Regulation of the prorenin angiotensin system by oxygen and miRNAs; parallels between placentation and tumour development?

Sarah J. Delforce[1,2,3], Eugenie R. Lumbers[1,2,3], *Kirsty G. Pringle[1,2,3]

[1]School of Biomedical Sciences and Pharmacy, University of Newcastle, Newcastle, NSW;

[2]Priority Research Centre for Reproductive Sciences, University of Newcastle, Newcastle, NSW;

[3]Mothers & Babies Research Centre, Hunter Medical Research Institute, Newcastle, NSW

*Corresponding Author:

Dr Kirsty G Pringle

Hunter Medical Research Institute

Lot 1 Kookaburra Circuit, New Lambton

NSW, Australia 2308

kirsty.pringle@newcastle.edu.au

Abstract

Tissue renin-angiotensin systems (RASs) are involved in tissue growth and development as they are important regulators of angiogenesis, cell proliferation and migration. The placental RAS is most highly expressed in early gestation, at a time when the oxygen tension within the conceptus is reduced, and plays a key role in placental growth and development. Similar to the placenta, tumour development relies on proliferation, angiogenesis and invasion in order to grow and metastasize. The RAS is known to be upregulated in a variety of solid tumours, including ovarian, endometrial, cervical, breast and prostate. This review explores the roles of oxygen and microRNAs in regulating the normal expression of the placental RAS providing insight into regulation of its development as well as the development of disease states in which the RAS is overexpressed. We propose that the placental RAS is downregulated by microRNAs that are suppressed during the physiologically normal 'hypoxic' phase of early placentation. Suppression of these miRNAs allows the placental RAS to stimulate placental growth and angiogenesis. We propose that similar mechanisms may be at play in solid tumours, which are characterised by hypoxia.

Introduction

The renin-angiotensin system (RAS) is a circulating endocrine system that controls blood pressure and salt and water homeostasis. However tissue renin-angiotensin systems also exist; many tissues express some or all components of the RAS and these systems can act independently of the circulating RAS. Tissue RASs play key roles in both

physiological and pathological tissue growth, invasion and angiogenesis [1]. Exploration of the key factors regulating the normal and abnormal expression of these tissues RASs could provide novel insight into both the normal physiological development and regeneration of tissues such as the placenta, as well as the pathogenesis of disease states in which the RAS is over expressed, such as cancer.

The RAS plays a critical role in placental development. It is most highly expressed in early gestation placentae [2] and expression is altered in pregnancies compromised by placental insufficiency [3]. Inadequate placentation causes placental insufficiency, and contributes to pregnancy complications such as preeclampsia, intrauterine growth restriction (IUGR) and spontaneous abortion [4-6]; major causes of fetal and maternal morbidity and mortality.

Like the placenta, tumour growth depends on cell proliferation, angiogenesis and invasion in order to grow and metastasize. The RAS is upregulated in many cancers including ovarian, endometrial, cervical, breast and prostate and most of this research focuses only on Angiotensin II (Ang II)/Ang II type 1 receptor (AT_1R) signaling mechanisms [7-11]. The potential roles of tissue RAS pathways in tumour development has been reviewed by George *et al.* [12].

The shared attributes of tumour and placental development have been reviewed by Holtan *et al.* [13]. The highly proliferative, angiogenic and invasive capacity of tumour cells are the result of regulatory mechanisms that promote autocrine regulation of growth, evasion of apoptosis, sustained angiogenesis, vascular mimicry and tissue invasion which all occur in trophoblast cells [13]. Furthermore, mechanisms regulating

62 immune evasion are shared between tumour and placental development, highlighting the
63 potential similarities between cancer and placental biology.

64 This review examines the regulation of the placental RAS by oxygen and miRNAs as
65 well as in other tissues where the RAS is known to be important for growth and
66 vascularisation. We also describe the potential roles for these pathways in regulating the
67 RAS in tumours.

68

69 ***Tissue renin-angiotensin systems***

70 In many tissues, prorenin, the inactive precursor to renin, is produced; it can be
71 activated by proteases [14] and by exposure to low pH or low temperature [15]. As well,
72 non-proteolytic activation of prorenin occurs when prorenin binds to the (pro)renin
73 receptor ((P)RR) also known as ATPase H(+)-transporting lysosomal accessory protein 2
74 (ATP6AP2)). This leads to a conformational change in prorenin so that it can now cleave
75 angiotensin I (Ang I) from angiotensinogen (AGT). Ang I is cleaved by angiotensin
76 converting enzyme (ACE) to Ang II, which exerts most of the known actions of the RAS by
77 binding to one of two receptors, angiotensin II type 1 and type 2 receptor (AT_1R and AT_2R)
78 (Fig. 1) [1]. In rodents, AT_1R exists in two forms (AT1a and AT1b, [16]). In tissues, the Ang
79 II/AT_1R interaction promotes proliferation, angiogenesis, migration, invasion and fibrosis
80 [17-20]. In addition, Ang II/AT_1R can stimulate intracellular signaling pathways including
81 mitogen activated protein kinase (MAPK)/extracellular signal-related kinase (ERK) and
82 p85α-phosphoinositol 3-kinase (p85α-PI3K), which promote growth and vascularization
83 in tissues [18]. AT_1R is also pro-inflammatory and can increase the expression of pro-

inflammatory mediators such as NF-κB, IL-6, IL-10 and TNF-α [21]. Ang II acting via the AT_2R mediates effects that are predominantly antagonistic to those of Ang II acting via the AT_1R; these include vasodilation and apoptosis (Fig. 1).

Additional RAS pathways exist. One pathway has actions that are opposite to the Ang II/AT_1R pathway. This pathway includes a homologue of ACE, ACE2, which cleaves a single amino acid from both Ang I and Ang II, forming Ang-(1-9) and Ang-(1-7), respectively [22]. Ang-(1-9) is subsequently cleaved by ACE to form Ang-(1-7). Ang-(1-7) acting on the Mas receptor has actions that antagonize the Ang II/AT_1R pathway, similar to Ang II/AT_2R. Another pathway involves the processing of Ang II by aminopeptidase A (APA) to produce either Ang III or Ang IV. Ang IV acting via the AT_4R, also known as insulin regulated amino peptidase (IRAP), induces vascularisation, inflammation, vasodilatation and hypertrophy (Fig. 1) [23].

The (P)RR contributes to proliferation, invasion, fibrosis and angiogenesis by stimulating intracellular signaling independent of Ang II production [24-28]. Additionally, (P)RR is the m8.9 segment of V-ATPase and is required for V-ATPase activity. The acidic extracellular environment created by H^+ secretion by this V-ATPase/(P)RR complex activates proteases (such as cathepsins) and matrix metalloproteases (MMPs), thus enhancing cellular invasion of adjacent tissues and stimulating angiogenesis [29]. The (P)RR/V-ATPase also activates the canonical Wnt/β-catenin signaling pathway which targets a multitude of genes also involved in proliferation, angiogenesis and invasion [30, 31]. Thus, tissue RASs play a role in tissue growth and remodeling.

106 ***Placental RAS Expression Across Gestation***

107 Expression of the placental RAS changes throughout gestation (Table 1, [32-36]).
108 The first trimester placenta has very high levels of expression of prorenin (*REN*), (P)RR
109 (*ATP6AP2*), *AGT* and *AGTR1* compared with term placentae. Placental Ang II acting via the
110 AT_1R has been shown to promote placental development. Ang II regulates
111 cytotrophoblast differentiation *in vitro,* promotes cellular outgrowth (proliferation into
112 cell columns) of human villous explant cultures [37], and promotes placental angiogenesis
113 through vascular endothelial growth factor (VEGF) production [38].

114 Thus Ang II has been shown to have a number of actions that promote placental
115 growth and development. There may also be a role for the (P)RR possibly activated by the
116 extremely high levels of prorenin occurring early in gestation. The spatio-temporal
117 pattern of the placental RAS, with high levels of prorenin and (P)RR co-localized to the
118 villous cytotrophoblast and extravillous trophoblast in early gestation suggest
119 paracrine/autocrine roles in placental growth (Table 1). The mechanisms responsible for
120 the high levels of expression of the placental RAS in early gestation however, have not
121 been fully elucidated.

122

123 ***Oxygen Regulation of Tissue RAS Expression***

124 *Oxygen Regulation of Placental RAS*

125 An important factor influencing placental growth and development is the oxygen
126 tension within the conceptus. During the first trimester, when RAS expression is the
127 highest [33], the placenta develops in a low oxygen environment [39]. This occurs because

from about two weeks after implantation, extravillous trophoblast (EVT) cells proliferate and invade the maternal decidua and form trophoblastic plugs within the maternal spiral arterioles, preventing maternal blood flow to the conceptus [40]. At 8 weeks the oxygen tension within the intervillous space is 17.9 mmHg (~2.5%) with a range of 5-30 mmHg (~0.7-4.3%) while the oxygen tension in the decidua is higher at 39.6 mmHg (~5.7%) with a range of 25-70 mmHg (~3.5-10%) [39]. This low oxygen environment stimulates proliferation and angiogenesis [41, 42]. Insufficient plugging of the maternal arterioles, resulting in early onset of maternal blood flow and early re-oxygenation of the placenta, results in the production of reactive oxygen species (ROS) [43] which are thought to cause abnormal placental development. Hypoxia is known to stabilize hypoxia inducible factors (HIFs), which promote angiogenesis and cell proliferation by stimulating the production of VEGF and angiopoietins. Ang II acting via the AT_1R also stabilizes HIFs and stimulates VEGF and angiopoietin expression [44, 45]. Thus a low oxygen environment is therefore critical for early placentation.

Alternatively, poor remodeling of the spiral arterioles in preeclampsia leads to ischemia-reperfusion events within placental lobules. These ischemia-reperfusion events lead to upregulation of the placental RAS later in gestation [46, 47] and are thought to be more detrimental in the pathogenesis of preeclampsia than the global ischemia induced in animal models [48].

The interaction between a low oxygen environment and the placental RAS has only recently been investigated. In a recent study on women at high altitude, placental levels of prorenin, (P)RR, AT_1R and AT_2R proteins were significantly higher in placentae

from normotensive women at altitude, despite their mRNA levels being unaffected [49]. In mice, maternal hypoxia in mid to late term pregnancy leads to decreased AGT, ACE, and ACE2 levels within the placenta and increased AT_1R expression [50]. Further studies demonstrated that mid to late term maternal hypoxia led to increased placental vascular density indicating that alterations in placental RAS expression may influence the placental vasculature [51]. In models of reduced utero-placental perfusion (RUPP) in rats and maternal hypoxia in mice, there were significant alterations in placental RAS expression indicating that the RAS may be one pathway by which the placenta adapts to compensate for inefficient placental perfusion [47, 52]. Similarly, models of placental insufficiency in sheep were associated with reduced expression of *AGT* and *REN* in late gestation fetal sheep kidneys. Interestingly, renal renin mRNA abundance was positively correlated to arterial pO_2 [53].

Incubation of HTR-8/SVneo cells (an immortalized primary trophoblast cell line) in low oxygen (1% O_2) increased *AGTR1* and *VEGF* expression, as well as ACE and VEGF protein levels, suggesting that there was activation of the pro-angiogenic RAS pathway [54]. Ang II treatment of first trimester explants (7-9 weeks) has also been shown to mimic the effects of low oxygen. Culture of explants in 3% O_2 promoted trophoblast outgrowth by stimulating trophoblast proliferation (as indicated by an increase in Ki67 staining) and inhibited trophoblast differentiation into an invasive phenotype, which was mimicked by Ang II treatment [37]. Placental explants incubated in a low oxygen environment or with Ang II remained positive for integrin α5, had increased matrix-metalloprotease-2 (MMP-2) activity and increased plasminogen activator inhibitor-1 (PAI-1) expression [37]. This

research highlights a potential role for the placental RAS in mediating the pro-angiogenic effects of low oxygen in placental development [54].

Oxygen Regulation of Tissue RAS

In other tissues compromised by hypoxic/ischaemic episodes such as the heart and kidney, losartan (an AT_1R antagonist) has been shown to reduce the deleterious effects of ischaemic injury. Local upregulation of the RAS in response to hypoxic events is deleterious, potentially because it causes increased inflammatory and/or pro-fibrotic activity through AT_1R signaling [55, 56]. Rats exposed to altered oxygen levels display altered renal, pulmonary and pancreatic RAS expression. Chronic intermittent hypoxia in rats (6-8% O_2) increased renal and pulmonary arterial ACE expression, decreased renal arteriole ACE2 and increased pulmonary arteriole ACE2 [57]. Furthermore, kidney and pulmonary Ang II was increased while Ang-(1-7) levels were decreased, demonstrating that oxygen altered the balance between the pro-inflammatory and anti-inflammatory RAS axes. These changes were also associated with increases in arterial thickness indicating that hypoxia driven dysregulation of tissue RASs may be driving vascular remodeling and disease severity [57]. Chronic hypoxia in rats is also associated with increased pancreatic AGT, AT_1b and AT_2R [58]. The pancreatic RAS is thought to regulate insulin release [59], thus in diseases such as diabetes alterations in pancreatic RAS may exacerbate disease. Therefore, there is reasonable evidence that oxygen regulates tissue RASs in both physiological and pathophysiological states, including placentation and

chronic kidney disease however its role in regulating the RAS in tumours is not well defined.

Does oxygen regulate the RAS in cancer?

While hypoxia in solid tumours is known to drive tumour proliferation and angiogenesis, only one study has investigated the role of hypoxia in RAS-mediated tumour development. Fan *et al.* showed that hypoxia caused upregulation of Ang II levels [60], ACE and AT_1R levels, in murine Lewis lung carcinoma cells, similar to that seen in the placenta [54], and decreased ACE2 levels. However, g Given the evidence that hypoxia regulates the expression of the RAS in the placenta, lung and heart [54, 61, 62], it may also play an important role in regulating the expression of the RAS in solid tumours. This requires further investigation.

Regulation of the placental RAS by MicroRNAs

MicroRNAs are small non-coding nucleotides that bind to specific sites in the 3'untranslated region (UTR) of mRNA, destabilizing the mRNA or impeding its translation, so that less encoded protein is synthesized. MicroRNAs are known to be important in both physiological and pathological regulation of molecular and hence cellular function. Although there are very few studies examining the role of miRNAs in regulating the placental RAS, miRNAs have been shown to be important in trophoblast invasion and as potential modulators of maternal immune tolerance [63, 64]. Goyal *et al.* demonstrated that several miRNAs that are predicted to target *REN*, *ACE* and AT_1R (miR-199b, miR-27a

and miR-468, respectively), are down regulated in the mouse placenta in response to maternal hypoxia and are associated with altered post-transcriptional regulation of their RAS targets [52]. Further studies have shown similar post-transciptional regulation of fetal brain RAS mRNAs following maternal protein restriction [65]. Goyal *et al.* demonstrated that a maternal low protein diet increased expression of *AGT* and *ACE1* mRNA and decreased AT_2R mRNA expression. AGT protein levels were unchanged while both ACE1 and AT_2R proteins were significantly reduced in fetal brains from protein restricted dams. These changes were associated with altered expression of miRNAs known to target both ACE1 and AT_2R (mir-27a and b and mir-330, respectively) [65].

One study has shown that over expression of miR-155 significantly reduced AT_1R protein expression in human vascular adventitial fibroblasts [66]. miR-155 is was significantly lower in human umbilical vein endothelial cells (HUVECs) from women with preeclampsia [67] which is characterised by increased placental expression of AT_1R [46]. Since both miRNAs and the RAS regulate placental development and may regulate the maternal response to pregnancy, it is imperative that further research be focused on the interaction between them. Placental miRNA profiles are significantly altered with both gestational age and pregnancy complications including preeclampsia [68-72] and we are currently exploring the potential role of miRNAs in regulating the placental RAS in these settings.

Do miRNAs and oxygen act together to regulate the placental RAS?

As outlined above, the placenta contains its own RAS that is expressed from at least 7 weeks gestation [2, 32, 34, 73-75], at a time when the oxygen tension within the placenta is at its lowest [39]. There is evidence that the low oxygen milieu stimulates the expression of some components of the placental RAS [54]. Ang II, acting through the AT_1R stimulates placental angiogenesis and trophoblast invasion [34, 74, 76] and may be mediating some of the effects of low oxygen. Thus regulation of the RAS by oxygen with its downstream effects are likely to be important in placental development.

Conversely, expression of some microRNAs that target key genes of the RAS are suppressed by hypoxia, leading to increased protein expression [52, 77]. MicroRNAs that target the placental RAS could therefore mediate the effects of low oxygen on RAS expression. In this way, oxygen and miRNAs may work together to alter placental development and might contribute to the abnormal placentation characteristic of preeclampsia and IUGR.

We propose that the placental RAS is regulated by microRNAs that are normally suppressed during the physiologically normal 'hypoxic' phase of early placentation. Suppression of these miRNAs stabilizes RAS components allowing the Ang II/AT_1R pathway to stimulate expression of genes controlling angiogenesis as well as trophoblast growth, invasion and transformation to an endovascular phenotype (Figure 2, [78]). If placentation is poor, as in IUGR and preeclampsia, and there is reduced uteroplacental perfusion, then there will be abnormal expression of the placental RAS and the microRNAs that target the RAS in late gestation (Figure 2).

Regulation of Tissue RASs by MicroRNAs

Chronic kidney diseases including hypertension and glomerulosclerosis which are associated with increased intrarenal renin mRNA, are shown to express significantly lower levels of miR-663 [79, 80]. Marques *et al.* found increased renal cortical expression of *REN* mRNA was associated with a downregulation of miR-663 and miR-181a. Furthermore, they found both miR-181a and miR-663 directly bind to 3'UTR constructs of *REN* [79]. Ramezani *et al.* also showed urinary miR-663 was downregulated in patients with focal segmental glomerulosclerosis (FSGS) when compared to patients with minimal change disease (MCD) [80]. Urinary miR-155, known to target AT_1R in human vascular adventitial fibroblasts [66], was also was significantly downregulated. Glomerulosclerosis is associated with increased both plasma renin and Ang II as well as increase renal ACE and AT_1R [81]. Interestingly, miRNA expression levels also correlated with clinical parameters including proteinuria and glomerular filtration rate. miR-663 inversely correlated with GFR and positively correlated with proteinuria whereas miR-155 positively correlated with proteinuria [80]. Thus miRNAs that target the RAS may play a role in the pathogenesis of renal disease and may provide insight into the regulation of the placental RAS by miRNAs in physiological and pathophysiological states.

Regulation of the RAS by microRNAs in cancer

There is an increasing body of evidence implicating a role for miRNAs in tumourigenesis [82, 83], however there are very few studies exploring the relationship between miRNAs and the RAS in cancer. miR-155 is one of the miRNAs most consistently

involved in neoplastic disease, being overexpressed in several solid tumours [84, 85] and as mentioned above, is known to repress *AGTR1* expression [66]. In endometrial carcinoma cells, abolishing the function of miR-155 or AGTR1 inhibited cell survival and the combined treatment showed synergistic effects [86]. This data is somewhat conflicting since inhibition of both miR-155 or AGTR1 would suggest that both miR-155 and AGTR1 overexpression were contributing to tumourigenesis but miR-155 is a negative regulator of AGTR1. Furthermore a more recent study has demonstrated that miR-410 suppresses pancreatic cancer growth, cell invasion, migration, and angiogenesis via the downregulation of *AGTR1*, acting as a tumor-suppressive miRNA [86]. Thus it appears that miRNAs and may be involved in regulating tissue RAS and that this could contribute to cancer progression, however further investigation is required.

Conclusion

Current research shows that tissue RASs play essential roles in promoting tissue growth, angiogenesis, migration, invasion and fibrosis, all important features of both placental and cancer growth. We don't fully understand how the activity and expression of these various RASs are regulated. There is good evidence that factors such as oxygen tension and miRNAs regulate the expression of tissue RASs such as those within the placenta as well as those in the kidney, heart, lung and pancreas. We hypothesise that oxygen and miRNAs act to together to regulate the placental RAS and that similar mechanisms might be at play in the regulation of the RAS in tumours. Since the RAS is overexpressed in many cancers [12], identifying these molecular pathways that regulate

302 the placenta RAS may allow us to gain further insight into the mechanisms of cancer onset
303 and progression.
304
305 ***Acknowledgements***
306 The authors would like to acknowledge project grant funding from the NHMRC to ERL
307 (GNT1043537). KGP is supported by an ARC Future Fellowship (FT150100179).
308

309 ***References***

310 [1] M. Paul, A. Poyan Mehr, R. Kreutz, Physiology of Local Renin-Angiotensin Systems,
311 Physiological Reviews 86(3) (2006) 747-803.
312 [2] K.G. Pringle, M.A. Tadros, R.J. Callister, E.R. Lumbers, The expression and localization
313 of the human placental prorenin/renin-angiotensin system throughout pregnancy: roles
314 in trophoblast invasion and angiogenesis?, Placenta 32(12) (2011) 956-62.
315 [3] L. Anton, K.B. Brosnihan, Systemic and uteroplacental renin--angiotensin system in
316 normal and pre-eclamptic pregnancies, Therapeutic advances in cardiovascular disease
317 2(5) (2008) 349-362.
318 [4] C.J. de Groot, T.J. O'Brien, R.N. Taylor, Biochemical evidence of impaired
319 trophoblastic invasion of decidual stroma in women destined to have preeclampsia,
320 American journal of obstetrics and gynecology 175(1) (1996) 24-9.
321 [5] R.B. Ness, B.M. Sibai, Shared and disparate components of the pathophysiologies of
322 fetal growth restriction and preeclampsia, American journal of obstetrics and
323 gynecology 195(1) (2006) 40-49.
324 [6] G.J. Burton, E. Jauniaux, Placental oxidative stress: from miscarriage to preeclampsia,
325 Journal of the Society for Gynecologic Investigation 11(6) (2004) 342-52.
326 [7] K. Shibata, F. Kikkawa, Y. Mizokami, H. Kajiyama, K. Ino, S. Nomura, S. Mizutani,
327 Possible involvement of adipocyte-derived leucine aminopeptidase via angiotensin II in
328 endometrial carcinoma, Tumor Biology 26(1) (2005) 9-16.
329 [8] T. Suganuma, K. Ino, K. Shibata, H. Kajiyama, T. Nagasaka, S. Mizutani, F. Kikkawa,
330 Functional Expression of the Angiotensin II Type1 Receptor in Human Ovarian
331 Carcinoma Cells and Its Blockade Therapy Resulting in Suppression of Tumor Invasion,
332 Angiogenesis, and Peritoneal Dissemination, Clinical Cancer Research 11(7) (2005) 2686-
333 2694.
334 [9] B. De Paepe, V.L. Verstraeten, C.R. De Potter, L.A. Vakaet, G.R. Bullock, Growth
335 stimulatory angiotensin II type-1 receptor is upregulated in breast hyperplasia and in
336 situ carcinoma but not in invasive carcinoma, Histochemistry and cell biology 116(3)
337 (2001) 247-254.

338 [10] F. Kikkawa, M. Mizuno, K. Shibata, H. Kajiyama, T. Morita, K. Ino, S. Nomura, S.
339 Mizutani, Activation of invasiveness of cervical carcinoma cells by angiotensin II,
340 American journal of obstetrics and gynecology 190(5) (2004) 1258-1263.
341 [11] H. Uemura, H. Hasumi, H. Ishiguro, J. Teranishi, Y. Miyoshi, Y. Kubota, Renin-
342 angiotensin system is an important factor in hormone refractory prostate cancer,
343 Prostate 66(8) (2006) 822-30.
344 [12] A.J. George, W.G. Thomas, R.D. Hannan, The renin–angiotensin system and cancer:
345 old dog, new tricks, Nature Reviews Cancer 10(11) (2010) 745-759.
346 [13] S.G. Holtan, D.J. Creedon, P. Haluska, S.N. Markovic, Cancer and Pregnancy:
347 Parallels in Growth, Invasion, and Immune Modulation and Implications for Cancer
348 Therapeutic Agents, Mayo Clinic Proceedings 84(11) (2009) 985-1000.
349 [14] B.J. Morris, Activation of human inactive ("pro-") renin by cathepsin D and pepsin, J
350 Clin Endocrinol Metab 46(1) (1978) 153-7.
351 [15] E.R. Lumbers, Activation of renin in human amniotic fluid by low pH, Enzymologia
352 40(6) (1971) 329-36.
353 [16] N. Iwai, T. Inagami, Identification of two subtypes in the rat type I angiotensin II
354 receptor, FEBS letters 298(2-3) (1992) 257-60.
355 [17] P. Pan, H. Fu, L. Zhang, H. Huang, F. Luo, W. Wu, Y. Guo, X. Liu, Angiotensin II
356 upregulates the expression of placental growth factor in human vascular endothelial
357 cells and smooth muscle cells, BMC Cell Biology 11(1) (2010) 36.
358 [18] M. de Gasparo, K.J. Catt, T. Inagami, J.W. Wright, T. Unger, International union of
359 pharmacology. XXIII. The angiotensin II receptors, Pharmacological reviews 52(3) (2000)
360 415-72.
361 [19] E. Napoleone, A. Cutrone, D. Cugino, C. Amore, A. Di Santo, L. Iacoviello, G. de
362 Gaetano, M.B. Donati, R. Lorenzet, Inhibition of the renin-angiotensin system
363 downregulates tissue factor and vascular endothelial growth factor in human breast
364 carcinoma cells, Thrombosis research 129(6) (2012) 736-42.

[20] M. Okazaki, S. Fushida, S. Harada, T. Tsukada, J. Kinoshita, K. Oyama, H. Tajima, I. Ninomiya, T. Fujimura, T. Ohta, The angiotensin II type 1 receptor blocker candesartan suppresses proliferation and fibrosis in gastric cancer, Cancer Lett 355(1) (2014) 46-53.
[21] X. Wang, M. Khaidakov, Z. Ding, S. Mitra, J. Lu, S. Liu, J.L. Mehta, Cross-talk between inflammation and angiotensin II: studies based on direct transfection of cardiomyocytes with AT1R and AT2R cDNA, Experimental biology and medicine (Maywood, N.J.) 237(12) (2012) 1394-401.
[22] S.R. Tipnis, N.M. Hooper, R. Hyde, E. Karran, G. Christie, A.J. Turner, A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase, The Journal of biological chemistry 275(43) (2000) 33238-43.
[23] M. Donoghue, F. Hsieh, E. Baronas, K. Godbout, M. Gosselin, N. Stagliano, M. Donovan, B. Woolf, K. Robison, R. Jeyaseelan, R.E. Breitbart, S. Acton, A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9, Circulation research 87(5) (2000) E1-9.
[24] A. Advani, D.J. Kelly, A.J. Cox, K.E. White, S.L. Advani, K. Thai, K.A. Connelly, D. Yuen, J. Trogadis, A.M. Herzenberg, M.A. Kuliszewski, H. Leong-Poi, R.E. Gilbert, The (Pro)renin receptor: site-specific and functional linkage to the vacuolar H+-ATPase in the kidney, Hypertension 54(2) (2009) 261-9.
[25] N. Koklanaris, J.C. Nwachukwu, S.J. Huang, S. Guller, K. Karpisheva, M. Garabedian, M.J. Lee, First-trimester trophoblast cell model gene response to hypoxia, American journal of obstetrics and gynecology 194(3) (2006) 687-93.
[26] Y. Huang, N.A. Noble, J. Zhang, C. Xu, W.A. Border, Renin-stimulated TGF-beta1 expression is regulated by a mitogen-activated protein kinase in mesangial cells, Kidney Int 72(1) (2007) 45-52.
[27] G. Nguyen, F. Delarue, C. Burckle, L. Bouzhir, T. Giller, J.D. Sraer, Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin, J Clin Invest 109(11) (2002) 1417-27.

393 [28] J.H. Schefe, T. Unger, H. Funke-Kaiser, PLZF and the (pro)renin receptor, Journal of
394 molecular medicine (Berlin, Germany) 86(6) (2008) 623-7.
395 [29] A. Ichihara, (Pro)renin receptor and vacuolar H(+)-ATPase, The Keio journal of
396 medicine 61(3) (2012) 73-8.
397 [30] G. Nguyen, D.N. Muller, The Biology of the (Pro)Renin Receptor, Journal of the
398 American Society of Nephrology 21(1) (2010) 18-23.
399 [31] Y. Shibayama, T. Fujimori, G. Nguyen, T. Hirose, K. Totsune, A. Ichihara, K. Kitada, D.
400 Nakano, H. Kobori, M. Kohno, T. Masaki, Y. Suzuki, S. Yachida, A. Nishiyama, (Pro)renin
401 receptor is crucial for Wnt/beta-catenin-dependent genesis of pancreatic ductal
402 adenocarcinoma, Scientific reports 5 (2015) 8854.
403 [32] F.Z. Marques, K.G. Pringle, A. Conquest, J.J. Hirst, M.A. Markus, M. Sarris, T. Zakar,
404 B.J. Morris, E.R. Lumbers, Molecular characterization of renin-angiotensin system
405 components in human intrauterine tissues and fetal membranes from vaginal delivery
406 and cesarean section, Placenta 32(3) (2011) 214-21.
407 [33] K.G. Pringle, M. Tadros, R. Callister, E.R. Lumbers, The expression and localization of
408 the human placental prorenin/renin-angiotensin system throughout pregnancy: roles in
409 trophoblast invasion and angiogenesis?, Placenta 32(12) (2011) 956-62.
410 [34] A.C. Cooper, G. Robinson, G.P. Vinson, W.T. Cheung, F. Broughton Pipkin, The
411 localization and expression of the renin-angiotensin system in the human placenta
412 throughout pregnancy, Placenta 20(5-6) (1999) 467-74.
413 [35] P.J. Williams, H.D. Mistry, B.A. Innes, J.N. Bulmer, F. Broughton Pipkin, Expression of
414 AT1R, AT2R and AT4R and their roles in extravillous trophoblast invasion in the human,
415 Placenta 31(5) (2010) 448-55.
416 [36] T. Morgan, C. Craven, L. Nelson, J.M. Lalouel, K. Ward, Angiotensinogen T235
417 expression is elevated in decidual spiral arteries, The Journal of clinical investigation
418 100(6) (1997) 1406-1415.
419 [37] M. Araki-Taguchi, S. Nomura, K. Ino, S. Sumigama, E. Yamamoto, T. Kotani-Ito, H.
420 Hayakawa, H. Kajiyama, K. Shibata, A. Itakura, F. Kikkawa, Angiotensin II mimics the

421 hypoxic effect on regulating trophoblast proliferation and differentiation in human
422 placental explant cultures, Life sciences 82(1-2) (2008) 59-67.
423 [38] Q. Zhao, M. Ishibashi, K. Hiasa, C. Tan, A. Takeshita, K. Egashira, Essential role of
424 vascular endothelial growth factor in angiotensin II-induced vascular inflammation and
425 remodeling, Hypertension 44(3) (2004) 264 - 270.
426 [39] F. Rodesch, P. Simon, C. Donner, E. Jauniaux, Oxygen measurements in endometrial
427 and trophoblastic tissues during early pregnancy, Obstetrics and gynecology 80(2)
428 (1992) 283-5.
429 [40] B. Huppertz, L.L. Peeters, Vascular biology in implantation and placentation,
430 Angiogenesis 8(2) (2005) 157-167.
431 [41] O. Genbacev, R. Joslin, C.H. Damsky, B.M. Polliotti, S.J. Fisher, Hypoxia alters early
432 gestation human cytotrophoblast differentiation/invasion in vitro and models the
433 placental defects that occur in preeclampsia, J Clin Invest 97(2) (1996) 540-50.
434 [42] D.S. Charnock-Jones, P. Kaufmann, T.M. Mayhew, Aspects of Human Fetoplacental
435 Vasculogenesis and Angiogenesis. I. Molecular Regulation, Placenta 25(2–3) (2004) 103-
436 113.
437 [43] F. Wu, F.J. Tian, Y. Lin, Oxidative Stress in Placenta: Health and Diseases, BioMed
438 research international 2015 (2015) 293271.
439 [44] C. Liu, J.W. Zhang, L. Hu, Y.C. Song, Activation of the AT1R/HIF-1 alpha /ACE axis
440 mediates angiotensin II-induced VEGF synthesis in mesenchymal stem cells, 2014 (2014)
441 627380.
442 [45] T. Tsuzuki, H. Okada, H. Cho, K. Shimoi, H. Miyashiro, K. Yasuda, H. Kanzaki,
443 Divergent regulation of angiopoietin-1, angiopoietin-2, and vascular endothelial growth
444 factor by hypoxia and female sex steroids in human endometrial stromal cells, European
445 Journal of Obstetrics & Gynecology and Reproductive Biology 168(1) (2013) 95-101.
446 [46] H.D. Mistry, L.O. Kurlak, F. Broughton Pipkin, The placental renin–angiotensin
447 system and oxidative stress in pre-eclampsia, Placenta 34(2) (2013) 182-186.
448 [47] R. Goyal, S.M. Yellon, L.D. Longo, E. Mata-Greenwood, Placental Gene Expression in
449 a Rat 'Model' of Placental Insufficiency, Placenta 31(7) (2010) 568-575.

[48] T.H. Hung, G.J. Burton, Hypoxia and reoxygenation: a possible mechanism for placental oxidative stress in preeclampsia, Taiwanese journal of obstetrics & gynecology 45(3) (2006) 189-200.
[49] L.O. Kurlak, H.D. Mistry, T. Cindrova-Davies, G.J. Burton, F. Broughton Pipkin, Human placental renin-angiotensin system in normotensive and pre-eclamptic pregnancies at high altitude and after acute hypoxia-reoxygenation insult, J Physiol 594(5) (2016) 1327-40.
[50] J.S. Cuffe, S.L. Walton, S.E. Steane, R.R. Singh, D.G. Simmons, K.M. Moritz, The effects of gestational age and maternal hypoxia on the placental renin angiotensin system in the mouse, Placenta 35(11) (2014) 953-61.
[51] J.S.M. Cuffe, S.L. Walton, R.R. Singh, J.G. Spiers, H. Bielefeldt-Ohmann, L. Wilkinson, M.H. Little, K.M. Moritz, Mid- to late term hypoxia in the mouse alters placental morphology, glucocorticoid regulatory pathways and nutrient transporters in a sex-specific manner, The Journal of Physiology 592(Pt 14) (2014) 3127-3141.
[52] R. Goyal, R. Lister, A. Leitzke, D. Goyal, C.P. Gheorghe, L.D. Longo, Antenatal maternal hypoxic stress: adaptations of the placental renin-angiotensin system in the mouse, Placenta 32(2) (2011) 134-9.
[53] D.Y. Zhang, E.R. Lumbers, G. Simonetta, J.J. Wu, J.A. Owens, J.S. Robinson, I.C. McMillen, Effects of placental insufficiency on the ovine fetal renin-angiotensin system, Exp Physiol 85(1) (2000) 79-84.
[54] S.J. Delforce, Y. Wang, M.E. Van-Aalst, C. Corbisier de Meaultsart, B.J. Morris, F. Broughton-Pipkin, C.T. Roberts, E.R. Lumbers, K.G. Pringle, Effect of oxygen on the expression of renin–angiotensin system components in a human trophoblast cell line, Placenta 37 (2016) 1-6.
[55] Y.M.D. Paz, J.M.D. Gurevitch, I.M.D. Frolkis, PhD, M.M.D. Matsa, A.M.D. Kramer, C.M.D. Locker, R.M.D. Mohr, G.M.D. Keren, Effects of an Angiotensin II Antagonist on Ischemic and Nonischemic Isolated Rat Hearts, The Annals of Thoracic Surgery 65(2) 474-479.

[56] Z. Miloradovic, M. Jerkic, D. Jovovic, N. Mihailovic-Stanojevic, J.G. Milanovic, G. Stosic, J. Markovic-Lipkovski, Bosentan and losartan ameliorate acute renal failure associated with mild but not strong NO blockade, Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 22(9) (2007) 2476-84.

[57] Y.H. Xiang, X.L. Su, R.X. He, C.P. Hu, Effects of different patterns of hypoxia on renin angiotension system in serum and tissues of rats, Zhonghua jie he he hu xi za zhi = Zhonghua jiehe he huxi zazhi = Chinese journal of tuberculosis and respiratory diseases 35(1) (2012) 33-6.

[58] W. Pong Chan, M. Lung Fung, R. Nobiling, P. Sing Leung, Activation of local renin-angiotensin system by chronic hypoxia in rat pancreas, Molecular and cellular endocrinology 160(1–2) (2000) 107-114.

[59] P.O. Carlsson, C. Berne, L. Jansson, Angiotensin II and the endocrine pancreas: effects on islet blood flow and insulin secretion in rats, Diabetologia 41(2) (1998) 127-33.

[60] L. Fan, Y. Feng, H.Y. Wan, L. Ni, Y.R. Qian, Y. Guo, Y. Xiang, Q.Y. Li, Hypoxia induces dysregulation of local renin-angiotensin system in mouse Lewis lung carcinoma cells, Genetics and molecular research : GMR 13(4) (2014) 10562-73.

[61] R.C. Passier, J.F. Smits, M.J. Verluyten, M.J. Daemen, Expression and localization of renin and angiotensinogen in rat heart after myocardial infarction, The American journal of physiology 271(3 Pt 2) (1996) H1040-8.

[62] V. Hampl, J. Herget, J. Bibova, A. Banasova, Z. Huskova, Z. Vanourkova, S. Jichova, P. Kujal, Z. Vernerova, J. Sadowski, L. Cervenka, Intrapulmonary activation of the angiotensin-converting enzyme type 2/angiotensin 1-7/G-protein-coupled Mas receptor axis attenuates pulmonary hypertension in Ren-2 transgenic rats exposed to chronic hypoxia, Physiological research 64(1) (2015) 25-38.

[63] M.T. Su, P.Y. Tsai, H.L. Tsai, Y.C. Chen, P.L. Kuo, miR-346 and miR-582-3p-regulated EG-VEGF expression and trophoblast invasion via matrix metalloproteinases 2 and 9, BioFactors (Oxford, England) (2016).

507 [64] A. Mori, H. Nishi, T. Sasaki, Y. Nagamitsu, R. Kawaguchi, A. Okamoto, M. Kuroda, K.
508 Isaka, HLA-G expression is regulated by miR-365 in trophoblasts under hypoxic
509 conditions, Placenta 45 (2016) 37-41.
510 [65] R. Goyal, D. Goyal, A. Leitzke, C.P. Gheorghe, L.D. Longo, Brain Renin-Angiotensin
511 System: Fetal Epigenetic Programming by Maternal Protein Restriction During
512 Pregnancy, Reproductive Sciences 17(3) (2010) 227-238.
513 [66] L. Zheng, C.-C. Xu, W.-D. Chen, W.-L. Shen, C.-C. Ruan, L.-M. Zhu, D.-L. Zhu, P.-J.
514 Gao, MicroRNA-155 regulates angiotensin II type 1 receptor expression and phenotypic
515 differentiation in vascular adventitial fibroblasts, Biochemical and Biophysical Research
516 Communications 400(4) (2010) 483-488.
517 [67] W. Cheng, T. Liu, F. Jiang, C. Liu, X. Zhao, Y. Gao, H. Wang, Z. Liu, microRNA-155
518 regulates angiotensin II type 1 receptor expression in umbilical vein endothelial cells
519 from severely pre-eclamptic pregnant women, International journal of molecular
520 medicine 27(3) (2011) 393-9.
521 [68] D.M. Morales-Prieto, W. Chaiwangyen, S. Ospina-Prieto, U. Schneider, J. Herrmann,
522 B. Gruhn, U.R. Markert, MicroRNA expression profiles of trophoblastic cells, Placenta
523 33(9) (2012) 725-34.
524 [69] B.L. Pineles, R. Romero, D. Montenegro, A.L. Tarca, Y.M. Han, Y.M. Kim, S. Draghici,
525 J. Espinoza, J.P. Kusanovic, P. Mittal, S.S. Hassan, C.J. Kim, Distinct subsets of microRNAs
526 are expressed differentially in the human placentas of patients with preeclampsia,
527 American journal of obstetrics and gynecology 196(3) (2007) 261.e1-6.
528 [70] G. Fu, G. Ye, L. Nadeem, L. Ji, T. Manchanda, Y. Wang, Y. Zhao, J. Qiao, Y.L. Wang, S.
529 Lye, B.B. Yang, C. Peng, MicroRNA-376c impairs transforming growth factor-beta and
530 nodal signaling to promote trophoblast cell proliferation and invasion, Hypertension
531 61(4) (2013) 864-72.
532 [71] L. Luo, G. Ye, L. Nadeem, G. Fu, B.B. Yang, E. Honarparvar, C. Dunk, S. Lye, C. Peng,
533 MicroRNA-378a-5p promotes trophoblast cell survival, migration and invasion by
534 targeting Nodal, J Cell Sci 125(Pt 13) (2012) 3124-32.

[72] A.M. Sheikh, H.Y. Small, G. Currie, C. Delles, Systematic Review of Micro-RNA Expression in Pre-Eclampsia Identifies a Number of Common Pathways Associated with the Disease, PloS one 11(8) (2016) e0160808.
[73] K.G. Pringle, T. Zakar, D. Yates, C.M. Mitchell, J.J. Hirst, E.R. Lumbers, Molecular evidence of a (pro)renin/ (pro)renin receptor system in human intrauterine tissues in pregnancy and its association with PGHS-2, J Renin Angiotensin Aldosterone Syst 12(3) (2011) 304-10.
[74] P.J. Williams, H.D. Mistry, B.A. Innes, J.N. Bulmer, F.B. Pipkin, Expression of AT1R, AT2R and AT4R and their roles in extravillous trophoblast invasion in the human, Placenta 31(5) (2010) 448-55.
[75] T. Morgan, C. Craven, L. Nelson, J.M. Lalouel, K. Ward, Angiotensinogen T235 expression is elevated in decidual spiral arteries, J Clin Invest 100(6) (1997) 1406-15.
[76] L. Hering, F. Herse, N. Geusens, S. Verlohren, K. Wenzel, A.C. Staff, K.B. Brosnihan, B. Huppertz, F.C. Luft, D.N. Muller, R. Pijnenborg, J.E. Cartwright, R. Dechend, Effects of circulating and local uteroplacental angiotensin II in rat pregnancy, Hypertension 56(2) (2010) 311-8.
[77] R. Goyal, A. Leitzke, D. Goyal, C.P. Gheorghe, L.D. Longo, Antenatal maternal hypoxic stress: adaptations in fetal lung Renin-Angiotensin system, Reproductive sciences (Thousand Oaks, Calif.) 18(2) (2011) 180-9.
[78] K.G. Pringle, K.L. Kind, A.N. Sferruzzi-Perri, J.G. Thompson, C.T. Roberts, Beyond oxygen: complex regulation and activity of hypoxia inducible factors in pregnancy, Human reproduction update 16(4) (2010) 415-31.
[79] F.Z. Marques, A.E. Campain, M. Tomaszewski, E. Zukowska-Szczechowska, Y.H. Yang, F.J. Charchar, B.J. Morris, Gene expression profiling reveals renin mRNA overexpression in human hypertensive kidneys and a role for microRNAs, Hypertension 58(6) (2011) 1093-8.
[80] A. Ramezani, J.M. Devaney, S. Cohen, M.R. Wing, R. Scott, S. Knoblach, R. Singhal, L. Howard, J.B. Kopp, D.S. Raj, Circulating and urinary microRNA profile in focal segmental

563 glomerulosclerosis: a pilot study, European Journal of Clinical Investigation 45(4) (2015)
564 394-404.
565 [81] J. Obata, T. Nakamura, H. Takano, A. Naito, H. Kimura, Y. Yoshida, F. Shimizu, D.F.
566 Guo, T. Inagami, Increased gene expression of components of the renin-angiotensin
567 system in glomeruli of genetically hypertensive rats, J Hypertens 18(9) (2000) 1247-55.
568 [82] T. Fan, W. Wang, B. Zhang, Y. Xu, L. Chen, S. Pan, H. Hu, Q. Geng, Regulatory
569 mechanisms of microRNAs in lung cancer stem cells, SpringerPlus 5(1) (2016) 1762.
570 [83] B. D'Angelo, E. Benedetti, A. Cimini, A. Giordano, MicroRNAs: A Puzzling Tool in
571 Cancer Diagnostics and Therapy, Anticancer Res 36(11) (2016) 5571-5575.
572 [84] H. Fang, D. Shuang, Z. Yi, H. Sheng, Y. Liu, Up-regulated microRNA-155 expression is
573 associated with poor prognosis in cervical cancer patients, Biomedicine &
574 pharmacotherapy = Biomedecine & pharmacotherapie 83 (2016) 64-69.
575 [85] Y.Q. Zhang, W.Y. Wang, J.X. Xue, Y. Xu, P. Fan, B.A. Caughey, W.W. Tan, G.Q. Cao,
576 L.L. Jiang, Y. Lu, K. Zhang, X. Hu, MicroRNA Expression Profile on Solid Subtype of
577 Invasive Lung Adenocarcinoma Reveals a Panel of Four miRNAs to Be Associated with
578 Poor Prognosis in Chinese Patients, Journal of Cancer 7(12) (2016) 1610-1620.
579 [86] C.H. Choi, Y. Park, J.-J. Choi, T. Song, S.Y. Song, Y.-Y. Lee, J.-W. Lee, T.-J. Kim, B.-G.
580 Kim, D.-S. Bae, Angiotensin II type I receptor and miR-155 in endometrial cancers:
581 Synergistic antiproliferative effects of anti-miR-155 and losartan on endometrial cancer
582 cells, Gynecologic oncology 126(1) (2012) 124-131.
583 [87] F.Z. Marques, K.G. Pringle, A.L. Conquest, J. Hirst, M.A. Markus, M. Sarris, T. Zakar,
584 B.J. Morris, E.R. Lumbers, Molecular characterization of renin-angiotensin system
585 components in human intrauterine tissues and fetal membranes from vaginal delivery
586 and cesarean section, Placenta 32(3) (2011) 214-221.
587 [88] M. Ito, A. Itakura, Y. Ohno, M. Nomura, T. Senga, T. Nagasaka, S. Mizutani, Possible
588 activation of the renin-angiotensin system in the feto-placental unit in preeclampsia, J
589 Clin Endocrinol Metab 87(4) (2002) 1871-1878.

590 [89] L. Anton, D.C. Merrill, L.A.A. Neves, K. Stovall, P.E. Gallagher, D.I. Diz, C. Moorefield,
591 C. Gruver, C.M. Ferrario, K.B. Brosnihan, Activation of local chorionic villi angiotensin II
592 levels but not angiotensin (1-7) in preeclampsia, Hypertension 51(4) (2008) 1066-1072.
593 [90] L. Anton, D.C. Merrill, L.A.A. Neves, D.I. Diz, J. Corthorn, G. Valdes, K. Stovall, P.E.
594 Gallagher, C. Moorefield, C. Gruver, K.B. Brosnihan, The Uterine Placental Bed Renin-
595 Angiotensin System in Normal and Preeclamptic Pregnancy, Endocrinology 150(9) (2009)
596 4316-4325.
597 [91] G. Valdés, L.A.A. Neves, L. Anton, J. Corthorn, C. Chacón, A.M. Germain, D.C. Merrill,
598 C.M. Ferrario, R. Sarao, J. Penninger, K.B. Brosnihan, Distribution of angiotensin-(1-7)
599 and ACE2 in human placentas of normal and pathological pregnancies, Placenta 27(2-3)
600 (2006) 200-207.
601 [92] C.L. Tower, S. Lui, N.R. Charlesworth, S.D. Smith, J.D. Aplin, R.L. Jones, Differential
602 expression of angiotensin II type 1 and type 2 receptors at the maternal-fetal interface:
603 potential roles in early placental development, Reproduction (Cambridge, England)
604 140(6) (2010) 931-42.
605
606

Tables

Table 1. Localisation of the Placental Renin Angiotensin System in early and late Gestation

Component	Protein/Peptide Localisation		mRNA Expression Across Gestation
Prorenin	Early	STB, CTB, EVT [2]	mRNA abundance is highest in early gestation (6-9 weeks) [33]
	Term	STB, Vascular Endothelium [87]	
(P)RR	Early	EVT, Vascular Endothelium, STB [2]	mRNA abundance is highest in early gestation (8-14 weeks) [33]
	Term	STB, Vascular Endothelium[87]	
AGT	STB, CTB, Villous Stroma [33] [87]		mRNA abundance is highest in early gestation (6-16 weeks) [33] Protein abundant at term [87]
ACE1	Fetal Vascular Endothelium [33, 87, 88]		mRNA abundance is higher at term than in early gestation [33]
ACE2	Early	STB, CTB, Villous Stroma [33]	mRNA abundance is highest in early gestation placentae (6-16 weeks) [33]
	Term	STB, CTB, Fetal Endothelium & Vascular Smooth Muscle [87]	
Angiotensin I and II	Placental bed and chorionic villi at term [89, 90]		Ang II is the dominant peptide in pregnancy, its expression decreases towards term
Ang-(1-7)	STB, CTB, Fetal Endothelium, EVT [89, 91]		Unknown
AT_1R	Chorionic Villi, STB, CTB, EVT, Fetal Endothelium [74, 87]		mRNA expression is highest in early gestation (6-13 weeks) [33, 34]
AT_2R	CTB, EVT [35, 87]		Expression is typically low but highest in early gestation [33, 34, 74, 89, 92]
AT_4R	STB, EVT [35]		Highest in early gestation decrease towards term
MasR	Chorionic Villi [89]		Typically undetectable [33, 87]

CTB, cytotrophoblast; EVT, extravillous trophoblast; STB, syncytiotrophoblast; (P)RR, (pro)renin receptor; AGT, angiotensinogen; ACE1, angiotensin converting enzyme 1; ACE2, angiotensin converting enzyme 2; AT_1R, angiotensin II type 1 receptor; AT_2R, angiotensin II type 2 receptor; AT_4R, angiotensin II type 4 receptor; MasR, Mas Receptor

Figure Legends

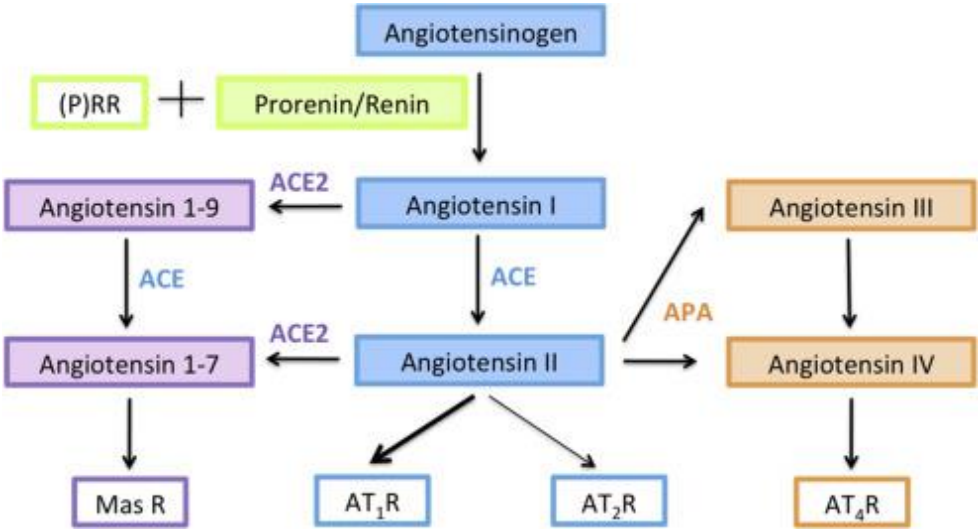

Figure 1. *The renin-angiotensin system cascade. Prorenin is activated by binding to the (pro)renin receptor ((P)RR) and possibly by proteolysis to cleave Angiotensin (Ang) I from Angiotensinogen (AGT). Angiotensin converting enzyme (ACE) then converts Ang I to the biologically active Ang II. Ang II can bind to angiotensin II type 1 receptor (AT_1R) to promote proliferation, angiogenesis, fibrosis, migration and invasion through stimulation of intracellular signaling pathways. Furthermore, Angiotensin (Ang) II binds to angiotensin I type 2 receptor (AT_2R) and antagonizes AT_1R activation. Aminopeptidase A (APA) allows conversion of Ang II to Ang III or Ang IV, which act on the AT_4R and can induce vascularisation, inflammation, vasodilation and hypertrophy. Ang I can also be further converted by angiotensin converting enzyme 2 (ACE2) to Ang-(1-7). Ang-(1-7) acts upon its receptor Mas. This results in antagonism of Ang II/AT_1R stimulation thus inhibiting proliferation, angiogenesis, fibrosis, migration and invasion.*

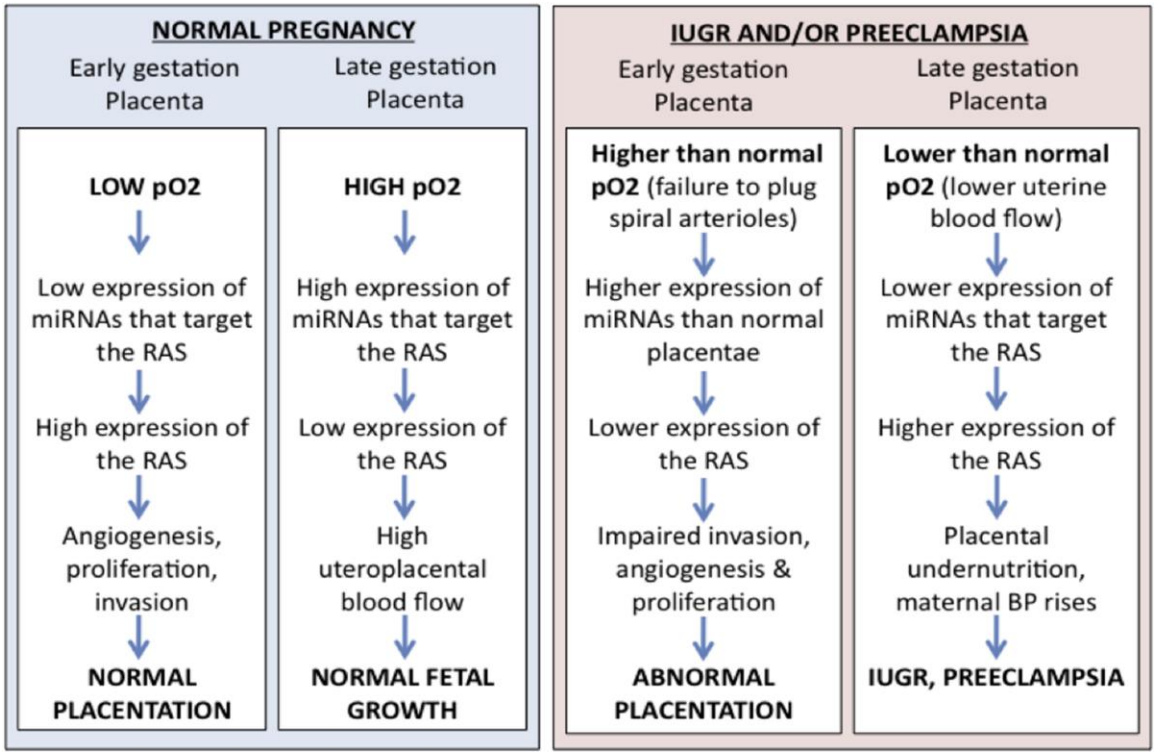

Figure 2. *Proposed model of placental RAS regulation in physiological and pathological pregnancies. In normal pregnancy (blue box), low oxygen in early gestation inhibits expression of miRNAs that target renin-angiotensin system components, promoting increased expression and appropriate placental development. At term, higher oxygen tensions result in higher expression of miRNAs and a subsequent inhibition of RAS components, resulting in high uteroplacental blood flow and normal fetal growth. In pathological pregnancies (red box), failure to plug spiral arterioles and a higher placental O_2 concentration in early pregnancy causes higher miRNA expression and thus lower expression of RAS components resulting in abnormal placentation. In late gestation, lower uterine blood flow causes lower expression of miRNAs and a higher expression of RAS components resulting in a lower uteroplacental blood flow and the onset of pregnancy complications.*