A role for the renin-angiotensin system in hematopoiesis

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The effects of the renin-angiotensin system (RAS) on blood pressure regulation were first described in 1898 by the physiologist Robert Tigerstedt, following observations that blood pressure increased in anesthetized animals following the injection of kidney extracts.[1-3] Since then, the RAS has been characterized extensively, not only for its roles in hypertension and atherosclerosis, but also for its relevance to the fields of diabetes, oncology, and hematology. It has been recently recognized that the RAS plays critical physiological roles beyond its originally recognized functions in cardiovascular and fluid homeostasis regulation. For example, in this issue of the journal, Heringer-Walther *et al.*[4] investigated the effects of angiotensin-(1-7) on *in vitro* proliferation and *in vivo* engraftment of human hematopoietic progenitor cells. These authors reported that the angiotensin II (Ang II) metabolite, Ang-(1-7) positively stimulates the proliferation of $CD34^+$ cord blood cells *in vitro*. They also demonstrated that coinjection of cord blood mononuclear cells with continuous post-transplantation supplementation of Ang-(1-7) drastically improved human hematopoietic progenitor engraftment. These results continue to highlight an important yet unappreciated role for RAS in regulating hematopoietic progenitor differentiation and self-renewal. Moreover, the use of Ang-(1-7), Ang II, or manipulation of Ang II receptor binding may have utility and clinical significance in bone marrow transplantation.

The RAS pathway mediates its effects primarily via angiotensinogen, angiotensin I (Ang I), and Ang II. The enzyme renin converts angiotensinogen to Ang I, which is transformed into Ang II by the surface ectoenzyme angiotensin I-converting enzyme (ACE; CD143). Ang II alternatively binds to either Ang II-type 1 receptors (AGTR1, a.k.a., AT1), or angiotensin II-type 2 receptors (AGTR2, a.k.a., AT2).[5] Although AGTR1 is expressed abundantly in renal and non-renal tissues from development to adulthood (where it functions as a key regulator of the cardiovascular system through G-protein-coupled interactions), AGTR2 is scarcely expressed in adult tissues,[6,7] suggesting a key role during early development. The AGTR1 and AGTR2 can act as antagonists, and mediate affects on cell migration and proliferation of cardiovascular cells, metastatic cancer cells, and hematopoietic stem-progenitor cells (HSC).[5,8] The regulation of the RAS on the hematopoietic system has been progressively documented over the last decade. In 1982, two independent groups first reported that high doses of ACE inhibitors could induce anemia and leucopenia in humans.[9,10] Ang II receptor antagonists and ACE inhibitors are commonly used for treatment of cardiovascular diseases, post-transplantation erythrocytosis, or polycythemia vera.[11] Nonetheless, the mechanisms by which RAS components and their inhibitors maintain balance in the hematopoietic and cardiovascular systems remain obscure.

ACE inhibitors and angiotensin receptor blockers are routinely used and clinically well-tolerated agents, despite sporadic reports of associated side effects that include severe anemia and bone marrow aplasia.[12,13] A review of the literature suggests that the effects of the RAS on regulation of hematopoietic differentiation are numerous and diverse. Renin, angiotensinogen, ACE, and/or AGTR1 are expressed in human umbilical cord blood,[14] bone marrow cells,[15] and $CD34^+$ HSC,[13,16] and Ang II peptide can modulate erythropoiesis either by upregulating erythropoietin levels, or by exerting mitogenic effects on erythroid progenitors and $CD34^+$ HSC. Furthermore, HSC can be differentiated toward the erythroid lineage by Ang II-AGTR1 interactions, and this effect can be abrogated by blocking Ang II binding using Losartan, an AGTR1 inhibitor.[17] ACE was also shown to increase the recruitment of primitive HSC into S-phase in the bone marrow via degradation of the tetrapeptide Ac-SDKP (acetyl-seryl-aspartyl- lysyl-proline), a potent inhibitor of HSC proliferation.[18,19] Aksu, *et al.* reported that surface ACE is overexpressed in leukemic myeloid blast cells, and such expression positively correlated with the number of bone marrow blast counts.[20]

Table 1 summarizes the most commonly used ACE inhibitors, and AGTR1/AGTR2 antagonists that are currently available for treatment of cardiovascular/renal disease, and their known effects on the hematopoietic system. Interestingly, treatment with AGTR1 antagonists in the adult resulted in side effects similar to those observed with ACE inhibitors (*e.g.* anemia), which was the opposite result obtained in human embryonic hematopoiesis.

Studies of knockout mice mutant for ACE (as well as other RAS components such as angiotensinogen, renin, AGTR1, and AGTR2) have further implicated a regulatory role for the RAS in hematopoiesis.[23] These mice have exhibited not only phenotypes related to blood pressure, but have also demonstrated defects in development, as well as in cardiovascular, reproductive, and hematopoietic systems. ACE null mice are mildly anemic (exhibiting hematocrit levels of about 40% when compared with normal levels of about 50%), and are also characterized by increased levels of serum and urinary potassium secondary to kidney developmental defects. The mechanisms of this anemia remain unclear, although it is presumed that the lack of systemic or local production of Ang II has a detrimental effect on erythropoiesis.[18,19,24,25] Interestingly, a mouse model (ACE 10/10 mice) with specific overexpression of ACE in monocytes and macrophages, but lacking expression in other cell

Table 1. Components of the RAS and summary of effects on the hematopoietic system.

RAS components	Medication	Original function	Effects on hematopoiesis
ACE inhibitors	Enalapril[1] Captopril[2] Zofenopri[1] Lisinopril[3] Bradykinin	- Reduce blood pressure - Treatment of congestive heart failure and renal disease - Stroke prevention	- Hyperkalemia - Anemia - Reduced Hb and RBC level[1] (10) - Inhibition of human hemangioblast colony expansion[2] (21) - Inhibition of cell cycling of HSC after irradiation in murine models[3] (22)
AGTR1 antagonist	Losartan[4] Irbesartan Olmesartan Telmisartan Valsartan	- Vasodilation	- Hyperkalemia - Hemoglobin level reduction - Reduced recovery from anemia - Augmented hematopoietic lineage commitment in human embryonic stem cell differentiation[4] (21)
AGTR2 antagonist	PD123-319	- Vasoconstriction	- Augmented vascular endothelial cell lineage commitment in human embryonic stem cell differentiation (21)

ACE: angiotensin I-converting enzyme, AGTR1: angiotensin II-type 1 receptor, AGTR2: angiotensin II-type 2 receptor, HSC: hematopoietic stem-progenitor cells. Superscript numbers are paired between medication component and the corresponding effect on hematopoiesis.

types (including endothelium and renal tissues) displayed significantly reduced tumor vascularization.[23] These studies suggested that abnormal ACE expression in hematopoietic cells can perturb angiogenesis mediated by myeloid cells.

The RAS has been further implicated in playing a critical role during the development of the hematopoietic system. The recent discovery that ACE (CD143) marks primitive human embryonic hemangioblasts[21] has now raised the possibility that the RAS plays a critical role in regulating the earliest stages of human hematoendothelial differentiation, as it does in avian embryos.[26] Not surprisingly, RAS components are locally expressed in all of the important sites of emerging angiohematopoiesis including the yolk sac, liver, kidney, embryonic aorta, and retinal/choroid regions.[27] Accordingly, the hemangioblast, a common precursor for both hematopoietic and endothelial cells, is first observed in the yolk sac blood islands and aorta gonad mesonephros (AGM) during embryogenesis in the same regions where these RAS components are also first expressed; Jokubaitis *et al.* confirmed that ACE (CD143) is expressed in human embryonic, fetal, and adult hematopoietic organs including the ventral wall of the aorta, fetal liver, and umbilical cord blood.[8]

Using a human embryonic stem cell (hESC) differentiation model, we recently reported that there was a dramatic upregulation of AGTR2 during expansion of human embryoid body (hEB)-derived ACE$^+$ hemangioblasts.[21] This observation suggested a unique role for the RAS in guiding the initial developmental phases of human hemato-endotheliogenesis. Moreover, hESC-derived hemangioblast colonies could be directed to differentiate into either hematopoietic or endothelial progeny by manipulating the signaling pathways normally mediated by the RAS. Manipulation of angiotensin II signaling with either AGTR1- or AGTR2-specific inhibitors resulted in pronounced skewing of hEB differentiation toward either endothelium, or multipotent hematopoietic progenitors. Since AGTR2 signaling is known to antagonize AGTR1 signaling directly,[28] these data implicated a general mechanism by which emerging hemangioblasts in the yolk sac or AGM may be directed to differentiate by the hematopoietic stem cell niche. One possibility is that antagonistic competition between AGTR1 and AGTR2 for Ang II binding on emerging hemangioblasts directs their development into either hematopoietic progenitors, or alternatively into vascularendothelial networks. Finally, these studies suggest that manipulation of AGTR2 signaling is an important mechanism by which multipotent hematopoietic progenitors expand from primitive HSC. Furthermore, it is also possible that AGTR1/AGTR2- regulated stem cell proliferation is a generalized phenomenon, since AGTR1 inhibition by losartan was reported to increase skeletal muscle regeneration in patients with primary muscular dystrophies,[29] and the renin-angiotensin axis critically influences normal fetal development, since both ACE and AGTR1 receptor blockers are teratogenic. Additionally, AGTR2 signaling is known to regulate cellular growth and apoptosis during vascular and neural development.[26,30]

In summary, these studies collectively implicate the involvement of the RAS in a broad spectrum of functions that surpass its known functions in cardiovascular homeostasis. ACE and its major enzymatic product, Ang II are recognized as critical determinants of angiogenesis, inflammation, tumor progression, and hematopoiesis. Modulation of RAS function may be determined through competitive interaction of Ang II with two antagonistic receptors, AGTR1 and AGTR2, and this intrinsic bivalence may regulate the fate of emerging hemangioblasts (which are present in the embryonic yolk sac or the AGM), or the adult HSC found in the bone marrow. The levels of regulation involved here are likely to be extremely complex and multilayered. For example, ACE is actively degraded by the vasoactive peptide bradykinin, and as a consequence, ACE inhibitors can act both upstream (by interfering with Ang II production), as well as downstream via direct bradykinin effects on the RAS pathway. Additionally, ACE inhibitors with thiol groups can also directly inhib-

it the activities of zinc metalloproteases MMP2 and MMP9,[5] which may connect the RAS to possible roles in migration of tumorigenic cells and HSC. A deeper understanding of how these multiple levels of the protean RAS regulate the development and differentiation of the hematopoietic system may provide new insights to help design improved therapies for hematologic disorders.

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