Short Communication

RHOA, *SEMA3B*, and *CKAP2* Expression in Leukaemia of Different Types: the Results of a Pilot Experiment

(leukaemia / gene expression / RT-PCR / *RHOA* / *SEMA3B* / *CKAP2*)

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Abstract. The transcriptional activity of *RHOA*, *SEMA3B*, and *CKAP2* genes was assessed in blood samples of leukaemia patients and healthy donors. In the blood of healthy donors, *RHOA* and *CKAP2* gene expression was not detected, and low *SEMA3B* gene expression was observed. Significant elevation of expression of all the three genes was shown in the case of acute myelogenous leukaemia. In cases of remission of acute lymphoblastic leukaemia and myelodysplastic syndrome, no expression of all three genes was detected. The long isoform of the *CKAP2* gene was highly expressed in most analysed types of leukaemia.

Introduction

Timely diagnosis of oncologic diseases is the key to successful treatment and survival of patients. Early alterations in the expression of genes that participate in carcinogenesis of various localizations may serve as markers of the disease progression. This approach may be particularly effective in monitoring different forms of leukaemia.

The ras homologue family member A *(RHOA)* gene encodes a protein that belongs to the well-known superfamily of small Ras GTPases (Etienne-Manneville and Hall, 2002). *RHOA* is known to take part in the processes related to formation of actin cytoskeleton, cell adhesion, and cellular motility (Vial et al., 2003; Parri and Chiarugi, 2010; Lessey et al., 2012). *RHOA* was shown to cause cellular transformation both *in vitro* and *in vivo*, and to participate in tumour invasion and metastasis (Kamai et al., 2003). Inhibition of activity of this gene at the level of transcription, translation, and/or at the post-translational level can revert the malignant phenotype of tumour cells, suppress their proliferation, and indirectly lead to apoptosis (Aznar et al., 2004; Liu et al., 2004). Elevated expression of both RHOA protein and mRNA was demonstrated in tumours of head and neck, mammary gland, stomach, colon, bladder, ovary, and testis (Pan et al., 2004). Growth of transcriptional activity of the *RHOA* gene in mammary gland and kidney tumours correlates with the increasing amount of gene copies in the genome, whereas a decrease in *RHOA* transcription is associated with the increase in the density of methylation of the 5'-regulatory region (Braga et al., 2006).

The semaphorin 3B *(SEMA3B)* gene encodes one of semaphorin proteins. Semaphorins are a large family of cytoplasmic and membrane-bound proteins that includes up to 20 members in the mouse and human (Yazdani and Terman, 2006). The *SEMA3B* gene is expressed in the lung, kidney, mammary gland, colon, etc. (Roche and Drabkin, 2001). The SEMA3B protein is an antagonist of receptors for neuropilins 1 and 2 (Np1 and Np2) that also act as receptors for several isoforms of vascular endothelial growth factor (VEGF), which is a general initiator of tumour angiogenesis, and thus *SEMA3B* suppresses vascular growth in tumours (Castro-Rivera et al., 2004). A decrease in transcriptional activity of this gene in tumours indirectly indicates that *SEMA3B* may participate in tumour cell growth suppression in the kidney, ovary, and colon (Pronina et al., 2009).

The cytoskeleton-associated protein 2 (*CKAP2)* gene was discovered (Maouche-Chrétien et al., 1998) and described (Rakhmanaliev et al., 2002) during the search

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Abbreviations: ALL rem1 – acute lymphoblastic leukaemia, remission 1, AML1 – acute myelogenous leukaemia, stage 1, AML rem1 – acute myelogenous leukaemia, remission 1, *CKAP2* – cytoskeleton-associated protein 2, CLL2 – chronic lymphocytic leukaemia, stage 2, CML3 – chronic myelogenous leukaemia, stage 3, GAPDH – glyceraldehyde-3-phosphate dehydrogenase, MDS5 – myelodysplastic syndrome, stage 5, Np – neuropilin, *RHOA* – ras homologue family member A, RT-PCR – reverse transcription PCR, *SEMA3B* – semaphorin 3B, VEGF – vascular endothelial growth factor.

for mRNA with increased expression in cells of diffuse B-cell lymphomas. The *CKAP2* gene product is a cytoplasmic protein that binds fibrils of the cytoskeleton (Maouche-Chrétien et al., 1998). For the *CKAP2* gene, two mRNA isoforms have been described – short (S isoform) and long (F isoform). The difference in length of the isoforms is caused by early termination of transcription after 6th exon in the S isoform (Bae et al., 2003). High activity of the *CKAP2* gene was revealed in primary stomach tumours in human. It is possible that protein CKAP2 is associated with cell cycle and cell proliferation control (Bae et al., 2003). Jin et al. (2004) showed that the mouse CKAP2 protein is localized in the cytoplasm, has a fibrillar appearance, and is co-localized with microtubules throughout the cell cycle. Presumably, the *CKAP2* gene may act as a regulator of aneuploidy, cell cycle, and p53-dependent apoptosis (Tsuchihara et al., 2005).

Material and Methods

In this study, the expression of *RHOA*, *SEMA3B*, and *CKAP2* mRNA was measured in blood samples obtained from patients with different types of leukaemia (six samples of total RNA extracted from the blood of adult patients): chronic lymphocytic leukaemia, stage 2 (CLL2); chronic myelogenous leukaemia, stage 3 (CML3); acute myelogenous leukaemia, stage 1 (AML1); myelodysplastic syndrome, stage 5 (MDS5); acute lymphoblastic leukaemia, remission 1 (ALL rem1); acute myelogenous leukaemia, remission 1 (AML rem1). As a control, lymphocytes of peripheral blood of six healthy adult donors were used. The study was approved by the Bioethics Committee of Lomonosov Moscow State University (record of April, 11, 2013) in accordance with the WMA Declaration of Helsinki regarding ethical principles for medical research involving human subjects.

Total RNA was extracted with commercially available kits "Trizol RNA Prep" (Isogene Lab Ltd, Moscow, Russia). Expression was assessed using semi-quantitative RT-PCR with commercially available kits "GenePak® RT Core" (Isogene Lab Ltd). For yielding cDNA, 60 ng of total RNA of each sample per reverse transcription reaction were used. As reverse transcription control and inner control, the expression of house-keeping gene glyceraldehyde-3-phosphate dehydrogenase *(GAPDH)* was assessed.

We have already described oligonucleotide primers and PCR conditions for the amplification of cDNA fragments of *RHOA*, *SEMA3B*, and *GAPDH* genes and procedures of data analysis for semi-quantitative RT-PCR (Braga et al., 2006; Pronina et al., 2009). The primers and PCR conditions for *CKAP2* cDNA fragment amplification were as follows: CKAP2-F: TTCTGAATGCCTGAACTTGAT, CKAP2f-R: TTAACATCATGGGTTGGATCT, CKAP2s-R: AATTCCGAATTGTCTACTACACTG; 95 °C/15", 52 °C/10", 72 °C/30" (F isoform) or 20" (S isoform). Electrophoretic analysis of PCR products was performed in 2% agarose gel. Gels were analysed using the ViTran utility and software (Biokom Ltd, St-Petersburg, Russia).

In all analysed samples, *GAPDH* expression was observed (the average value is shown in Fig. 1; dispersion is 13 %).

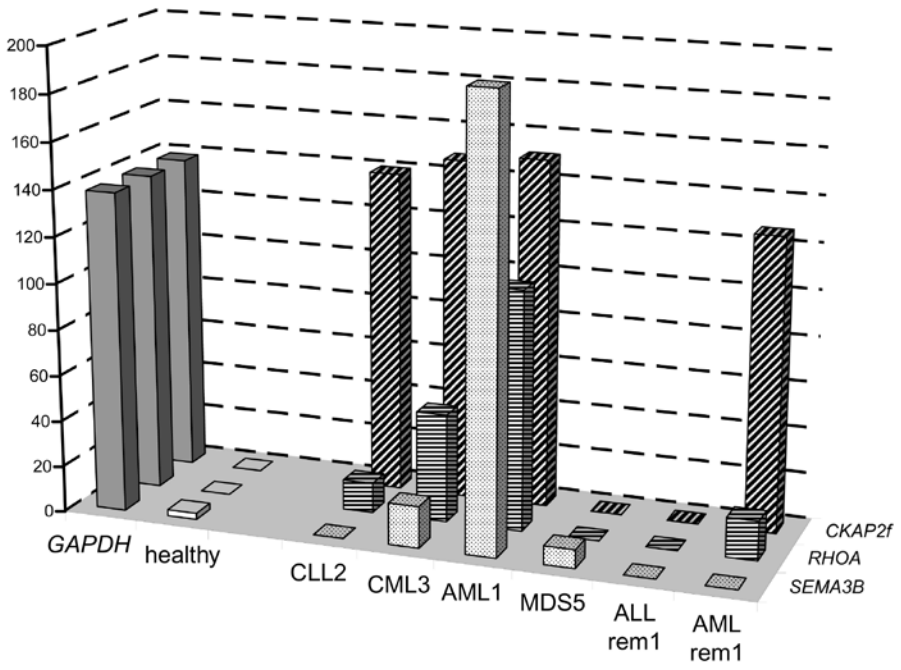

Fig. 1. Expression of genes *RHOA*, *SEMA3B* and *CKAP2* (long isoform) in blood samples of leukaemia patients. Mean value is shown for expression of the studied genes in healthy blood samples. Mean value between all samples is shown for control housekeeping gene expression (*GAPDH*). Vertical axis scale reflects normalized fluorescence intensity for each PCR product in agarose gel.

Results and Discussion

The expression of the *RHOA* gene and *CKAP2* long isoform was not detected in blood samples of healthy individuals. The *SEMA3B* gene retained low expression in the blood samples of healthy donors (as compared to *GAPDH* expression). In blood samples of healthy donors, the expression of the *CKAP2* short isoform was detected, which is why we considered inexpedient its comparative analysis in normal samples and in samples with different types of leukaemia.

We observed a significant increase in mRNA amounts of all three genes in AML. In the case of ALL remission, no expression of all three genes was detected. In the case of MDS, the expression of *RHOA* and *CKAP2* long isoform was not observed either, and the transcriptional activity of the *SEMA3B* gene slightly increased. The long isoform of *CKAP2* was expressed at a high level comparable to that of *GAPDH* in the case of AML and remission of AML, as well as in chronic types of leukaemia. This could indicate lower specificity of the transcriptional activation of *CKAP2* in blood cells in the studied types of leukaemia.

Our results may provide the basis for further research of the involvement of the studied genes (*RHOA*, *SEMA3B*, and *CKAP2*) in the development of leukaemia of different types. Today, the possibility of using the expression of these genes either at the mRNA or protein level for early diagnosis and subsequent monitoring of leukaemia is just as attractive as ever.

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