

## RESEARCH ARTICLE

# miR-1267 Induces Tumorigenicity and Contributes to Risk of Clear Cell Renal Cell Carcinoma

Sercan Ergün<sup>1</sup>, Kalbiye Konanç<sup>2</sup>

<sup>1</sup> Department of Medical Biology, Faculty of Medicine, Ordu University, Ordu, Turkey

<sup>2</sup> Laboratory and Veterinary Health, Ulubey Vocational Higher School, Ordu University, Ordu, Turkey

Received: 23 May 2019, Accepted: 22 June 2019, Published online: 28 August 2019

© Ordu University Institute of Health Sciences, Turkey, 2019

## Abstract

**Objective:** Dysregulated microRNA signatures in different cancer types are being uncovered continually implying their significance in cancer pathogenesis. miR-1267 was not previously associated with RCC. In this study, it is aimed to obtain the expression profile of miR-1267 in patients with ccRCC and its correlation with patient parameters.

**Methods:** Kidney Cancer cDNA Array consisting of cDNA samples obtained from healthy kidney tissues of 4 healthy individuals and tumoral kidney tissues of 5 Stage I, 5 Stage II, 3 Stage III and 2 Stage IV ccRCC patients was used. Hsa-miR-1267 and SNORD48 (as housekeeping gene) expressions were analyzed. miR-1267 expression was statistically correlated with the clinical parameters of patients. miRgator 3.0 database was used to compare miR-1267 expression patterns of different urological cancer types.

**Results:** The expression of miR-1267 was significantly higher in male than female ( $p=0.027$ ). Also, there were statistically significant increase in miR-1267 expression in stage IV when compared to stage I ( $p<0.001$ ). Moreover, increased platelet/lymphocyte ratio and calcium level, which were parameters giving information about the occurrence of ccRCC, are significantly associated with increased miR-1267 expression ( $p<0.001$  and  $p=0.003$ , respectively). The expression of miR-1267 in kidney tumor tissues was higher approximately three times than normal kidney tissues ( $p>0.05$ ).

**Conclusion:** miR-1267 could have oncogenic function, have predictive value for RCC development and be predictive about aggressiveness in ccRCC.

**Key words:** miR-1267; ccRCC; platelet/lymphocyte ratio; high calcium level

**Suggested Citation:** Ergun S, Konanc K. miR-1267 Induces Tumorigenicity and Contributes to Risk of Clear Cell Renal Cell Carcinoma. Middle Black Sea Journal of Health Science, 2019; 5(2):100-105

---

## Address for correspondence/reprints:

Sercan Ergün

**Telephone number:** +90 (452) 226 5214 5251

**E-mail:** sercanergun@msn.com

**DOI:** 10.19127/mbsjohs.569404

## Introduction

Renal cell carcinoma (RCC) is different from kidney cancer with the involvement of renal pelvis or renal medullary and it is also the only type of cancer that occurs in cells (renal tubules) that extend into the kidney bed. RCC includes a range of heterogeneous cancers arising from renal tubular cells. RCC is the third most common cause of death after prostate and bladder cancer among the urological cancers and accounts for about 2% of all adult cancer patients. Moreover, its clinical course is the most fatal one among urological cancers. RCC is caused by the accumulation of many

genetic and epigenetic alterations as similar to other cancer types (Shingarev and Jaimes, 2017).

Clear cell renal cell carcinomas (ccRCCs) are ordinarily globular masses that may originate anywhere in the renal cortex and frequently extrude beyond the normal form of the kidney. ccRCC oftentimes attacks the renal venous system, sometimes filling the renal vein and growing longer into the vena cava or even the right atrium. ccRCC is the most frequent type of kidney cancer in adults by far (Znaor et al., 2015).

In the last twenty years, genetic and clinical researches have presented that ccRCC is both heterogeneous in its histology and clinical course, and heterogeneous in its genetic changes. The identification of various histological subtypes of ccRCC ensures a better comprehension of the molecular mechanism of these distinct subtypes of cancer and one or more crucial mutations were defined for each subtype. Sporadic cancers originate from multiple (epi)genetic alterations. Therefore, promoter hypermethylation of genes is considered to be involved in sporadic or hereditary forms of ccRCC. The epigenetic alterations that regulate the formation and progression of ccRCC are in the initial stages of reconnaissance yet. More detailed specification of epigenetically changed genes and pathways in ccRCC may canalize to the development of new and minimally invasive diagnostic and prognostic tools for ccRCC. For the future, epigenetic therapies may provide an additional treatment preference for advanced ccRCC that does not respond to standard therapy (Shingarev and Jaimes, 2017). Factors are potentially related with the pathophysiology of ccRCC and probably usable as biomarkers in the development of the disease need to be researched.

MicroRNAs (miRNAs) are functional RNA molecules of 18-28 nucleotides in length which are transcribed from exonic or intronic regions of protein coding genes and non-coding regions of the genome. miRNAs perform their functions by virtue of their ability to recognize complement genes to their nucleotide sequences. RISC complex formed by the addition of miRNA to the structure binds to mRNA and causes the inhibition of the protein translation of interested gene and / or the destruction of the mRNA (Garzon et al., 2009). Some studies have shown that miR-21, which has proven oncogenic properties in many cancers, triggers the emergence of tumorigenic properties by targeting tumor suppressor genes such as RCC-specific PTEN, SATB1, PDCD4, TCF21 and KISS1 (Yu et al., 2014; Kowalczyk et al., 2016;

Asangani et al., 2008; Zhang et al., 2012). miR-21 is a miRNA that has been extensively studied. However, the exact location in the pathological pathways of RCC molecular mechanism cannot be determined, and it is necessary to identify new miRNA intermediate molecules in which miR-21 and its targets interact. Upon that, we identified miR-1267, another miRNA that targets all tumor suppressor genes such as PTEN, SATB1, PDCD4, TCF21 and KISS1 in RCC like miR-21 by using biostatistical approaches. Relying on biostatistical data and literature review, we know that miR-1267 was not previously associated with RCC, and we estimate that it may have oncogenic function for RCC like miR-21. This suggests that this miRNA can be a reliable agent for the diagnosis and treatment of disease, indicating that it is an informative molecule for RCC. In this study, the expression profiles of miR-1267 in patients with RCC, and its correlation with patient parameters, were investigated. In this way, it is aimed to determine the new candidate molecules that may play a role in the pathology and progression of RCC.

### Methods

In this study, Kidney Cancer cDNA Array (Origene Technologies Inc., Rockville, MD, USA) consisting of cDNA samples obtained from healthy kidney tissues of 4 healthy individuals and tumoral kidney tissues of 5 Stage I, 5 Stage II, 3 Stage III and 2 Stage IV ccRCC patients (15 ccRCC patients and 4 healthy controls in total) was used. Upon this cDNA panel, Real-Time PCR (qRT-PCR) method was used for hsa-miR-1267 and SNORD48 expression analyzes and Rotor-Gene Q (Qiagen GmbH, Manheim, Germany) was used for this purpose. As the procedure, hsa-miR-1267 and SNORD48 expression primers (Origene Technologies Inc., Rockville, MD, USA) were added separately to the panels containing all Real-Time PCR reaction ingredients except the primers and the device was switched on under the conditions specified in kit procedure. All Real-Time PCR experiments were performed in three replicates. Since the study was carried out using the commercially available ccRCC cDNA panel, the approval of the ethics committee was not required. The number of patients to be included in the study was detected with 80% test power and 95% confidence interval. The statistically significant number of patients was calculated as at least 19.

In the method based on comparative expression,

measured values of hsa-miR-1267 were normalized with SNORD48. In qRT-PCR method, Ct (Cp, Crossing points) values were obtained. The comparison between healthy and ccRCC tumor samples with different stages in cDNA panel was performed. The concerned miRNA expression levels were statistically compared using Ct values obtained from the groups defined via  $2^{-\Delta\Delta Ct}$  formula.

#### Formula 1. $2^{-\Delta\Delta Ct}$ calculation

$$2^{-\Delta\Delta Ct} = 2^{-[\text{Tumor } \Delta Ct (\text{miRNA} - \text{Reference}) - \text{Control } \Delta Ct (\text{miRNA} - \text{Reference})]}$$

The statistical significance analysis of differences in miRNA expressions between tumor and normal samples was performed and statistically correlated with the clinical parameters of patients. All demographic and histopathological data of the patients were obtained with the purchased panel.

SPSS 21 program (IBM software, Pointe Claire, Quebec, Canada) was used in the statistical analysis of measured hsa-miR-1267 expression levels. Normal distribution of data was evaluated statistically by Kolmogorov-Smirnov test. It was decided to use non-parametric tests because the data were not suitable for normal distribution ( $p < 0.05$ ) and the number of samples was below 30. Wilcoxon Signed Rank Test was used in binary comparisons and Kruskal Wallis Test was applied in multi-comparisons.  $p < 0.05$  was accepted as a statistical significant value and the evaluation was made at 0.95 confidence interval.

Finally, miRgator 3.0 database, which collected 73 deep sequencing datasets on human samples from Gene Expression Omnibus (GEO), Sequence Read Archive (SRA) and The Cancer Genome Atlas (TCGA) archives, was used to compare miR-1267 expression patterns of different urological cancer types, including ccRCC, papillary renal cell carcinoma (PRCC), bladder urethelial carcinoma and prostate adenocarcinoma (Cho et al., 2012).

## Results

Expression levels of miR-1267 in ccRCC tumor tissues and the healthy kidney tissues were compared and the possible association between the clinical parameters of the patients and miRNA expression levels were investigated.

Demographic (gender, age) and clinicopathological [Tumor node metastasis (TNM) staging, Fuhrman nuclear grade,

platelet/lymphocyte ratio, calcium level] characteristics of patients enrolled in this study were presented in Table 1. The expression of miR-1267 was significantly higher in male than female (20.12-fold) ( $p = 0.027$ ). Also, there were statistically significant associations between stage I-IV with respect to miR-1267 expression (32.45 fold higher in stage IV than stage I) ( $p < 0.001$ ). Moreover, increased platelet/lymphocyte ratio and calcium level, which were parameters giving information about the occurrence of ccRCC, are significantly associated with increased miR-1267 expression ( $p < 0.001$  and  $p = 0.003$ , respectively).

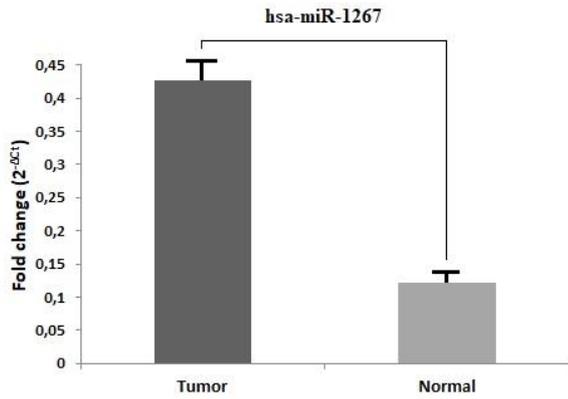
**Table 1.** Demographic and clinicopathological characteristics of patients and the statistical significance of the associations of these data with hsa-miR-1267 expressions of the patients

		Patients (n=19)	p value
Gender	Male	10 (52.6%)	0.027
	Female	9 (47.4%)	
Age	35-44	6 (31.6%)	0.642
	45-64	8 (42.1%)	
	>65	5 (26.3%)	
TNM staging	Healthy	4 (21.1%)	< 0.001 (between stage I and IV)
	Stage I	5 (26.3%)	
	Stage II	5 (26.3%)	
	Stage III	3 (15.7%)	
Fuhrman nuclear grade	Stage IV	2 (10.6%)	0.082
	Healthy	4 (21.1%)	
	Grade 2	8 (42.1%)	
	Grade 3	5 (26.2%)	
	Grade 4	2 (10.6%)	

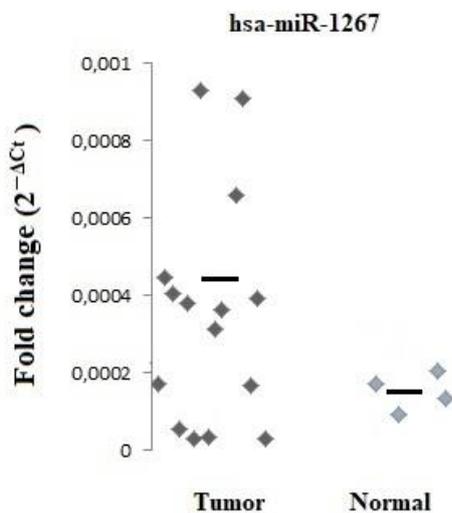
(Abbreviations. TNM: Tumor Node Metastasis)

Tumor samples of patients with ccRCC were compared to healthy kidney tissues in cDNA panel with respect to the expression levels of miR-1267 (Figure 1-2). The expression of miR-1267 in kidney tumor tissues was increased approximately three times compared to normal kidney tissues ( $p > 0.05$ ).

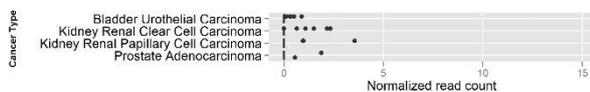
According to miRgator 3.0 database, miR-1267 expression patterns of different urological cancer types, including ccRCC, papillary renal cell carcinoma (PRCC), bladder urethelial carcinoma and prostate adenocarcinoma, were compared. Among these urological cancer types, ccRCC showed the highest miR-1267 expression profile (Figure 3).



**Figure 1.** Quantitative expression levels of hsa-miR-1267 in tumor tissues as compared to adjacent healthy kidney tissues of patients with ccRCC (Approximately 3-fold increase in hsa-miR-1267 expression) (p=0.084).



**Figure 2.** Distribution of patients' quantitative expression levels of hsa-miR-1267 in tumor tissues as compared to adjacent healthy kidney tissues.



**Figure 3.** Comparative expression levels of hsa-miR-1267 in tumor tissues as compared to adjacent healthy tissues of patients with different types of urological cancer according to deep sequencing datasets provided by miRGator v3.0 database

**Discussion**

Renal cell carcinoma is one of fifteen most frequent cancer types arising globally. The most aggressive subtype, ccRCC comprises about 70% of all kidney tumors. ccRCC is potentially medicable by resection, however approximately

30% of patients show recurrence after first nephrectomy. Unhappily, ccRCC is often non-symptomatic in the early stages, and is repeatedly stated in advanced phase frequently with metastases. In case of metastasis, ccRCC is radiation- and chemo- resistant and remains incurable in most cases, resulting in a 95% mortality ratio. Up to now, no effective ccRCC therapy has been created and none of the probable biomarkers have been approved for clinical administration (Moch, 2013).

In our study, tumor tissues of the patients with ccRCC were compared with healthy kidney tissues in terms of expression levels of miR-1267 gene and the possible association between the clinical parameters of the patients and miR-1267 expression levels were analyzed.

According to the association analysis between demographic (gender, age) and clinicopathological (TNM staging, Fuhrman nuclear grade, platelet/lymphocyte ratio, calcium level) parameters, and the expression level of miR-1267, interesting results were obtained. The expression of miR-1267 was significantly higher in male than female (p=0.027). This significant expression change of miR-1267 between genders might be caused by different hormonal status between male and female. According to the study performed by Znaor et al., RCC incidence in men varied from approximately 1/100 000 in African countries to >15/100 000 in several Northern and Eastern European countries and among US blacks. Similar patterns were observed for women, although incidence rates were commonly half of those for men. Moreover, kidney cancer is currently the ninth most common cancer in men and the 14th most common in women worldwide (Znaor et al., 2015). This data shows consistency with high miR-1267 expression in men when compared to women in our study because of the fact that we suggest an oncogenic function to miR-1267 for ccRCC.

Moreover, platelet/lymphocyte ratio and calcium level, which were parameters giving information about the occurrence of ccRCC, are significantly associated with miR-1267 expression (p<0.001 and p=0.003, respectively). All studies correlating platelet/lymphocyte ratio and calcium level with RCC in the literature are consistent to our results. According to a study realized by Wang et al (2018) on a total of 1528 patients with RCC, a high preoperative platelet/lymphocyte ratio is correlated with poor prognosis in RCC patients. Also, the pooled analysis showed that an elevated platelet/lymphocyte ratio is an effective prognostic

marker of both overall survival (OS) and progression free survival (PFS). When we look at another study, high platelet/lymphocyte ratio was associated with shorter survival of metastatic RCC (mRCC) patients receiving first-line TKI (Park et al., 2016). So, miR-1267 may be an effective independent prognostic factor in this setting, like platelet/lymphocyte ratio, based on their significant association in our cohort. If we pass to the association of high calcium level and ccRCC in our study, there are some investigations correlating high calcium levels with ccRCC progression. For example, Motzer et al. (1999) reported in their study conducted on 670 RCC patients that high serum calcium level (>10 mg/dL) was one of the pretreatment features associated with a shorter survival in the multivariate analysis. So, all these findings support that miR-1267 could have predictive value for RCC development in accordance with its correlation to platelet/lymphocyte ratio and calcium level in RCC.

Tumor samples of patients with ccRCC were compared to healthy kidney tissues in cDNA panel with respect to the expression levels of miR-1267 (Figure 1-2). The expression of miR-1267 in kidney tumor tissues was increased approximately three times compared to normal kidney tissues ( $p>0.05$ ). Also, there were statistically significant associations between stage I-IV with respect to miR-1267 expression. The expression of miR-1267 was significantly higher than in stage IV than that of stage I ( $p<0.001$ ). According to our results, miR-1267 not only has an oncogenic feature but also provide significantly discrimination between ccRCC stages consistently. All these results are consistent with the literature. For instance, the mitochondrial-processed miRNAs are likely to contribute to some post-transcriptional regulation of gene expression related to the mitochondrial functions. It was obtained a list of 33 human pre-miRNAs and 25 miRNAs. The most significant alignments with human miRNAs were obtained with four pre-miRNAs (pre-mir-302a, pre-let-7b, pre-mir-1267 and pre-mir-1296) (Barrey et al., 2011). Also, low mitochondrial respiratory chain content correlates with tumor aggressiveness in ccRCC (Simonnet et al., 2002). So, this mitochondrial dysfunction could be related with miR-1267, one of the most mitochondrial function related miRNAs, consistently with our study findings. Moreover, some studies have shown that miR-21, which has proven oncogenic properties in many cancers, triggers the emergence of

tumorigenic properties by targeting tumor suppressor genes such as RCC-specific PTEN, SATB1, PDCD4, TCF21 and KISS1 genes (Yu et al., 2014; Kowalczyk et al., 2016; Asangani et al., 2008; Zhang et al., 2012). Like miR-21, miR-1267 also targets all of these tumor suppressor genes. Relying on biostatistical data and literature review, miR-1267 was not previously associated with RCC, and we estimate that it may have oncogenic function for RCC like miR-21.

### Conclusion

ccRCC is the most common and apparently most aggressive RCC subtype with the highest rates of local invasion, metastasis and mortality (Protzel et al., 2012). In our study, we found that ccRCC showed the highest miR-1267 expression profile in ccRCC among different urological cancer types, including ccRCC, papillary renal cell carcinoma (PRCC), bladder urethelial carcinoma and prostate adenocarcinoma (Figure 3). Upon this consistency with the literature finding, we can suggest that miR-1267 could also be predictive about aggressiveness of RCC.

Consequently, the changes in the expression levels of miR-1267 analyzed and RCC-specific parameters are needed to be confirmed in the larger study groups. If verified, the expression changes of miR-1267 may be possible to be used as biomarkers for the prognosis of ccRCC. The results of this study may propose that molecular applications can be designed to change the level of the expression of miR-1267 to affect the development of ccRCC in future projects.

---

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept– S. E; Design S. E; Supervision S. E; Materials – S. E; Data Collection and/or Processing – S. E, K. K; Analysis and/or Interpretation- S. E, K. K; Literature Review – S. E, K. K; Writing- S. E, K. K; Critical Review – S. E, K. K.

**Conflict of Interest:** No conflict of interest was declared by the author.

**Financial Disclosure:** This study has been financed partially by Scientific Research Projects Commission of Ordu University (Project number: HD-1718).

---

**References**

- Asangani IA, Rasheed SA, Nikolova DA, Leupold JH, Colburn NH, Post S, Allgayer H. MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. *Oncogene* 2008; 27: 2128.
- Barrey E, Saint-Auret G, Bonnamy B, Damas D, Boyer O, Gidrol X. Pre-microRNA and mature microRNA in human mitochondria. *PLoS One* 2011; 6: e20220.
- Cho S, Jang I, Jun Y, Yoon S, Ko M, Kwon Y, et al. MiRGator v3. 0: a microRNA portal for deep sequencing, expression profiling and mRNA targeting. *Nucleic Acids Res* 2012; 41: D252-D57.
- Garzon R, Calin GA, Croce CM. MicroRNAs in cancer. *Annu Rev Med* 2009; 60: 167-79.
- Kowalczyk AE, Krazinski BE, Godlewski J, Grzegorzolka J, Kiewisz J, Kwiatkowski P, et al. SATB1 is Down-regulated in Clear Cell Renal Cell Carcinoma and Correlates with miR-21-5p Overexpression and Poor Prognosis. *Cancer Genomics Proteomics* 2016; 13: 209-17.
- Moch H. An overview of renal cell cancer: pathology and genetics. *Semin Cancer Biol* 2013; 23(1): 3-9
- Motzer RJ, Mazumdar M, Bacik J, Berg W, Amsterdam A, Ferrara J. Survival and prognostic stratification of 670 patients with advanced renal cell carcinoma. *J Clin Oncol* 1999; 17: 2530-30.
- Park TJ, Cho YH, Chung HS, Hwang EC, Jung SH, Hwang JE, et al. Prognostic significance of platelet-lymphocyte ratio in patients receiving first-line tyrosine kinase inhibitors for metastatic renal cell cancer. *Springerplus* 2016; 5(1):1889.
- Protzel C, Maruschke M, Hakenberg OW. Epidemiology, aetiology, and pathogenesis of renal cell carcinoma. *Eur Urol Suppl* 2012; 11: 52-59.
- Shingarev R, Jaimes EA. Renal cell carcinoma: new insights and challenges for a clinician scientist. *Am J Physiol Renal Physiol* 2017; 313: F145-F54.
- Simonnet H, Alazard N, Pfeiffer K, Gallou C, Bérout C, Demont J et al. Low mitochondrial respiratory chain content correlates with tumor aggressiveness in renal cell carcinoma. *Carcinogenesis* 2002; 23: 759-68.
- Wang Z, Peng S, Wang A, Xie H, Guo L, Jiang N, Niu Y. Platelet-lymphocyte ratio acts as an independent predictor of prognosis in patients with renal cell carcinoma. *Clin Chim Acta* 2018; 480: 166-72.
- Yu G, Yao W, Gumireddy K, Li A, Wang J, Xiao W. Pseudogene PTENP1 functions as a competing endogenous RNA to suppress clear-cell renal cell carcinoma progression. *Mol Cancer Ther* 2014; 13: 3086-97.
- Zhang H, Guo Y, Shang C, Song Y, Wu B. miR-21 downregulated TCF21 to inhibit KISS1 in renal cancer. *Urology*, 2012; 80: 1298-302.
- Znaor A, Lortet-Tieulent J, Laversanne M, Jemal A, Bray F. International variations and trends in renal cell carcinoma incidence and mortality. *Euro Urol* 2015; 67: 519-30.