzuki S, Nakao K. Adipose tissue-specific
530 regulation of angiotensinogen in obese humans and mice: Impact of nutritional
531 status and adipocyte hypertrophy. *Amer J Hyperten.* 2010;23(4):425-431.
- 532 32. Johnston CI. Biochemistry and pharmacology of the renin-angiotensin system.
533 *Drugs.* 1990;39(Supple 1):21-31.
- 534 33. Karamyan VT, Speth RC. Enzymatic pathways of the brain renin-angiotensin
535 system: Unsolved problems and continuing challenges. *Regul Pept.* 2007;143(1-
536 3):15-27.
- 537 34. Speth RC, Karamyan VT. The significance of brain aminopeptidases in the
538 regulation of the actions of angiotensin peptides in the brain. *Heart Fail Rev.*
539 2008;13(3):299-309.
- 540 35. Ferrario CM, Strawn WB,. Role of the renin-angiotensin-aldosterone system and
541 proinflammatory mediators in cardiovascular disease. *Amer J Cardiol.*
542 2006;98(1):121-128.
- 543 36. Kokubu T, Ueda E, Joh T, Nishimura K. Purification and properties of angiotensin I-
544 converting enzyme in human lung and its role on the metabolism of vasoactive
545 peptides in pulmonary circulation. *Adv Exp Med Biol B.* 1979;120(3):467-475.
- 546 37. Unger T. The role of the renin-angiotensin-aldosterone system in heart failure. *J*
547 *Renin Angiotensin Aldosterone Syst.* 2004;5(Supple. 1):S7-S10.
- 548 38. Rich DH, Moon BJ, Harbeson S. Inhibition of aminopeptidases by amastatin and
549 bestatin derivatives, effect of inhibitor structure on slow-binding processes. *J Med*
550 *Chem.* 1984;27(4):417-422.

- 551 39. Ramírez M, Arechaga G, García S, Sanchez B, De Gandarias JM. Mn²⁺-activated
552 aspartate aminopeptidase activity, subcellular localization in young and adult rat
553 brain. *Brain Res.* 1990;522(1):165-167.
- 554 40. Wilk S, Healy DP. Glutamyl aminopeptidase (aminopeptidase A), the BP-1/6C3
555 antigen. *Adv Neuroimmunol.* 1993;3(1):195-207.
- 556 41. Chauvel EN, Llorens-Cortes C, Coric P, Wilk S, Roques BP, Fournie-Zaluski MC,
557 Differential inhibition of aminopeptidase A and aminopeptidase N by new -amino
558 thiols. *J Med Chem.* 1994;37(18):2950-2957.
- 559 42. Unger T, Badoer E, Ganten D, Lang RE, Rettig R. Brain angiotensin: Pathways and
560 pharmacology. *Circulation.* 1988;77(6 Pt. 2):140-154.
- 561 43. Saavedra JM. Brain and pituitary angiotensin. *Endocr Rev.* 1992;13(2):329-380.
- 562 44. Speth RC, Brown TE, Barnes RD, Wright JW. Brain angiotensinergic activity: The
563 state of our current knowledge. *Proc Western Pharmacol Soc.* 2003;46(1):11-15.
- 564 45. Reudelhuber TL. The renin-angiotensin system: Peptides and enzymes beyond
565 angiotensin II. *Curr Opin Neph Hypertens.* 2005;14(2):155-159.
- 566 46. Banegas I, Prieto I, Vives F, Alba F, de Gasparo M, Segarra AB, Hermoso F, Duran
567 R, Ramírez M. Brain aminopeptidases and hypertension. *Journal Renin Angiotensin*
568 *Aldosterone Syst.* 2006;7(3):129-134.
- 569 47. Wright JW, Harding JW. Important roles for angiotensin III and IV in the brain renin-
570 angiotensin system. *Brain Res Rev.* 1997;25(1):96-124.
- 571 48. Glossmann H, Baukal A, Catt, KJ. Angiotensin II receptors in bovine adrenal cortex.
572 Modification of angiotensin II binding by guanyl nucleotides. *J Biol Chem.*
573 1974;249(2):664-666.
- 574 49. Bennett, Jr. JP, Snyder SH. Angiotensin II binding to mammalian brain membranes.
575 *J Biol Chem.* 1976;251(23):7423-7430.
- 576 50. Harding JW, Cook VI, Miller-Wing AV, Hanesworth JM, Sardinia MF, Hall KL, Stobb
577 JW, Swanson GN, Coleman JK, Wright JW, Harding EC. Identification of an AII (3-
578 8) [AIV] binding site in guinea pig hippocampus. *Brain Res.* 1992;583(1-2):340-343.
- 579 51. Jarvis, MF, Gessner GW, C.G. Ly CG. The angiotensin hexapeptide 3-8 fragment
580 potently inhibits [¹²⁵I] angiotensin II binding to non-AT₁ or -AT₂ recognition sites in
581 bovine adrenal cortex. *Eur J Pharmacol.* 1992;219(2):319-322.
- 582 52. Swanson GN, Hanesworth JM, Sardinia MF, Coleman JK, Wright JW, Hall KL,
583 Miller-Wing AV, Stobb JW, Cook VI, Harding EC, Harding JW. Discovery of a distinct
584 binding site for angiotensin II (3-8), a putative angiotensin IV receptor. *Regul Pept.*
585 1992;40(3):409-419.
- 586 53. Bernier SG, Bellemare JM, Escher E, Guillemette, G. Characterization of AT₄
587 receptor from bovine aortic endothel photosensitive analogues of angiotensin IV.
588 *Biochemistry.* 1998;37(12):4280-4287.
- 589 54. Wright JW, Krebs LT, Stobb JW, Harding JW. The angiotensin IV system: Functional
590 implications. *Front Neuroendocrinol.* 1995;16(1):23-52.
- 591 55. Wright JW, Bechtholt AJ, Chambers SL, Harding JW. Angiotensin III and IV
592 activation of the brain AT₁ receptor subtype in cardiovascular function. *Peptides.*
593 1996;17(8):1365-1371.
- 594 56. Lochard N, Thibault G, Silversides DW, Touyz RM, Reudelhuber TL. Chronic
595 production of angiotensin IV in the brain leads to hypertension that is reversible with
596 an angiotensin II AT₁ receptor antagonist. *Circ Res.* 2004;94(11):1451-1457.
- 597 57. Yang R, Smolders I, De Bundel D, Fouyn R, Halberg M, Demaegdt H,
598 Vanderheyden P, Dupont AG. Brain and peripheral angiotensin II type 1 receptors
599 mediate renal vasoconstrictor and blood pressure responses to angiotensin IV in the
600 rat. *J Hypertens* 2008;26(5):998-1007.
- 601 58. Yang R, Walther T, Gembardt F, Smolders I, Vanderheyden P, Albiston AL, Chai
602 SY, Dupont AG. Renal vasoconstrictor and pressor responses to angiotensin IV in
603 mice are AT_{1a}-receptor mediated. *J Hypertens.* 2010;28(3):487-494.

- 604 59. Bosnyak S, Jones ES, Christopoulos A, Aguilar MI, Thomas WG, Widdop RE.
605 Relative affinity of angiotensin peptides and novel ligands at AT₁ and AT₂ receptors.
606 Clin Sci. 2011;121(7):297-303.
- 607 60. Kakuta H, Sudoh K, Sasamata M, Yamagishi S. Telmisartan has the strongest
608 binding affinity to angiotensin II type 1 receptor: Comparison with other angiotensin
609 II Type 1 receptor blockers. Int J Clin Pharm Res. 2005;25(1):41-46.
- 610 61. Sayeski PP, Ali MS, Semeniuk DJ, Doan TN, Bernstein KE. Angiotensin II signal
611 transduction pathways,. Regul Pept. 1998;78(1-3):19-29.
- 612 62. Dinh DT, A.Grauman AG, Johnston CI, Fabiani ME. Angiotensin receptors:
613 distribution, signaling and function. Clin Sci. 2001;100(5):481-492.
- 614 63. Iwai N, Inagami T. Identification of two subtypes in the rat type 1 angiotensin II
615 receptor. FEBS Letters. 1992;298(2-3):257-260.
- 616 64. Kakar SS, Sellers JD, Devor DC, Musgrove LC, Neill JD. Angiotensin II type-1
617 receptor subtype cDNAs: Differential tissue expression and hormonal regulation.
618 Biochem Biophysics Res Commun. 1992;183(3):1090-1096.
- 619 65. Sadamura H, Hein L, Krieger JE, Pratt RE, Kobilka BK, Dzau VJ. Cloning,
620 characterization, and expression of two angiotensin receptor (AT-1) isoforms from
621 the mouse genome. Biochem Biophysics Res Commun. 1992;185(1):253-259.
- 622 66. Konoshi H, Kuroda S, Inada Y, Fujisawa Y. Novel subtype of human angiotensin II
623 type 1 receptor: cDNA cloning and expression. Biochem Biophysics Res Commun.
624 1994;199(2):467-474.
- 625 67. Guo DF, Inagami T. The genomic organization of the rat angiotensin II receptor
626 AT_{1B}. Biochem Biophysics Acta. 1994;1218(1):91-94.
- 627 68. Speth RC, Thompson SM, Johns SJ. Angiotensin II receptors: structural and
628 functional considerations," In: Mukhopadhyay, AK, Raizada, MK., eds., *Current*
629 *Concepts: Tissue Renin Angiotensin Systems as Local Regulators in Reproductive*
630 *and Endocrine Organs*. New York: Plenum Press. 1995:169-192.
- 631 69. Saavedra JM. Emerging features of brain angiotensin receptors. Regul Pept.
632 1999;85(1):31-45.
- 633 70. Thomas WG, Mendelsohn FAO. Molecules in focus: Angiotensin receptors: form
634 and function and distribution. Internat J Biochem Cell Biol. 2003;35(6):774-779.
- 635 71. Bottari SP, Raylor V, King IN, Bogdal S, Whitebrea S, DeGasparo M. Angiotensin II
636 AT₂ receptors do not interact with guanine nucleotide binding proteins. Eur J
637 Pharmacol. 1991;207(2):157-163.
- 638 72. Kambayashi Y, Bardhan S, Takahashi K, Tsuzuki S, Inui T, Hamakubo T, Inagami
639 Y. Molecular cloning of a novel angiotensin II receptor isoform involved in
640 phosphotyrosine phosphatase inhibition. J Biol Chem. 1993;268(33):24543-24546.
- 641 73. Hanesworth JM, Sardinia JF, Krebs LT, Hall KL, Harding JW. Elucidation of a
642 specific binding site for angiotensin II(3-8), angiotensin IV, in mammalian heart
643 membranes. J Pharmacol Exp Therapeut. 1993;266(2):1036-1042.
- 644 74. Sardinia MF, Hanesworth JM, Krebs LT, Harding JW. AT₄ receptor binding
645 characteristics: D-amino acid- and glycine-substituted peptides. Peptides.
646 1993;14(5):949-954.
- 647 75. Sardinia MF, Hanesworth JM, Krishnan F, Harding JM. AT₄ receptor structure-
648 binding relationship: N-terminal-modified angiotensin IV analogues. Peptides.
649 1994;15(8):1399-1406.
- 650 76. Krishnan R, Hanesworth JM, Wright JW, Harding JW. Structure-binding studies of
651 the adrenal AT₄ receptor: Analysis of position two- and three-modified angiotensin IV
652 analogs. Peptides. 1999;20(8):915-920.
- 653 77. Sernia C. A critical appraisal of the intrinsic pancreatic angiotensin-generating
654 system. J Physical Agents. 2001;2(1):50-55.

- 655 78. Price DA, Porter LE, Gordon M, Fisher ND, De'Oliveira JM, Laffel LM, Laffel DR,
656 Williams GH, Hollenberg NK. The paradox of the low-renin state in diabetic
657 nephropathy. *J Amer Soc Nephrol.* 1999;10(11):2382-2391.
- 658 79. Franken AA, Derkx FH, Schalekamp MA, Man in t'Veld, AH, Hop WC, van Rens EH,
659 de Jong PT. Association of high plasma prorenin with diabetic retinopathy. *J*
660 *Hypertens.* 1988;6 (Suppl. No. 6):S461-463.
- 661 80. Luetscher JA, Kraemer FB, Wilson DM, Schwartz HC, Bryer-Ash M. Increased
662 plasma inactive renin in diabetes mellitus. A marker of microvascular complications.
663 *New Engl J Med.* 1985;312(22):1412-1417.
- 664 81. Ichihara A, Hayashi M, Kaneshiro Y, Suzuki F, Nakagawa T, Tada Y, Koura Y,
665 Nishiyama A, Okada H, Uddin MN, Nabi AH, Ishida Y, Inagami T, Saruta T.
666 Inhibition of diabetic nephropathy by a decoy peptide corresponding to the "handle"
667 region for nonproteolytic activation of prorenin. *J Clin Invest.* 2004;114(8):1128-
668 1135.
- 669 82. Scheffe JH, Menk M, Reinemund J, Effertz K, Hobbs RM, Pandolfi PP, Ruiz P,
670 Unger T, Funke-Kaiser H. A novel signal transduction cascade involving direct
671 physical interaction of the renin/prorenin receptor with the transcription factor
672 promyelocytic zinc finger protein. *Circ Res.* 2006;99(12):1355-1366
- 673 83. Carey RM, Siragy, HM. The intrarenal renin-angiotensin system and diabetic
674 nephropathy. *Trends Endocrinol Metab.* 2003;14(6):274-281.
- 675 84. Ebrahimian TG, Tamarat R, Clergue M, Durie M, Levy BI, Silvestre JS. Dual effect of
676 angiotensin-converting enzyme inhibition on angiogenesis in type 1 diabetic mice.
677 *Arterioscl Thromb Vasc Biol.* 2005;25(1):65-70.
- 678 85. Danser AH, van den Dorpel MA, Deinum J, de Jong P, Schalekamp MA. Renin,
679 prorenin, and immunoreactive renin in vitreous fluid from eyes with and without
680 diabetic retinopathy," *J Clin Endocrinol Metab.* 1989;68(1):160-167.
- 681 86. Danser AH, Derkx FH, Admiraal PJ, Deinum J, de Jong PT, Schalekamp MA.
682 Angiotensin levels in the eye," *Invest Ophthalmol Vis Sci.* 1994;35(3):1008-1018.
- 683 87. H. Funatsu H, H. Yamashita H, T. Ikeda T, Y. Nakanishi Y, S. Kitano S, and S. Hori
684 S. Angiotensin II and vascular endothelial growth factor in the vitreous fluid of
685 patients with diabetic macular edema and other retinal disorders," *Amer J*
686 *Ophthalmol.* 2002;133(4):537-543.
- 687 88. Forstermann U. Oxidative stress in vascular disease: Causes, defense mechanisms
688 and potential therapies. *Natl Clin Pract CardiovascMed.* 2009;5(6):338-349.
- 689 89. Yamagishi S, Amano S, Inagaki Y, Okamoto T, Inoue H, Takeuchi M, Choei H,
690 Sasaki N, Kikuchi S. Angiotensin II-type 1 receptor interaction upregulates vascular
691 endothelial growth factor messenger RNA levels in retinal pericytes through
692 intracellular reactive oxygen species generation. *Drugs Exp Clin Res.*
693 2003;29(2):75-80.
- 694 90. Chen P, Guo AM, Edwards PA, Trick G, Scicli AG. Role of NADPH oxidase and
695 ANG II in diabetes-induced retinal leukostasis. *Amer J Physiol Regul Integ Comp*
696 *Physiol.* ;293(4):R1619-1629.
- 697 91. Fukumoto M, Takai S, Ishizaki E, Sugiyama T, Oku H, Jin D, Sakaguchi M, Sakonjo
698 H, Ikeda T, Miyazaki M. Involvement of angiotensin II-dependent vascular
699 endothelial growth factor gene expression via NADPH oxidase in the retina in a type
700 2 diabetic rat model. *Curr Eye Res.* 2008;33(10):885-891.
- 701 92. Steckelings UM, Rompe F, Kaschina E, Unger T. The evolving story of the RAAS in
702 hypertension, diabetes and CV disease – moving from macrovascular to
703 microvascular targets," *Fund Clin Pharmacol.* 2009;23(6):693-703.
- 704 93. Onozato ML, Tojo A, Goto A, Fujita T, Wilcox CS. Oxidative stress and nitric oxide
705 synthase in rat diabetic nephropathy: Effects of ACEI and ARB. *Kidney Internatl.*
706 2002;61(1):186-194.

- 707 94. Chiarelli F, Di Marzio D, Santilli F, Mohn A, Blasetti A, Cipollone F, Meazzeti A,
708 Verrotti A. Effects of irbesartan on intracellular antioxidant enzyme expression and
709 activity in adolescents and young adults with early diabetic angiopathy. *Diabetes*
710 *Care*. 2005;28(7):1690-1697.
- 711 95. Izuhara Y, Sada T, Yanagisawa H, Koike H, Ohtomo S, Dan T, Ito S, Nangaku M,
712 van Ypersele de Strihou C, Miyata T. A novel sartan derivative with very low
713 angiotensin II type 1 receptor affinity protects the kidney in type 2 diabetic rats.
714 *Arterioscl Thomb Vasc Biol*. 2008;28(10):1767-1773.
- 715 96. Mauer M, Zinman B, Gardiner R, Drummond KN, Suissa S, Donnelly SM, Strand
716 TD, Kramer MS, Klein R, Sinaiko AR. ACE-I and ARBs in early diabetic
717 nephropathy. *J Renin Angiotensin Aldosterone Syst*. 2002;3(4):262-269.
- 718 97. Chaturvedi N, Porta M, Klein R, Orchard T, Fuller J, Parving HH, Bilous R, Sjolie AK.
719 DIRECT Programme Study Group, Effect of candesartan on prevention (DIRECT-
720 Prevent 1) and progression (DIRECT-Protect 1) of retinopathy in type 1 diabetes:
721 Randomised, placebo-controlled trials. *Lancet*. 2008;372(9647):1394-1402.
- 722 98. Bichu P, Nistala R, Khan A, Sowers JR, Whaley-Connell A. Angiotensin receptor
723 blockers for the reduction of proteinuria in diabetic patients with overt nephropathy:
724 Results from the AMADEO study. *Vasc Health Risk Manag*. 2009;5(1):129-140.
- 725 99. Sjolie AK, Klein R, Porta M, Orchard T, Fuller J, Parving HH, Bilous FR, Chaturvedi
726 N. DIRECT Programme Study Group, Effect of candesartan on progression and
727 regression of retinopathy in type 2 diabetes (DIRECT-Protect2): A randomized
728 placebo-controlled trial. *Lancet*. 2008;372(9647):1385-1393.
- 729 100. Ma PC, Maulik G, Christensen J, Salgia R. C-Met: structure, functions and potential
730 for therapeutic inhibition. *Cancer Metast Rev*. 2003;22(4):309-325.
- 731 101. Liu Y. Hepatocyte growth factor and the kidney. *Curr Opin Neph Hypertens*.
732 2002;11(1):23-30.
- 733 102. Liu Y, Bell AW, Michalopoulos GK, Zarnegar R. The mouse hepatocyte growth
734 factor-encoding gene: Structural organization and evolutionary conservation. *Gene*.
735 1994;144(2):179-187.
- 736 103. Yang J, Dai C, Liu Y. Hepatocyte growth factor gene therapy and angiotensin II
737 blockage synergistically attenuate renal interstitial fibrosis in mice. *J Amer Soc*
738 *Nephrol*. 2002;13(10):2464-2477.
- 739 104. Comoglio PM, Giordano S, Trusolino L. Drug development of MET inhibitors:
740 Targeting oncogene addiction and expedience. *Nat Rev Drug Disc*. 2008;7(6):504-
741 516.
- 742 105. Jiang WG, Martin TA, Parr C, Davies G, Matsumoto K, Nakamura T. Hepatocyte
743 growth factor, its receptor, and their potential value in cancer therapies. *Crit Rev*
744 *Oncol/Hematol*. 2005;53(1):35-69.
- 745 106. Shimamura M, Sato N, Waguri S, Uchiyama Y, Hayashi T, Iida H, Nakamura T,
746 Ogiwara T, Kaneda Y, Morishita R. Gene transfer of hepatocyte growth factor gene
747 improves learning and memory in the chronic stage of cerebral infarction.
748 *Hypertension*. 2006;47(4):742-51.
- 749 107. Akimoto M, Baba A, Ikeda-Matsuo Y, Yamada MK, Itamura R, Nishiyama N, Ikegaya
750 Y, Matsuki N. Hepatocyte growth factor as an enhancer of NMDA currents and
751 synaptic plasticity in the hippocampus. *Neuroscience*. 2004;128(1):155-62.
- 752 108. Date I, Takagi N, Takagi K, Kago T, Matsumoto K, Nakamura T, Takeo S.
753 Hepatocyte growth factor attenuates cerebral ischemia-induced learning
754 dysfunction. *Biochem Biophys Res Comm*. 2004;319(4):1152-1158.
- 755 109. Date I, Takagi N, Takagi K, Kago T, Matsumoto K, Nakamura T, Takeo S. Hepatocyte
756 growth factor improved learning and memory dysfunction of microsphere-embolized
757 rats. *J Neurosci Res*. 2004;78(3):442-453.
- 758 110. Tada R, Zhan H, Tanaka Y, Hongo K, Matsumoto K, Nakamura T. Intraventricular
759 administration of hepatocyte growth factor treats mouse communicating

hydrocephalus induced by transforming growth factor beta1. *Neurobiol Dis.* 2006; 21(3):576-585.

111. Takeo S, Takagi N, Takagi K. Ischemic brain injury and hepatocyte growth factor. *Yakugaku Zasshi.* 2007;127(11):1813-1823.

112. Shang J, Deguchi K, Ohta Y, Liu N, Zhang X, Tian T, Yamashita T, Ikeda Y, Matsuura T, Funakoshi H, Nakamura T, Abe K. Strong neurogenesis, angiogenesis, synaptogenesis, and antifibrosis of hepatocyte growth factor in rats brain after transient middle cerebral artery occlusion. *J Neurosci.* 2011;89(1):86-95.

113. Tsuboi Y, Kakimoto K, Nakajima M, Akatsu H, Yamamoto T, Ogawa K, Ohnishi T, Daikuhara Y, Yamada T. Increased hepatocyte growth factor level in cerebrospinal fluid in Alzheimer's disease. *Acta Neurol Scand.* 2003;107(2):81-86.

114. Martins GJ, Plachez C, Powell EM. Loss of embryonic MET signaling alters profiles of hippocampal interneurons. *Dev Neurosci.* 2007;29(1-2):143-158.

115. Yamamoto BJ, Elias PD, Masino JA, Hudson BD, McCoy AT, Anderson ZJ, Varnum MD, Sardinia MF, Wright JW, Harding JW. The angiotensin IV analog Nle-Tyr-Leu-psi-(CH₂-NH₂)3-4-His-Pro-Phe (norleual) can act as a hepatocyte growth factor/c-Met inhibitor. *J Pharmacol Exp Therapeut.* 2010;333(1):161-173.

116. Kawas LH, Yamamoto BJ, Wright JW, Harding JW. Mimics of the dimerization domain of hepatocyte growth factor exhibit anti-Met and anticancer activity. *J Pharmacol Exp Therapeut.* 2011;339(2):509-518.

117. Stuart KA, Riordan SM, Lidder S, Crostella L, Williams R, Skouteris GG. Hepatocyte growth factor/scatter factor-induced intracellular signaling. *Internat J Exp Path.* 2000;81(1):17-30.

118. Furge KA, Zhang YW, Vande Woude GF. Met receptor tyrosine kinase: Enhanced signaling through adapter proteins. *Oncogene.* 2000;19(49):5582-5589.

119. Johansson M, Mattsson G, Andersson A, Jansson L, Carlsson PO. Islet endothelial cells and pancreatic beta-cell proliferation: Studies in vitro and during pregnancy in adult rats. *Endocrinology.* 2006;147(5):2315-2324.

120. Roccisana J, Reddy V, Vasavada RC, Gonzalez-Pertusa JA, Magnuson MA, Garcia-Ocana A. Targeted inactivation of hepatocyte growth factor receptor c-met in beta-cells leads to defective insulin secretion and GLUT-2 downregulation without alteration of beta-cell mass. *Diabetes.* 2005;54(7):2090-2102.

121. Suzuki A, Y. Zheng YW, Fukako K, Nakauchi H, Taniguchi H. Liver repopulation by c-Met-positive stem/progenitor cells isolated from the developing rat liver. *Hepatogastroenterology.* 2004;51(56):424-426.

122. Otonkoski T, Beattie GM, Rubin JS, Lopez AD, Baird A, Hayek A. Hepatocyte growth factor/scatter factor has insulinotropic activity in human fetal pancreatic cells. *Diabetes.* 1994;43(7):947-953.

123. Otonkoski T, Cirulli V, Beattie M, Mally MI, Soto G, Rubin JS, Hayek A. A role for hepatocyte growth factor/scatter factor in fetal mesenchyme-induced pancreatic beta-cell growth. *Endocrinology* 1996;137(7):3131-3139.

124. Hayek A, Beattie GM, Cirulli V, Lopez AD, Ricordi C, Rubin JS. Growth factor/matrix-induced proliferation of human adult beta-cells. *Diabetes.* 1995;44(12):1458-1460.

125. Mellado-Gil J, Rkosa TC, Dmirci C, Gkonzalez-Pertusa JA, Velazquez-Garcia S, Ernst S, Valle S, Vasavada RC, Stewart AF, Alonso LC, Garcia-Ocana A. Disruption of hepatocyte growth factor/c-Met signaling enhances pancreatic beta-cell death and accelerates the onset of diabetes. *Diabetes.* 2011;60(2):525-536.

126. Fafallios A, Ma J, Tan X, Stoops J, Luo J, Defrances MC, Zarnegar R. A hepatocyte growth factor receptor (Met)-insulin receptor hybrid governs hepatic glucose metabolism. *Natl Med.* 2011;17(12):1577-1588.

127. Kagawa T, Takemura G, Kosai K, Murata I, Ohno T, Takahashi T, Esaki M, Maruyama R, Fujiwara T, Ohashi H, Fujiwara H. Hepatocyte growth factor gene

813 therapy slows down the progression of diabetic nephropathy in db/db mice. *Nephron*
814 *Physiol.* 2006;201(3-4):92-102.

815 128. Dai C, Li Y, Yang J, Liu Y. Hepatocyte growth factor preserves beta cell mass and
816 mitigates hyperglycemia in streptozotocin-induced diabetic mice. *J Biol Chem.*
817 2003;278(29):27080-27087.

818 129. Izumida Y, Aoki T, Yasuda D, Kouzumi T, Suganuma C, Saito K, Murai N, Shimizu
819 Y, Hayashi K, Odaira M, Kusano T, Kushima M, Kudano M. Hepatocyte growth
820 factor is constitutively produced by donor-derived bone marrow cells and promotes
821 regeneration of pancreatic beta-cells. *Biochem Biophys Res Comm.*
822 2005;333(1):273-282.

823 130. Carey RM, Siragy HM. The intrarenal renin-angiotensin system and diabetic
824 nephropathy. *Trends Endocrinol Metab.* 2003;14(6):274-281.

825 131. Ebrahimian TG, Tamarat R, Clergue M, Duriez M, Levy BK, Silvestre JS. Dual effect
826 of angiotensin-converting enzyme inhibition on angiogenesis in type 1 diabetic mice.
827 *Arterioscl Thromb Vasc Biol.* 2005;25(1):65-70.

828 132. Paul M, Poyan Mehr A, Kreutz R. Physiology of local renin-angiotensin systems.
829 *Physiol Rev.* 2006;86(3):747-803.

830 133. Ichihara A, Hayashi M, Kaneshiro Y, Suzuki F, Nakagawa T, Tada Y, Koura Y,
831 Nishiyama A, Okada H, Uddin MN, Nabi AH, Ishida Y, Inagami, T, Saruta T.
832 Inhibition of diabetic nephropathy by a decoy peptide corresponding to the "handle"
833 region for nonproteolytic activation of prorenin. *J Clin Invest.* 2004;114(8):1128-
834 1135.

835 134. Scheffe JH, Menk M, Reinemund J, Effertz K, Hobbs RM, Pandolfi PP, Ruiz P, Unger
836 T, Funke-Kaiser H. A novel signal transduction cascade involving direct physical
837 interaction of the renin/prorenin receptor with the transcription factor promyelocytic
838 zinc finger protein. *Circ Res.* 2006;99(12):1355-1366.

839 135. H. Funatsu H, H. Yamashita H, T. Ikeda T, Y. Nakanishi Y, S. Kitano S, and S. Hori
840 S. Angiotensin II and vascular endothelial growth factor in the vitreous fluid of
841 patients with diabetic macular edema and other retinal disorders. *Amer J*
842 *Ophthalmol.* 2002;133(4):537-543.

843 136. Cao Z, Cooper ME. Efficacy of renin-angiotensin system (RAS) blockers on
844 cardiovascular and renal outcomes in patients with type 2 diabetes. *Acta Diabetol.*
845 Epub ahead of print, September 2011.

846 137. Jeganathan VS. The therapeutic implications of renin-angiotensin system blockade
847 in diabetic retinopathy. *Curr Pharmacol Biotechnol.* 2011;12(3):392-395.

848 138. Weir MR. Effects of renin-angiotensin system inhibition on end-organ protection:
849 Can we do better? *Clin Ther.* 2007;29(9):1803-1824.

850 139. Benoist CC, Wright JW, Zhu M, Appleyard SM, Wayman GA, Harding JW.
851 Facilitation of hippocampal synaptogenesis and spatial memory by C-terminal
852 truncated Nle¹-angiotensin IV analogues. *J Pharmacol Exper Therapeut.*
853 2011;339(1):35-44.

854 140. Wright JW, Tamura-Myers E, Wilson WL, Roques BP, Llorens-Cortes C, Speth RC,
855 Harding JW. Conversion of brain angiotensin II to angiotensin III is critical for
856 pressor response in rats. *Amer J Physiol: Regul Integr Comp Physiol.*
857 2002;284(3):R725-733.

858 141. Wright JW, Yamamoto BJ, Harding JW. Angiotensin receptor subtype mediated
859 physiologies and behaviors: New discoveries and clinical targets. *Prog Neurobiol.*
860 2008;84(2):157-181.

861 142. Wright JW, Harding JW. The brain RAS and Alzheimer's disease. *Exp Neurol.*
862 2010;223(2):326-333

863 143. Bogaert YE, Schrier RW. Into the future: prevention of diabetes. *Contrib Nephrol.*
864 2011;170(1):56-263.

865 144. Koulis C, de Hann JB, Allen TJ. Novel pathways and therapies in experimental
866 diabetic atherosclerosis. *Expert Rev Cardiovasc Ther.* 2012;10(3):323-335.
867 145. Porta M , Maldari M, Mazzaglia F. New approaches to the treatment of diabetic
868 retinopathy. *Diabetes Obesity Metab.* 2011;13(9):784-790.
869 146. Flaquer M, Franquesa M, Vidal A, Bolanos N, Torras J, Lloberas N, Herrero-
870 Fresneda I, Grinyo JM, Cruzado JJ. Hepatocyte growth factor gene therapy
871 enhances infiltration of macrophages and may induce kidney repair in db/db mice as
872 a model of diabetes. *Diabetologia.* Epub ahead of print March , 2012.
873 147. Hoesli CA, Johnson JD, Piret JM. Purified human pancreatic duct cell culture
874 conditions defined by serum-free high-content growth factor screening. *Plos One.*
875 2012;7(3):e33999.
876 148. Demirci C, Ernst S, Alvarez-Perez JD, Rosa T, Valle, Shridhar C, Casinelli GP,
877 Alonso LC, Vasavada RC, Garcia-Ocana A. Loss of HGF/c-Met signaling in
878 pancreatic β cells leads to incomplete maternal β -cell adaptation and gestational
879 diabetes mellitus. *Diabetes.* Epub ahead of print, March, 2012.
880 149. Fafalios A, Ma J, Tan X, Stoops J, Luo J, DeFrances MC, Zarnegar R. A hepatocyte
881 growth factor receptor (Met) insulin receptor hybrid governs hepatic glucose
882 metabolism. *Nature Med.* 2011;17(12):1577-1584.
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