Deregulation of RBP4 and RBP7 Genes in Clear Cell Renal Cell Carcinoma

Anna Viktorovna Kudryavtseva1,2, Kirill Mikhailovich Nyushko2, Andrew Rostislavovich Zaretsky3,4, Dmitriy Alekseevich Shagin3, Boris Yakovlevich Alekseev2, Andrey Dmitrievich Kaprin2, Anastasiya Vladimirovna Snezhkina1

1Department of Postgenomic Research, Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia, 2Department of Pathology, National Medical Research Radiological Center, Ministry of Health of the Russian Federation, Moscow, Russia, 3Institute of Translational Medicine, Pirogov Russian National Research Medical University, Moscow, Russia, 4Evrogen Lab LLC, Moscow, Russia

Abstract

Background: The incidence of clear cell renal cell carcinoma (ccRCC) steadily increases each year, while the molecular mechanisms involved in the initiation and progression of the disease remain unclear. Therefore, the search for genes that could be potential targets for therapy as well as diagnostic and prognostic markers is an important task. In this work, we studied the expression of genes, encoding retinol binding proteins, in ccRCC. Materials and Methods: Using quantitative polymerase chain reaction, we analyzed the expression of RBP2, RBP4, and RBP7 genes in a representative set of ccRCC samples. Results: We identified deregulation of RPB4 and RBP7 genes in ccRCC. Significant decrease in the expression of the RBP4 gene was shown in most ccRCC samples. In contrast, the increase in mRNA level of the RBP7 gene was observed in almost all cases. Conclusions: Obtained results allow us to assume that RBP4 and RBP7 could be involved in ccRCC carcinogenesis. Moreover, our data confirm alterations in retinoid metabolism in ccRCC.

Key words: Clear cell renal cell carcinoma, gene expression, qPCR, retinol binding proteins, retinol metabolism

INTRODUCTION

Renal cancers account for approximately 2-3% of all malignancies in adults, and renal cell carcinoma (RCC) is diagnosed in 80-90% of the cases. However, the origin and evolution of RCC are poorly understood. Most RCCs are sporadic, but around 2-4% of cases result from familial susceptibility. The disease is almost two times more common in men than in women. Clear cell RCC (ccRCC) represents the largest subgroup of RCC and has the highest rates of local invasion, metastasis, and mortality.1,2 It is known that ccRCC is a metabolic disorder. Numerous genetic and epigenetic changes, occurring in ccRCC, effect transcription of many genes and lead to alterations in different metabolic pathways.3-8 Deregulation of metabolic pathways in ccRCC determines its aggressive phenotype.9 Understanding the molecular mechanisms leading to altered metabolic pathways in ccRCC could provide novel targets for therapies and diagnostic biomarkers.

Vitamin A (retinol) and its derivatives play an important role in many biological processes and influence cell differentiation, proliferation, and apoptosis.10 A number of studies have suggested an association between development of cancer and dietary intake of total vitamin A.11 Retinoids were shown to be active in the treatment of preneoplastic diseases12,13 and different malignancies, including breast, lung, skin, kidney, hepatic, and other cancers.14-19 These provide a basis for the use of retinoids in cancer treatment and chemoprevention.

In this work, we have analyzed the expression of several genes encoding retinol binding proteins in ccRCC using quantitative polymerase chain reaction (qPCR). Deregulation in the expression of RBP4 and RBP7 genes was revealed.

Address for correspondence:
Anna Viktorovna Kudryavtseva, Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia. E-mail: rhizamoeba@mail.ru

Received: 30-01-2017
Revised: 08-03-2017
Accepted: 14-03-2017
Obtained results may indicate the alterations in retinoid metabolism during ccRCC carcinogenesis and involvement of RBP4 and RBP7 genes in the process.

MATERIALS AND METHODS

Tissue samples

A total of 38 samples of ccRCC (I-IV Stages) and adjacent morphologically normal tissues (conventional “normal” tissues) were obtained after surgical resection before radiation or chemotherapy. The samples were frozen immediately after surgery and stored in liquid nitrogen. The diagnosis was verified by histopathology, and only samples containing 70-80% or more tumor cells were used in the study. Each patient provided written consent and gave permission for the use of their samples for research purposes. The study was approved by the Ethics Committee of Herzen Moscow Cancer Research Institute, the Ministry of Health of the Russian Federation. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki (1964).

RNA isolation and cDNA synthesis

RNA was isolated from the samples using mikro dismembrator S (Sartorius, Germany) and RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer’s protocols. RNA was quantified on Nanodrop spectrophotometer (Nanodrop Tech., USA). The Agilent RNA 6000 Nano Kit and Agilent 2100 Bioanalyzer system (Agilent Tech., USA) were used to determine the RNA integrity number (RIN). The isolated RNA was treated with DNAsa I (Thermo Fisher Scientific, USA) according to manufacturer’s instructions. cDNA synthesis was performed using M-MuLV Reverse Transcriptase (Thermo Fisher Scientific, USA) and random hexamers according to the standard manufacturer’s protocol.

qPCR and statistical analysis

qPCR reactions were carried out on applied biosystems 7500 real-time PCR System (Thermo Fisher Scientific, USA). TaqMan Gene Expression Assays (RBP2: Hs00188160_m1, RBP4: Hs00198830_m1, RBP7: Hs00364812_m1) (Thermo Fisher Scientific, USA) were used to estimate the mRNA level of target genes. qPCR procedure was performed as described.\textsuperscript{[20]} PCR products were analyzed in 2% agarose gels, purified and submitted for Sanger sequencing on an ABI Prism 3100 Genetic Analyzer (Thermo Fisher Scientific, USA). qPCR data were analyzed using two reference genes, GUSB and RPN1,\textsuperscript{[21,22]} and ΔΔCt-method with ATG program (Analysis of Transcription of Genes).\textsuperscript{[23-25]} Each reaction was repeated three times. At least 2-fold mRNA changes were considered as significant because of reference genes variability.

RESULTS

Down-regulation of RBP4

We revealed more than 100-fold down-regulation of the RBP4 gene in 87% (33 of 37, P < 0.05) of ccRCC samples [Figure 1]. Decreased expression of the RBP4 gene was particularly observed in ccRCC of stages II and III. The mRNA level of RBP4 was increased in two cases by 3- and 20-fold. The mean value of relative mRNA level decrease was 32.6.

Up-regulation of RBP7

A significant increase in the mRNA level of the RBP7 gene was observed in 79% (30 of 38, P < 0.05) of examined ccRCC samples [Figure 2]. Up-regulation of RBP7 was mainly found in early-stage ccRCC. Decreased expression of RBP7 was detected in three cases (from 4- to 8-fold). The mean value of relative mRNA level increase was 15.4.

The mRNA level of RBP2 gene was very low and could not be detected by qPCR method.

DISCUSSION

Vitamin A is obtained from a diet in the form of retinyl esters (animal sources) or carotenoids (plant food). In the intestine, retinyl esters/carotenoids are converted to retinol, which is secreted through the lymphatic system into the blood and is taken up by hepatocytes. The liver stores the retinol and provides it to the body. Retinol is secreted from the liver into the blood and bound to plasma RBP4. Although the liver is the main site of RBP4 synthesis, the protein can also be expressed and presumably secreted by other tissues, such as adipose, lung, kidney, testis, brain, and retinal pigment epithelium in the eye.\textsuperscript{[26]} Cellular RBPs (RBP2, RBP5, and RBP7) play a role in the cellular metabolism of retinol and vitamin A stability. For example, the vitamin A metabolite, retinoic acid, can regulate the transcription of numerous genes, and its effect is mediated by cellular retinoid binding proteins.\textsuperscript{[27-29]}

Recent studies have reported that RBPs may be involved in the development of cancer. The plasma level of RBP4 was shown to be associated with risk of colon adenoma,\textsuperscript{[30]} and the increased serum level of RBP4 was detected in pancreatic cancer patients.\textsuperscript{[31]} It has been suggested that the decrease in the serum level of RBP4 might be a potential biomarker of ovarian cancer.\textsuperscript{[32]}
Kudryavtseva, et al.: Deregulation of RBP4 and RBP7 genes in ccRCC

was shown in esophageal squamous cell carcinoma. In addition, other RBPs have been studied to a lesser extent, and their participation in cancer was not shown.

Using qPCR, we first revealed deregulation of RBP4 and RBP7 genes in ccRCC. A decreased mRNA level of the RBP4 gene was demonstrated in the examined samples, in contrast, a significant increase in RBP7 expression was shown in most ccRCC cases. These data indicate that alterations of retinoid metabolism, effecting both intra- and extracellular transport of retinol, may be associated with the development of cancer. Moreover, the findings implicate RBP4 and RBP7 expression levels and their correlation with the disease stage as potential diagnostic or prognostic biomarkers of ccRCC.

CONCLUSION

Our study revealed a significant deregulation of RBP4 and RBP7 gene expression in ccRCC. These proteins play an important role in retinoid metabolism. Extracellularly and intracellularly, they bind to retinol, solubilize and stabilize it, and then transport retinol from retinoid stores to target tissues. Thus, the obtained results allow us to assume that alterations in the regulation of the transport, metabolism, and action of retinol may occur in ccRCC carcinogenesis.

ACKNOWLEDGMENTS

The authors thank the National Medical Research Radiological Center for the collection and characterization of ccRCC samples, Evrogen Lab LLC for RNA isolation and Sanger sequencing, and Engelhardt Institute of Molecular Biology for the opportunity to perform qPCR experiments using the equipment of EIMB RAS “Genome” center (http://www.eimb.ru/ru/ckp/ccu_genome_c.php).

REFERENCES


Source of Support: This work was financially supported by the Russian Science Foundation grant no. 14-35-00105. Conflict of Interest: None declared.