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Report

Renin-angiotensin system expression in the K562 human erythroleukaemic cell line

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Abstract

Local renin-angiotensin system (RAS) may affect leukaemic cell production within the bone marrow microenvironment. Angiotensin-converting enzyme (ACE), renin, and angiotensin could influence leukaemogenesis. In this study, mRNA expressions of the major RAS components (ACE, renin, and angiotensinogen) in K562 human erythroleukaemia cell line have been searched by Real Time quantitative polymerase chain reaction. K562 blasts are multipotential, haematopoietic malignant cells that spontaneously differentiate into recognisable progenitors of the erythrocyte, granulocyte and monocytic series. We observed significant expressions of ACE, renin, and angiotensinogen in K562 leukaemic blast cells. Therefore, K562 human erythroleukaemia cell line may serve as an *in vitro* model to elucidate the role of RAS in leukaemia and to test the effects of RAS-affecting drugs on leukaemic cellular proliferation.

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The local bone marrow renin-angiotensin system (RAS) affects neoplastic blood cell production as an autocrine-paracrine-intracrine network.[1-4] RAS peptides are involved in the control of cellular proliferation.[5,6] For instance, angiotensin-converting enzyme (ACE) hyperfunction may lead to the acceleration of the negative, the metabolism of the haematopoietic regulator peptide N-acetyl-Seryl-Aspartyl-Lysyl-Proline =Goralatide (AcSDKP) which in turn lowers its level in the bone marrow microenvironment, finally removing the anti-proliferative effect of the peptide on the haematopoietic cells and blasts.[1,7] ACE is positively correlated with bone marrow blast count.[1] *In vitro* incubation of acute myeloid leukaemia (AML) cells with an ACE inhibitor decreased the growth and colony-forming ability of AML cells in a dose-dependent manner. The addition of angiotensin II (Ang II) peptide to AML cells partially rescued their colony-forming ability.[8] Hence, local bone marrow RAS has been researched in acute and chronic myeloproliferative disorders.[3,9] Renin expression could have a role in disease development and could be used as a marker of leukaemia.[10] Furthermore, angiotensin has been suggested to act as an autocrine growth factor for AML cells.[8]

We searched for the messenger ribonucleic acid (mRNA) expression of the major RAS components (ACE, renin, and angiotensinogen) in K562 human erythroleukaemia cell line by Real Time quantitative polymerase chain reaction (RT-PCR). K562 blasts are multipotential, haematopoietic malignant cells that spontaneously differentiate into recognisable progenitors of the erythrocyte, granulocyte and monocytic series.[11] RT-PCR analyses for ACE, renin and angiotensinogen gene expressions were done by using LightCycler™ instrument (Roche Diagnostics, Germany) and results were analysed by LC software 3.0. All PCR primers and probes were designed using Primer Premier 5 software (Premier Biosoft International, Palo Alto, CA, USA). The primers and probes were carefully designed to avoid amplification of genomic DNA. All PCR products were sequenced to ensure product validity (data not shown). The sequences of the primers and TaqMan probes are shown in table 1. The β-actin mRNA was quantified to adjust the amount of mRNA in each sample with

Table 1
The primer and probe sequences of angiotensin-converting enzyme (ACE), renin and angiotensinogen genes.

	ACE	Renin	Angiotensinogen
Forward	5′TCGGCCATGTTGAGCTACTTC 3′	5′GCCACCTTCATCCGAAAGTTC 3′	5′GCACCTCAGTGTCTGTTCCCAT 3′
Reverse	5′TCCCCATGCAGCTCGTTC 3′	5′GCCAAGGCGAAGCCAAT 3′	5′ACCGAGAAGTTGTCCTGGATGT 3′
TaqMan Probe	5′ AGCCGCTGCTGGACTGGCTCC 3′	5′ ACACAGAGTTTGATCGG CGTAACAACCG 3′	5′ TGGCATGGGCACCTTCCAGCA 3′

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Table 2
Relative gene expression results of the major renin-angiotensin system (RAS) components namely angiotensin-converting enzyme (ACE), renin (REN), and angiotensinogen (ANG) genes in K562 human erythroleukaemia cell line by quantitative real time quantitative PCR (RT-PCR). β-actin (ACTIN) gene expression of the sample was used for calculations.

	ACTIN	ACE	ACE INDEX		INDEX	ANG	ANG INDEX		INDEX	REN	REN INDEX		INDEX
K562 leukaemic cell line	20,42	26,83	6,41	41,0881	0,024	26,13	5,71	32,6041	0,031	33,51	13,09	171,3481	0,006

a 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 quencer; 5´-FAM- ATCCTGCGTCTGGACCTGGCT-TAMRA (Tibmolbiol, Germany). Amplifications were performed in 20 µl volume including 2 µl cDNA, 4 nM of each primers, 2 nM of TaqMan probe and LightCycler DNA master hybridisation master mix. The cycling parameters were two minutes at 95°C for denaturation, 40 cycles of 15 seconds at 95°C, 30 seconds at 60°C for amplification and quantification. The same RT-PCR conditions described above were used for ACE, renin and angiotensinogen gene expression. The assays used β-actin as the endogenous internal housekeeping gene that revealed less variability and better reproducibility in our method. Real-time expression values were calculated using the relative standard curve method. Standard curves were generated for each mRNA using ten-fold serial dilutions for both the target of interest and the endogenous control (β-actin) by measuring the cycle number at which exponential amplification occurred in a dilution series of samples. Values were normalised to the relative amounts of β-actin mRNA, which were obtained from a similar standard curve. In every PCR reaction, PCR grade water was used as a negative control, and standards of the targets were used as a positive control. In RT-PCR reactions the same initial amounts of target molecules were used, and the Cp values of β-actin mRNA were constant in all samples. A new software tool was used, named REST (relative expression software tool), which compared all samples of each group. The mathematical model used is based on the PCR efficiencies and the crossing point deviation between the samples. REST uses the pair-wise fixed reallocation randomisation test to calculate the significance of results. Differences at $p<0.001$ were considered significant. The software used for statistical analyses is an established method and analyses RT-PCR results directly.

We observed significant expression of the all major RAS components (ACE, renin and angiotensinogen) in the K562 human erythroleukaemia cell line (table 2). RAS expression in K562 was comparable to that in human leukaemic bone marrow samples of our previous experiments.[3] Locally produced RAS peptides may be active in the pathobiological basis of leukaemias. This issue is not just academic, since the pharmacological modification of the RAS affects the course of neoplastic diseases.[12]

ACE is a mostly membrane-bound ectoenzyme that plays a major role in numerous physiological activities including cellular proliferation. ACE is also involved in the metabolism of several other biologically active peptides. ACE expression in cells is genetically determined. ACE is present in human monocytes/macrophages, and in the T-lymphocyte population. ACE may participate like other ectopeptidases in the regulation of lymphocyte functions. We have recently investigated ACE insertion/deletion (I/D) gene polymorphisms, which may affect the behaviour of the local RAS in haematological neoplastic disorders.[13] We suggested that the ACE ID/II gene polymorphism may be linked to the development of leukaemia as a clue of activated local RAS in leukaemogenesis. The overexpression of a critical RAS component, ACE (CD 143), surface antigen was shown to be over-expressed in leukaemic myeloid blast cells.[7] The level of expression of membrane-bound ACE in blast cells is associated with the insertion/deletion polymorphism of the ACE gene. ACE ID/II genotype frequency is increased in leukaemic patients.[13] The regulation of ACE synthesis in cells may also be genetically determined in leukaemic patients probably at the transcriptional level. ACE is produced in higher quantities in the leukaemic bone marrow. ACE degrades a tetrapeptide called AcSDKP (goralatide), a negative haematopoietic regulator. Furthermore, while peripheral blood ACE levels increase, the percentage of blasts in the bone marrow increases and they migrate into the circulation. Therefore, ACE hyperfunction may lead to the acceleration of AcSDKP metabolism, which in turn lowers its level in the bone marrow

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microenvironment, finally removing the antiproliferative effect of goralatide on the haematopoietic cells and blasts.[1,14] The negative haematopoietic regulator peptide, AcSDKP, may be an ideal starting point for the alteration of local RAS kinetics in the bone marrow microenvironment. A new class of organometallic compounds, the Biphosphinic Palladacycle Complex [Pd (C2, N-S(-) (dmpa) (dppf)] Cl (BPC), which has been developed as an ACE-inhibitor produces increased levels of AcSDKP and has already been shown to act in haematological regulation.[15] Ang II antagonists and ACE-inhibitors have shown some anti-neoplastic actions. The Ang II receptor blocker losartan antagonises platelets, which is thought to be modulated via vascular endothelial growth factor. They may even protect the patient from the major toxicity of chemotherapy and/or radiotherapy, myelotoxicity, enabling us to give higher doses resulting in higher clinical success rates.[14] The pathobiological consequences of the genetic polymorphism of ACE levels for leukaemic cell regulation and other ACE-mediated functions within the local bone marrow RAS remain to be investigated. Based on this biological background, the K562 human erythroleukaemia cell line may serve as an *in vitro* model to test the effects of RAS-affecting drugs on leukaemic cellular proliferation.

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