Molecular Biology Reports (2021) 48:5561–5569
https://doi.org/10.1007/s11033-021-06573-w

ORIGINAL ARTICLE

Methylation and expression levels of microRNA-23b/-24-1/-27b, microRNA-30c-1/-30e, microRNA-301a and let-7g are dysregulated in clear cell renal cell carcinoma

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Received: 30 September 2020 / Accepted: 15 July 2021 / Published online: 24 July 2021

Abstract

Background Renal cell carcinoma is the most common form of kidney cancer in adults. DNA methylation of regulatory sequences at the genomic level and interaction between microRNAs and the messenger RNAs of target genes at the post-transcriptional level contribute to the dynamic regulation of gene activity. Aberrations in these mechanisms can result in impaired functioning of cell signaling pathways, such as that observed in malignant tumors. We hypothesized that microRNA genes methylation may be associated with renal cancer in patients.

Methods and results We examined methylation levels of 22 microRNA genes in tumor and normal kidney tissue of 30 patients with TNM Stage III clear cell renal cell carcinoma using a pathway-specific real-time polymerase chain reaction array (EpiTect Methyl II PCR Arrays, Qiagen). MicroRNA expression analysis by quantitative polymerase chain reaction was also performed. Significant differences in methylation levels were found in two genes and in two clusters of microRNA genes. MicroRNA-23b/-24-1/-27b, microRNA -30c-1/-30e and let-7 g was hypermetylated in clear cell renal cell carcinoma tissue, microRNA -301a was hypomethylated in tumor compared with the adjacent normal tissues. Expression of microRNA-301a, microRNA-23b in the clear cell renal cell carcinoma tissues was significantly overexpressed when compared with the adjacent normal tissues and let-7 g was significantly downregulated in tumor.

Conclusions Our results may indicate the contribution of microRNA-301a, microRNA-23b and let-7 g in the pathogenesis of renal cancer, but further studies are needed to determine the functional significance of the detected changes.

Keywords Renal cell carcinoma · microRNA · DNA methylation

Introduction

Oncological diseases of the genitourinary system are one of the most important problems of modern clinical medicine. The most common forms of oncourological diseases are prostate, bladder and kidney cancer, which account for about a quarter of all malignant human tumors [1]. The first place in terms of mortality is occupied by kidney cancer [2]—a heterogeneous group of malignant tumors, the vast majority of which are renal cell carcinomas (RCC) of various morphological types. Most often, the clear cell variant of renal cell carcinomas (ccRCC) occurs, which accounts for about 80% of cases of kidney cancer [3]. According to the GLOBOCAN platform in 2018, more than 400,000 new cases of kidney cancer and 175,000 deaths from this disease were registered in the world [4]. The incidence of RCC in the world varies widely from region to region. The highest incidence rates are in the Czech Republic (standardized ratio 16.7 per 100,000 population), Lithuania (13.2), Slovakia (12.5) and North America (11.7), and the lowest in Central Africa (0.6), West Africa (0.7) and sub-Saharan Africa (0.9) [5]. In 2018, 23,157 cases of primary renal cancer were diagnosed in Russia [6, 7].

The asymptomatic course of the disease leads to the locoregional disease in about 15% of patients with kidney cancer at the time of diagnosis. In addition, about half of

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these patients are prone to relapses of the disease [8], and the resistance of kidney cancer to chemotherapy and radiation therapy, as well as the absence of specific targeted therapy, increases the mortality rate. In this regard, studies aimed to investigate the molecular mechanisms of RCC development will contribute to the improvement of the prevention, diagnosis and therapy of this disease.

To date, a change in the regulation of microRNAs, small non-coding RNAs that carry out post-transcriptional regulation of genes activity, is considered to be an important phenomenon in the onset and development of a malignant tumor [9, 10]. Numerous studies demonstrate that dysregulation of microRNA is often associated with promoter methylation or a change in the number of gene copies [11, 12].

One of the most studied epigenetic mechanisms of the expression regulation of coding and non-coding genes is DNA methylation [13, 14]. Methylation of microRNA genes affects the regulation of gene activity at several levels at once, modify the ability of microRNAs to post-transcriptional regulation. There are many studies confirming the contribution of microRNA genes promoter methylation regions to the development of various types of tumors, including renal cell carcinomas. A number of studies have demonstrated the promoter hypermethylation of various microRNAs in renal cell carcinoma. In tumor tissues of renal cell carcinoma, hypermethylation of microRNA-9–1, known as a tumor suppressor in breast and stomach cancer, has been shown [15]. In various types of cancer, an increase expression of oncogenic microRNA-21 is often described, which is closely associated with cell growth, invasion, and metastasis [16]. Interestingly, the target of microRNA-21 is the tumor suppressor PTEN gene, a negative regulator of the PI3K/AKT/mTOR signaling pathway [17]. In addition, the expression level of microRNA-21 is associated with the activity of the tumor suppressor p53 [18]. TCGA studies have demonstrated the association of hypomethylation of the microRNA-21 promoter with increased expression in clear cell renal cell carcinoma [19]. In addition, this mechanism of regulation of microRNA-21 activity was demonstrated for other types of cancer—breast cancer, lung adenocarcinoma, hepatic carcinoma, papillary renal cell carcinoma, and pancreatic adenocarcinoma [16].

MicroRNA promoter methylation is often associated with the prognosis of the disease, progression, overall and disease-free survival of patients with ccRCC. The promoter hypermethylation in renal cell carcinoma is observed for microRNA-429, microRNA-30a-5p, microRNA-34a, microRNA-34b / c, microRNA-124, microRNA-182, etc. It was shown that a decrease of the microRNA-429 promoter methylation and a return to the normal level of expression promoted inhibition of proliferation and invasion, stimulation of apoptosis and cell adhesion. It is assumed that the restoration of the normal level of microRNA-429 activity in renal cell carcinoma may be promising as a therapeutic target [20]. Similar data were obtained when studying microRNA-34a in renal cell carcinoma cell lines and in mouse models. Restoration of the expression level as a result of demethylation of the promoter regions of microRNA-34a led to a decrease in cell growth, tube formation, migration, and invasion. It was observed that the CD44 gene, which plays a role in intercellular interactions, cell adhesion and migration, can act as a target of microRNA-34a [21].

In addition, genes of the Notch pathway involved in the regulation of apoptosis, proliferation, angiogenesis, metastasis, and other cellular processes that induce the emergence and development of malignant tumors can act as a target of microRNA-34a [22]. In turn, microRNA-34a can be a target for the action of the p53 tumor suppressor gene product. Increased expression of microRNA-34a causes cell cycle arrest in G1, senescence, and apoptosis. At the same time, inhibition of microRNA-34a as a result of methylation prevailed over its transactivation by p53 after DNA damage [23]. MicroRNA-124 can also participate in the regulation of apoptosis and cell proliferation, targeting the apoptosis inhibitor protein LIVIN. It is noted that in renal cell carcinoma, a decrease in the expression of microRNA-124 occurs as a result of hypermethylation of its promoter. In addition, expression levels correlate with response to chemotherapy, namely, an increase in expression promoted the chemotherapeutic sensitivity of tumor cells, while a decrease in microRNA-124 levels produced the opposite effect [24].

Another microRNA, microRNA-182, was also hypermethylated in renal cell carcinoma, which was accompanied by a decrease in the level of its expression in tumor tissues as compared to the adjacent normal kidney tissue. MicroRNA-182, like many other microRNAs, in various types of cancer can exhibit either a tumor suppressor or oncogene properties. In renal cell carcinoma, microRNA-182 has been shown to inhibit tumor cell proliferation, at least in part, by targeting the target gene FLOT1 [25].

The promoter hypermethylation in renal cell carcinoma is also demonstrated by microRNA-30a. It was shown that the levels of microRNA-30a methylation correlate with the duration of disease-free survival and metastasis. In addition, the methylation level of this microRNA can be assessed in urine samples from patients, which is an extremely promising tool for minimally invasive diagnosis and prognosis of the disease [26].

In a recent study based on omix data from TCGA, hypermethylation of promoters of microRNA genes-25, -26b, -10b, -30c1, -let7e, -219a1, -125a, -125b1 was also shown in papillary renal cell carcinoma [27]. Thus, despite the active accumulation of data on the contribution of microRNA gene methylation to the development of renal cell carcinomas, further study of this issue is required. In the present study, we studied the methylation and expression profile

of a number of microRNAs involved in the development of various types of cancer in patients with clear cell renal cell carcinoma. We found hypermethylation of promoters of microRNA-23b/-24-1/-27b, microRNA-30c-1/-30e, and microRNA let-7 g clusters, as well as a decrease in methylation of the promoter region of microRNA-301a.

Materials and methods

Clinical specimens

A total of 30 pairs of matched ccRCCs and normal renal parenchyma tissues from patients who underwent radical nephrectomy at Bashkir State Medical University Clinic were studied. The study was performed according to the ethical standards of Bioethics Committee developed the Declaration of Helsinki of the World Association "The ethical principles of medical research involving human subjects" and with the ethical standards of the Research Ethics Committee of the Institute of Biochemistry and Genetics—Subdivision of the Ufa Federal Research Centre of the Russian Academy of Sciences. Informed written consent was obtained from all individual participants included in the study. All patients included in the study had clear cell renal cell carcinoma at the third tumor stage according to TNM classification, confirmed histologically after surgery, and did not receive chemotherapy and radiation therapy in the preoperative period. Tumor tissue was taken from areas without necrosis and hemorrhage and from three points for the same tumor to compensate for tumor heterogeneity. Samples of tumor and normal kidney tissue included in the study were received and examined by two independent pathologists at the pathology department of the Bashkir State Medical University Clinic. Tumor tissue samples contained more than 80% of tumor cells. Normal renal parenchyma did not contain tumor cells. Samples were processed and stored at − 70 °C until DNA/RNA extraction. Tissue samples for RNA extraction were stored in EverFresh RNA reagent.

Tissue DNA and RNA extraction

Genomic DNA was extracted from frozen tissue samples using the phenol–chloroform methodology. Total RNA was extracted from 20 mg tissue samples using Direct-zol™ RNA MiniPrep (Zymo Research, Irvine, CA, USA) following manufacturer's instructions. The concentration and purity of DNA and RNA was determined by measuring its optical density (A260/280 > 2.0; A260/230 > 1.8) using NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA).We used DNA samples for methylation analysis with ratios and values A260/A230: > 1.7, A260/A280: > 1.8, A260 concentration: > 4 μg/ml, according manufactorer recommendations. For each reaction, 5 ng of total RNA was used in the microRNA expression analysis (manufacturer's instructions recommended use 1 to 10 ng total RNA per reaction). Samples of nucleic acids were stored at − 70 °C, or were used immediately for subsequent analysis.

Treatment of tissue DNA with restriction enzymes

In preparation for the subsequent methylation analysis, DNA isolated from kidney tissue was treated with methylation-dependent and methylation-sensitive restriction enzymes, capable of digesting methylated and unmethylated DNA, respectively, using the EpiTect Methyl DNA Restriction Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions.

Methylation qPCR array

Methylation analysis of CpG islands of 22 primary microRNAs (let-7 g, let-7i, microRNA-10a, microRNA-1–1, microRNA-124–2, microRNA-126, microRNA-149, microRNA-155, microRNA-15b, microRNA-16–2, microRNA-17 Cluster, microRNA-191, microRNA-425, microRNA-193b, microRNA-210, microRNA-218–1, microRNA-218–2, microRNA-23b, microRNA-24-1, microRNA-27b, microRNA-301a, microRNA-30c-1, microRNA-30e, microRNA-32, microRNA-34c, microRNA-34b, microRNA-378, microRNA-7–1) involved in the development of various types of cancer, including kidney cancer was performed. Among the targets of these microRNAs are genes the products of which are involved in cell proliferation, regulation of transcription and apoptosis. After restriction enzyme digestion, DNA was quantified using real-time polymerase chain reaction using array plate (EpiTect Methyl II PCR Arrays, Qiagen) containing pre-aliquoted primer mixes in a Bio-Rad CFX96™ real-time system.

MicroRNA expression analyses

The expression analysis for the microRNA genes with differential methylation levels was conducted. Complementary DNA was synthesized using total RNA and a reverse transcription reaction with TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems, Waltham, MA, USA).

Reactions were run in a 20-mL final volume using TaqMan® Small RNA Assays (Applied Biosystems) targeting let-7 g, cluster microRNA-23b/-24-1/-27b, cluster microRNA-30c-1/-30e and microRNA-301a genes. U6 was used as internal control gene for normalization. All reactions were carried out in duplicate and in the presence of a negative control lacking cDNA. The PCR conditions were chosen according to the manufacturer's protocol.

Statistical analysis

Data analysis was performed using a Microsoft Excel-based data analysis template for EpiTect Methyl II PCR (Qiagen, www.sabiosciences.com/dna_methylation_data_analysis.php), and GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA). P values were determined by Mann Whitney test, $P \leq 0.05$ were considered statistically significant. Values are given as median with range of two experiments.

Table 1 Clinical characteristics of patients with clear cell renal cell carcinoma

Age (years)	
Range; median	37–79; 58
Sex [n (%)]	
Male	15 (50.0)
Female	15 (50.0)
Tumor size (cm)	2.4–6.5
Fuhrman grade [n (%)]	
1–2	17 (56.7)
3–4	13 (43.3)
Metastasis classification [n (%)]	
M0	30 (100.0)
M1	0 (0.00)
Pathological lymph node status [n (%)]	
N0	29 (96.7)
N1	1 (3.3)

Results

A study of microRNA genes methylation was performed on 30 paired fresh frozen samples of normal and tumor kidney tissue of patients with clear cell renal cell carcinoma. All patients were in clinical stage III according to TNM classification. The clinical characteristics of the specimens are summarized in Table 1.

DNA methylation analysis

We determined the methylation levels of 22 microRNA genes. Figure 1 represents the methylation levels in tumor and normal kidney tissue samples. Overall, the percentage of promoter methylation of microRNA genes was obviously low in renal tissue samples both in normal and in tumor. Significant differences were found in two genes and in two clusters of microRNA genes. MicroRNA-23b/-24-1/-27b, microRNA-30c-1/-30e and let-7 g was hypermetylated in ccRCC tissue, while microRNA-301a was hypomethylated in tumor compared with the adjacent normal tissues. Among studied microRNA genes, the highest methylation levels were noted for cluster microRNA23b/-24-1/-27b.

Gene expression analyses

We performed expression analysis to evaluate the effect of promoter methylation on the activity of the investigated microRNAs genes. The expression levels of let-7 g, cluster microRNA-23b/-24-1/-27b, cluster microRNA-30c-1/-30e

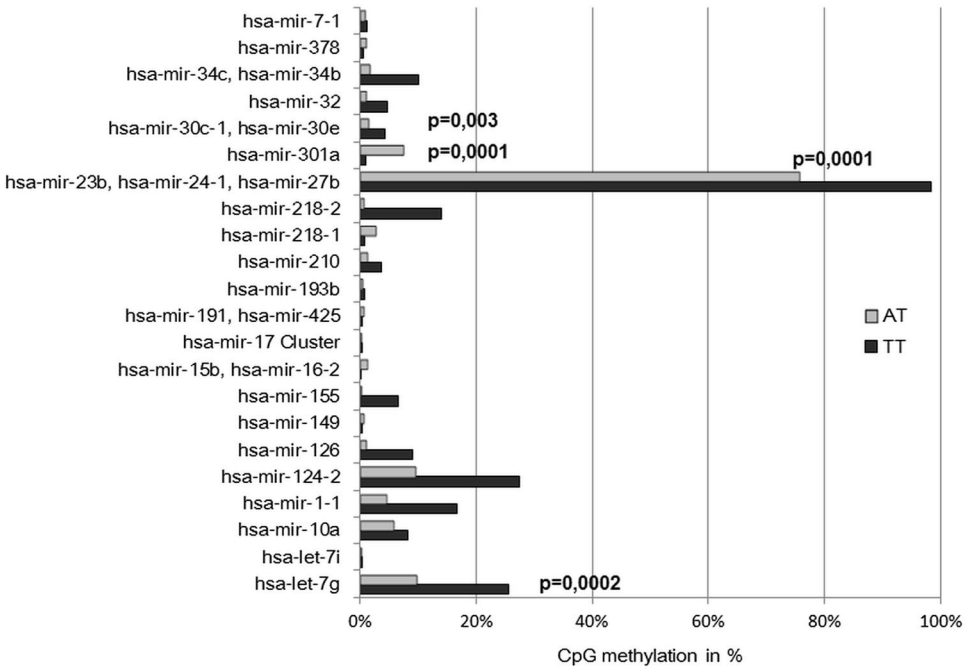

Fig. 1 Frequency of the promoter methylation of 22 microRNA genes. P-value was calculated with Mann Whitney test. $P \leq 0.05$ were considered statistically significant. *AT* adjacent tissue, *TT* tumor tissue

and microRNA-301a were determined in 30 pairs of fresh frozen tumor and normal renal tissue of ccRCCs patients by quantitative real-time PCR. In order to avoid random results and outliers, samples falling into the + 3sd area were excluded from the analysis. After exclusion significant differences in microRNA expression were revealed between tumor and normal kidney tissue. Thus, a significant decrease in let-7 g expression levels was detected in the renal tumor tissue compared to adjacent normal tissues (P = 0.0178), as shown in Fig. 2a.

In contrast, microRNA-23b (Fig. 2b) and microRNA-301a (Fig. 2c), showed increased expression levels in tumor compared with normal tissue. MicroRNA-24-1, microRNA-27b, microRNA-30e and microRNA-30c, did not show any statistically significant differences between tumor and normal tissues (Online Resource 1).

Discussions

This study examined the methylation profiles of 22 microRNA genes in patients with clear cell renal cell carcinoma. It is well known that methylation inhibits gene transcription, thus regulating their activity [28].

We found a significant alteration in the methylation and expression levels of the microRNA-23b/-24-1/-27b cluster in renal tumor tissue samples compared to normal renal parenchyma. Thus, a significant increase in the cluster microRNA-23b/-24-1/-27b promoter methylation level and microRNA-23b expression in the renal tumor tissue compared with normal adjacent tissue was shown. There was a clear mismatch in gene activity relative to the epigenetic regulatory mechanism. This phenomenon may be related to the specific organization of the microRNA-23b/-24-1/-27b gene, which represents an intron cluster structure in the region of human chromosome 9q22.32 [29]. The expression levels of microRNA-24-1 and microRNA-27b were lower in the tumor, but were not statistically significant.

The microRNA-23b/27b/24 cluster consists of three microRNA genes located in the intron of the C9orf3 gene. Previous studies have reported on the conflicting functions of this cluster in the onset and progression of malignant tumors. Most studies of the microRNA-23b-27b-24 cluster are focused on the study of individual microRNAs of this cluster and specific target genes. At the same time, the effects of the whole cluster on tumor progression, as well as the exact mechanism of regulation of cluster expression, have been little studied [30].

In a study by Hovestadt et al., it was proposed that there is a short transcript of the C9orf3 gene, the methylation of which, along with methylation of the microRNA-23b-27b-24 cluster, negatively correlates with their expression [31]. Whereas in colon cancer, DNA methylation has been shown to be involved neither in the regulation of transcription of the short mRNA C9orf3, nor in the expression of the microRNA-23b-27b-24 cluster [30].

It is known that one of the targets of microRNA-23b is the NOTCH2 gene, an important participant in the Notch pathway [32]. Hypermethylation of microRNA-23b was noted in glioma, which led to the silence of this microRNA [33], as well as in prostate cancer [34] and cervical cancer [35]. It is assumed that in the oncogenesis of the prostate gland microRNA-23b acts as a tumor suppressor, helps to reduce migration, invasion, proliferation of tumor cells, and also inhibits the epithelial-mesenchymal transition [34].

The study of the microRNA-23b/27b cluster in renal cell carcinoma showed a significant decrease in expression in renal tumor tissues as compared to normal tissues, as well as an association of the expression level of this cluster with the pathological stage and overall patient survival. In addition, restoration of microRNA-23b and microRNA-27b expression levels inhibited cell proliferation,

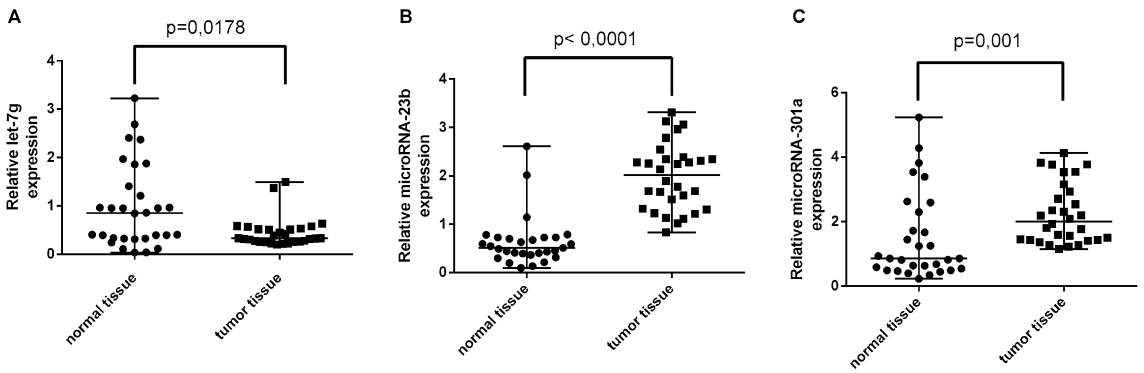

Fig. 2 Expression analysis of microRNA genes in ccRCC tissue showing differential methylation levels. Values are given as means with range. Let-7 g (**a**) expression levels were significantly lower in ccRCC tissues in comparison with matched adjacent tissues. MicroRNA-23b (**b**) and microRNA-301a (**c**) expression levels were significantly higher in ccRCC tissues in comparison with matched adjacent tissues. P-value was calculated with Mann Whitney test. $P \leq 0.05$ were considered statistically significant

migration, and invasion [36]. It is also assumed that increased expression of microRNA-23b and microRNA-27b is associated with a prolonged response to sunitinib therapy in patients with clear cell renal cell carcinoma [37]. On the other hand, there is information about the oncogenic nature of microRNA-23b. Zaman et al. reported that microRNA-23b is capable of inhibiting the expression of the tumor suppressor gene PTEN. Moreover, it has been shown that a high level of microRNA-23b expression correlates with a lower 5-year survival rate in patients with clear cell renal cell carcinoma [38]. In addition, experiments on RCC cell lines demonstrated an increase in apoptosis and a decrease in the invasive potential of cells with a decrease of microRNA-23b expression. Thus, the question of the role of microRNA-23b in renal cell carcinoma remains open.

Another microRNA cluster that demonstrated significant alterations in methylation levels in our study was the microRNA-30c/-30e cluster. An increase of the microRNA-30c/-30e cluster methylation level in the renal tumor tissue compared with normal renal parenchyma was shown, while the expression analysis of microRNA-30c and microRNA-30e did not show statistically significant differences in the expression levels in tumor and normal renal tissue.

It is known that the microRNA-30 family is involved in the formation of pronephros [39], podocyte homeostasis [40] and is widely expressed in the kidneys. The microRNA-30c/30e cluster is located in one intron of the Nfyc gene. It is known that the microRNA-30 family is highly conserved and targets the Notch1 ligand DLL4 [41]. MicroRNA-30c, as shown in various studies, is capable of inhibiting the invasion of tumor cells and metastasis, and hypermethylation of its promoter causes a sharp decrease in activity [42]. One of the targets of microRNA-30c is the GALNT2 gene, which encodes an O-glycosylating enzyme that regulates the EGFR receptor, a well-known participant in tissue malignancy [43]. Another important target for microRNA-30c is predicted to be HIF2a. In clear cell renal cell carcinoma, it was shown that microRNA-30c, along with microRNA-30a, are able to regulate the protooncogene HIF2a [44]. In our study, hypermethylation of the microRNA-30c / -30e cluster was found; however, the expression levels of these microRNAs did not show statistically significant differences in the tumor and normal kidney tissue. Nevertheless, in the works of other authors, it was found that in renal cell carcinoma, there is a significant decrease in the expression of microRNA-30c in the tumor compared to normal kidney tissue [45]. In addition, it was suggested that a decrease in the expression of microRNA-30c is a specific event specifically for clear cell renal cell carcinoma, but not for tumors of other localization [46]. At the same time, increased expression of microRNA-30c inhibits the progression of clear cell renal cell carcinoma, invasion, migration and growth of tumor cells, and also improves the sensitivity of the tumor to anticancer agents [47].

The second member of the microRNA-30 cluster, microRNA-30e, changes expression levels in many types of cancer. In clear cell renal cell carcinoma, microRNA-30e as well as microRNA-30c demonstrates the properties of a tumor suppressor. It has been shown that microRNA-30e can inhibit cell proliferation and invasion, possibly by targeting the SOX9 [48] and Snail1 [49] gene. In addition, the therapeutic potential of microRNA-30e in combination with proanthocyanidin in glioblastoma was noted. The use of a combination of these molecules promoted the induction of internal and external pathways of apoptosis by inhibiting the activation of caspases AVEN and BIRC6 [50]. Hypermethylation of microRNA-30e has been shown in hepatoma cells compared to normal liver cells [51].

In the present study, we found a higher level of let-7 g methylation in ccRCC samples compared to the normal renal tissue, while let-7 g expression levels were significantly reduced ($p = 0.016$) in tumor tissues compared to corresponding non-cancerous tissues. MicroRNA let-7 g belongs to the well-known tumor suppressive family Let-7, the targets of which are the well-known proto-oncogenes of solid tumors c-Myc, K-Ras, N-Ras, HMGA2, etc.[52]. One of the targets for let-7 g is predicted the type I collagen gene COL1A2. Early research showed that COL1A1 and COL1A2 can enhance cancer progression [53]. In this regard, a decrease in the activity of microRNA let-7 g as a result of promoter hypermethylation can lead to the activation of its targets and cancer progression.

Hypomethylation and an increase in the expression level of let-7 g microRNA were noted in ovarian cancer [54]. In mouse models of non-small cell lung cancer, it was demonstrated that the expression of microRNA let-7 g contributes to a decrease in the number of tumor foci and their size [52]. A decrease in let-7 g levels is observed in hepatocellular carcinoma cells. In addition, let-7 g expression was significantly lower in metastatic liver cancer compared with non-metastatic tumors [55].

Interesting data were obtained by studying the relationship between let-7 g expression and disease progression in clear cell renal cancer. It was found that the expression levels of this microRNA were reduced in tumor tissue compared with normal renal parenchyma; however, let-7 g was sequentially activated with an increase in the stage, grade and progression of the disease [56].

We also found a hypomethylation of microRNA-301a and an increased expression of it in the tumor compared to normal renal tissue. The role of microRNA-301a is also controversial in various types of cancer. On the one hand, it was shown that the expression level of microRNA-301a is significantly increased in the tumor tissue of non-small cell lung cancer compared to normal tissue [57]. At the same time,

downregulation of microRNA-301a was accompanied by a decrease in cell proliferation, migration, and invasion. On the other hand, in hepatocellular carcinoma, microRNA-301a is involved in inhibiting tumor growth and metastasis.

In the study of acute kidney injury, it was demonstrated that the expression of microRNA-301a can be regulated by methyl-CpG-binding domain protein 2 (MDB2) by demethylation of the promoter region. At the same time, it was shown that microRNA-301a is capable of inducing apoptosis by suppressing antiapoptotic genes (HDGF and MITF) and, thus, can be considered as a potential therapeutic target for VAN-induced AKI [58]. The ability of microRNA-301a to stimulate cell apoptosis in acute kidney injury contradicts somewhat the results of the analysis of its expression both in our study and in studies of other authors, which showed a correlation between increased expression of microRNA-301a with the development of tumors. Nevertheless, this may indicate a complex role of microRNA-301a, which can vary depending on the type of tissue, cells, and microenvironment.

It is known that microRNA-301a has increased expression levels under hypoxic conditions, which often accompanies the development of solid tumors. In addition, increased levels of expression of microRNA-301a promoted an increase in autophagy and radioresistance under hypoxic conditions in prostate cancer, which also testifies to the oncopromotor role of microRNA-301 [59].

One of the targets of microRNA-301a is the PTEN gene, a well-known tumor suppressor involved in the regulation of the cell cycle and proliferation. In mouse models, it was shown that the introduction of anti-microRNA-301a helps to reduce the growth of renal cell carcinoma tumors. It is assumed that high levels of microRNA-301a expression can disrupt the control of the cell cycle and provoke cell proliferation and, as a consequence, the development of cancer, including renal cell carcinoma [60].

The main limitation of our study is the relatively small number of clear cell renal cell carcinoma samples tested. Moreover, accuracy might be improved by adding additional microRNA to the panel. Further, we plan to perform investigations of microRNA genes methylation in lager and independent series to compare the clinical and pathological characteristics of tumors, which would be promising for predicting the course of the disease. These findings have provided some basis for further investigation of microRNA-23b/-24-1/-27b, cluster microRNA-30c/-30e, let-7 g, microRNA-301a and for evaluating the prognostic value by analyzing these microRNAs status in ccRCC patients.

Conclusions

In this study, we performed analysis of methylation levels of microRNA genes in clear cell renal cell carcinoma patients from Russia. We identified hypermetylation of cluster microRNA-23b/-24-1/-27b, cluster microRNA-30c/-30e, let-7 g and hypomethylation of microRNA-301a in ccRCC samples compared with normal renal tissue. Further expression analysis showed significant upregulation of microRNA-23b, microRNA-301a and downregulation of let-7 g in tumor compared with normal renal tissue. Our results may indicate the contribution of microRNAs in the pathogenesis of clear cell renal cell carcinoma, but further studies are needed to determine the functional significance of the detected changes.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-021-06573-w.

Author contribution GI and IE designed experiments and performed methylation, expression analyses, data analysis and interpretation and drafted manuscript. GG and SI contributed clinical material and collected data. IA and SR provided counseling on the clinical and pathological characteristics of the samples and drafted manuscript. KE and PV coordinated the study, and drafted manuscript. All authors read and approved the final manuscript.

Funding This work was supported by the Ministry of Education and Science of the Russian Federation [№AAA-A16-116020350032–1] and by the megagrant from the Government of Russian Federation No. 075-15-2021-595. In the study, DNA samples from the "Collection of Biological Materials of Human Beings" of the IBG UFRC RAS were used, supported by the Program of Bioresource Collections of the FASO of Russia [Agreement No. 007–030164/2].

Data availability The dataset supporting the conclusions of this article is included within the article.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The current study was performed according to the ethical standards of Bioethics Committee developed the Declaration of Helsinki of the World Association "The ethical principles of medical research involving human subjects" and approved by the Research Ethics Committee of the Institute of Biochemistry and Genetics—Subdivision of the Ufa Federal Research Centre of the Russian Academy of Sciences. A signed informed consent was collected from every study participant who agreed to participate in the study.

Informed consent Informed written consent was obtained from all individual participants included in the study.

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