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Local umbilical cord blood renin–angiotensin system

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Abstract Local bone marrow (BM) renin–angiotensin system (RAS) is an autocrine–paracrine system affecting normal and neoplastic hematopoiesis. Angiotensin II type 1a (AT1a) receptors are present on the CD34⁺ hematopoietic stem cells (HSC). Angiotensin II stimulates the proliferation and differentiation of the HSC populations through the activation of AT1 receptors on HSC. Umbilical cord blood (UCB) is a rich source of HSC. The existence of a complete local UCB RAS has not been previously investigated. In this study, local synthesis of the major RAS components, namely, angiotensin-converting enzyme (ACE), renin, and angiotensinogen, was identified by demonstrating their corresponding mRNAs using quantitative reverse transcriptase polymerase chain reaction (RT-PCR) in human UCB. Local RAS could regulate cellular growth in a variety of tissues including the BM. Major RAS peptides can exert significant effects on primitive pluripotential HSC populations. Further studies should focus on the interactions between possible autocrine, paracrine, endocrine, and intracrine actions of the local UCB RAS and growth, engraftment, differentiation, and plasticity functions of HSC of UCB origin.

Keywords Umbilical cord blood · Renin–angiotensin system · Hematopoiesis · Stem cells · Renin · Angiotensinogen · ACE

Introduction

The new frontiers of the renin–angiotensin system (RAS) include organ-, tissue-, and even cell-based systems exhibiting autocrine, paracrine, endocrine, and intracrine actions within the human body [1–4]. The presence of a local RAS in the hematopoietic bone marrow, affecting physiological and neoplastic blood cell production, was initially suggested in 1996 [5]. After a long debate, as documented in the literature [6–9], a local hematopoietic bone marrow RAS [2] was recently demonstrated as an autocrine–paracrine system within the rat hematopoietic lineage and marrow stromal cells [10].

Angiotensin II type 1a (AT1a) receptors are present on the CD34⁺ hematopoietic stem cells [11], in the context of the local hematopoietic bone marrow RAS [2]. Likewise, angiotensin II stimulates the proliferation of bone marrow hematopoietic progenitors [11] and umbilical cord blood cells [12, 13]. Angiotensin II could serve in the erythroid and myeloid differentiation of stem cells [11]. Umbilical cord blood is a rich source of hematopoietic stem cells. However, the existence of a complete local umbilical cord blood RAS has not yet been explored. The local synthesis of a particular molecule can be deduced from the presence of the corresponding messenger ribonucleic acid (mRNA) in a given local tissue [5]. In this study, the expression of the mRNAs of the major RAS components, namely, angiotensin-converting enzyme (ACE), renin, and angiotensinogen, in human umbilical cord blood samples was quantified by reverse transcriptase polymerase chain reaction (RT-PCR) to search for a local umbilical cord blood RAS. Currently, the clinical benefit of using umbilical cord blood hematopoietic stem cells for allogeneic transplants is well established [14]. Therefore, elucidation of the presence of

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a local autocrine-paracrine RAS-mediated regulation of human umbilical cord blood hematopoietic stem cells is both (patho)biologically and clinically important. Identification of local umbilical cord blood RAS could open new avenues of investigations in stem cell biology.

Materials and methods

Isolation of RNA and synthesis of cDNA from human umbilical cord blood samples

Twenty human umbilical cord blood samples were collected from the umbilical vein on tubes containing ethylenediaminetetraacetate (EDTA) after written informed consents had been obtained from the mothers. None of those pregnant women included in the study had had any systemic diseases such as hypertension, heart diseases, anemia, diabetes, and cancer. They were not receiving any medication during the pregnancy such as antihypertensives, antiaggregants, or vasodilators, which might have affected the results of the study. All of the subjects had normal vaginal spontaneous deliveries and none of the 20 pregnancies were accompanied by complications such as preeclampsia or eclampsia. The Ethical Committee of Hacettepe University Medical School had approved the study design. Twenty EDTA-treated human umbilical cord blood samples were then stored at -196°C until analysis. Total RNA was extracted from 200 μl cord blood samples by the High Pure RNA Isolation Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. RNA integrity was electrophoretically verified by ethidium bromide staining and by an $\text{OD}_{260}/\text{OD}_{280}$ nm absorption ratio of >1.95 . One microgram of total RNA was used for cDNA synthesis using the 1st Strand cDNA Synthesis Kit for RT-PCR (AMV) (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's protocol.

Quantitative real-time PCR analysis for major RAS components (ACE, renin, and angiotensinogen) in human umbilical cord blood samples

Real-time quantitative PCR analyses for ACE, renin, and angiotensinogen gene expressions were done using the LightCycler instrument (Roche Diagnostics, Mannheim, Germany) and results were analyzed by LC software 3.0.

Amplifications were performed in 20 μl volume including 2 μl cDNA, 4 nM of each primer, 2 nM of TaqMan probe, and LightCycler DNA master hybridization master mix. The cycling parameters were 2 min at 95°C for denaturation, 40 cycles of 15 s at 95°C , and 30 s at 60°C for amplification and quantification. The primer and probe sequences used in this study are shown in Table 1. The β -actin mRNA was quantified to adjust the amount of mRNA in each sample with the β -actin probe and primer set. The upstream and downstream primer sequences were 5' TCACCCACACTGTGCCCAT and 5' TCCTTAATGTCACGCACGATTT 3', respectively, and the TaqMan probe selected between the primers was fluorescence labeled at the 5' end with 6-carboxyfluorescein (FAM) as the reporter dye and at the 3' end with 6-carboxytetramethylrhodamine (TAMRA) as the quencher; 5'-FAM-ATCCTGCGTCTGGACCTGGCT-TAMRA (TibMolBiol, Berlin, Germany). In every PCR reaction, the level of the housekeeping gene β -actin was also quantified to normalize the ACE, renin, and angiotensinogen gene expression values of each sample with the same PCR conditions described above. Relative expressions were calculated according to the mathematical model [15] based on the PCR efficiencies and the crossing points.

Results

Corresponding mRNAs of all of the major RAS components, namely, ACE, renin, and angiotensinogen, were identified in human umbilical cord blood samples. Specific mRNAs for all three components of the RAS in the cord blood samples could be detected by real-time PCR analysis (Fig. 1). ACE and angiotensinogen gene expressions were observed in all of the human umbilical cord blood samples. Renin gene expression was also demonstrated in all but one sample. Relative expressions of each target were calculated according to the expression of the housekeeping gene β -actin. Relative gene expressions of the quantitative real-time PCR are shown in Table 2. The median ratio was found to be 0.078 for ACE, 0.084 for angiotensinogen, and 0.003 for renin.

Discussion

In this study, local syntheses of the major RAS components, namely, ACE, renin, and angiotensinogen, were

Table 1 The primer and probe sequences of ACE, renin, and angiotensinogen genes [16]

	ACE	Renin	Angiotensinogen
Forward	5' TCGCCATGTTGAGCTA CTTC 3'	5' GCCACCTTCATCCGAAAGT TC 3'	5' GCACCTCAGTGTCTGTT CCCAT 3'
Reverse	5' TCCCATGCAGCTCGT TC 3'	5' GCCAAGGCGAAGCCAAT 3'	5' ACCGAGAAGTTGTCCTG GATGT 3'
TaqMan probe	5' AGCCGCTGCTGGACTG GCTCC 3'	5' ACACAGAGTTTGATCGGCG TAACAACCG 3'	5' TGGCATGGGCACCTTCC AGCA 3'

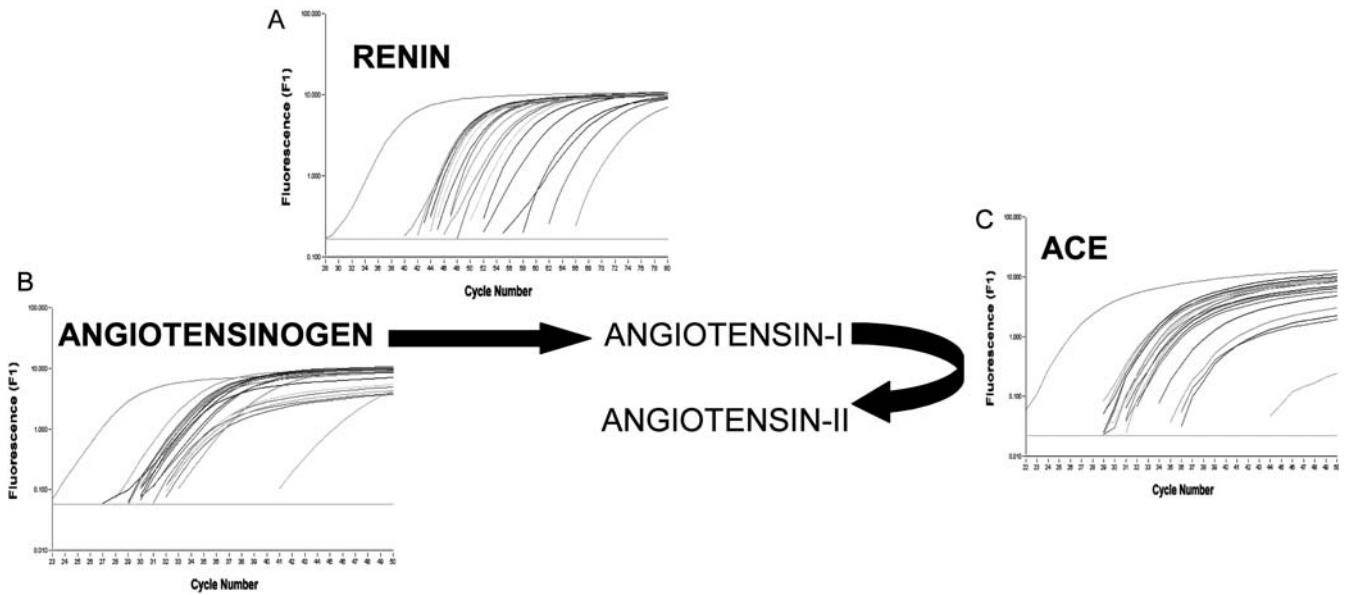


Fig. 1 Real-time quantitative PCR for gene expression analysis. cDNA from human umbilical cord blood samples were PCR amplified in the presence of TaqMan probes. Plots of fluorescence signals (*X axis*) vs cycle number (*Y axis*) using each region's primers/probes and cDNA samples are shown in diagrams [renin (**a**), an-

giotensinogen (**b**), and ACE (**c**) amplification curves]. The expression of renin, angiotensinogen, and ACE genes were compared with β -actin expression of each sample (data not shown). The negative controls without target cDNA showed no signal increase and are not visible in the diagrams.

Table 2 Relative gene expression results of ACE, angiotensinogen, and renin (β -actin gene expressions of the same samples were used for calculations). *ND* not determined

Cord blood sample #	ACE ratio	Angiotensinogen ratio	Renin ratio
1	0.012	0.156	0.0010
2	0.201	0.271	0.0044
3	0.013	0.148	0.0008
4	0.081	0.058	0.0033
5	0.016	0.038	0.0007
6	0.492	0.330	0.0039
7	0.037	0.049	0.0015
8	0.004	0.007	ND
9	0.980	0.463	0.0052
10	0.143	0.070	0.0041
11	0.080	0.078	0.0022
12	0.266	0.090	0.0044
13	0.049	0.063	0.0028
14	0.411	0.189	0.0050
15	0.860	0.670	0.0029
16	0.020	0.036	0.0018
17	0.013	0.030	0.0013
18	0.059	0.147	0.0019
19	0.076	0.094	0.0031
20	0.056	0.073	0.0056

identified by demonstrating their corresponding mRNAs in human umbilical cord blood. These findings support the existence of a local RAS in the human umbilical cord blood. Quantitative mRNA expression levels of the individual cord RAS components disclosed variations between

the samples. That variability may be due to the dynamic interactions between uteroplacental and umbilical cord stem cells during ongoing biological events [17–19]. Hematopoietic stem/progenitor/precursor cells and mesenchymal progenitor or stem cells [18, 20] are resident in umbilical cord blood. Recent investigations revealed several RAS elements and receptors on normal and neoplastic hematopoietic cells [2, 11, 21–23]. Strawn et al. [21] detected the presence of ACE, angiotensin II, angiotensin AT1 and AT2 receptors in rat unfractionated bone marrow cells (BMC), hematopoietic lineage BMC, and cultured marrow stromal cells (MSC). The mRNAs for angiotensinogen, renin, ACE, and AT1a and AT2 receptors were found to be present in BMC and in cultured MSC, while ACE2 mRNA was detected only in BMC in their study. Flow fluorocytometry analyses by Strawn et al. also showed immunodetectable angiotensinogen, ACE, AT1 and AT2 receptors, and angiotensin II, as well as binding of angiotensin II to AT1 and AT2 receptors, in CD4⁺, CD11b/c⁺, CD45R⁺, and CD90⁺ BMC and cultured MSC. Renin was found to be present in almost all cell types except CD4⁺ BMC, and angiotensin II was detected in MSC homogenates. Strawn et al. [21] concluded that the presence of angiotensin II receptors in both hematopoietic lineage BMC and MSC and the de novo synthesis of angiotensin II by MSC suggest a potential autocrine–paracrine mechanism for local RAS-mediated regulation of hematopoiesis.

Identification of the “local umbilical cord blood RAS” could shed further light on the presence and (patho)biological functions of the “local hematopoietic bone marrow RAS [2].” Hematopoietic stem and progenitor cells represent the major basis for the common discussion of those two distinct local RASs. Both hematopoietic cells of bone marrow and umbilical cord blood origin share key RAS

elements as indicated in previous studies [2, 11, 22, 24] in accordance with our present results. RAS regulates cellular growth in a variety of tissues [1, 3, 4] including the bone marrow [2]. RAS can exert significant effects on erythropoietic progenitors and primitive pluripotential hematopoietic stem cell populations [2, 11]. Angiotensin II acts as a systemic and “locally active” hematopoietic growth factor or cytokine through activation of CD34⁺ cell AT1 receptors [2, 11–13, 23, 25]. Angiotensin II may directly act on the stem cell compartment by promoting entry into the cell cycle and regulate the hematopoietic niche [26]. Angiotensin peptides can alter the differentiation of erythroid and myeloid precursors, while ACE inhibitors inhibit the proliferation of hematopoietic stem and progenitor cells [26]. Angiotensin signaling pathways such as the JAK-STAT pathway represent the point of cross-talk between RAS and hematopoiesis [27]. Angiotensin II can affect the proliferation and differentiation of CD34⁺ cells of umbilical cord blood and bone marrow origin [11–13]. Therefore, the functions of angiotensin peptides and RAS in ex vivo umbilical cord blood expansion could be further investigated for potential clinical benefit. On the other hand, there are preliminary data that RAS may be involved in pathologic/neoplastic hematopoiesis and leukemogenesis [2, 22–24]. Renin mRNA is expressed in leukemic blast cells [2, 23]. Angiotensin gene is aberrantly expressed in leukemic cells. Angiotensin may act as an autocrine growth factor for acute myeloid leukemia (AML) cells [28]. ACE is produced at higher quantities in the leukemic bone marrow. ACE degrades a tetrapeptide called AcSDKP (Goralatide), a negative hematopoietic regulator, and ACE hyperfunction may remove the antiproliferative effect of Goralatide on the hematopoietic cells and blasts [2, 24]. Thus, probable confounding effects of hematopoietic RAS on atypical blood cell production during umbilical cord blood transplantation in leukemic patients should also be considered.

RAS components are expressed in uteroplacental tissues. Angiotensin receptor is present on placenta-derived cells [29]. Umbilical cord releases angiotensin II in vitro [30]. Effector angiotensin peptides of local RASs are located at the critical crossroads of in utero hemo-vasculogenesis and embryonic hematopoiesis [19, 31–33]. Many embryonic tissues produce their own blood cells. Hematopoiesis is, therefore, widespread in the embryo probably in relation to the angiotensin peptides [31]. For instance, the kidney represents a model for hematopoiesis during organogenesis [19]. Likewise, mesenchymal progenitor or stem cells isolated from fetal blood, bone marrow, and cord blood can proliferate and differentiate into multiple mesodermal tissues including bone, cartilage, muscle, ligament, tendon, fat, and stroma [20]. Angiotensin II has a pivotal role in hemo-vasculogenesis and kidney embryogenesis [31]. Locally produced angiotensin peptides correspond to hematopoietic/embryonic stem cells and hematopoiesis in the tissue microenvironment. Therefore, local umbilical cord blood RAS could act in the differentiation to hematopoietic cells during transplantation [14] and in umbilical cord blood stem cell

plasticity [34–40] for nonhematopoietic tissues [41]. It is hoped that further investigations will reveal the enigmatic relations between local RAS and the hematopoietic potential of the umbilical cord.

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