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Association of Abl interactor 2, *ABI2*, with platelet/lymphocyte ratio in patients with renal cell carcinoma: A pilot study

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Summary

There are many unknown aspects of the pathogenesis of renal cell carcinoma (RCC). The aim of the current study was to define new RCC-related genes and measure their associations with RCC and clinical parameters, especially platelet/lymphocyte ratio which may be an independent predictor of prognosis in patients with RCC and other forms of cancer. Via in silico analysis upon RCC-specific deleted genes in chromosome 3, four possible ceRNAs (*ATXN3*, *ABI2*, *GOLGB1* and *SMAD2*) were identified. Then, the expression levels of these genes in tumour and adjacent healthy kidney tissues of 19 RCC patients were determined by real-time PCR. *ATXN3* and *GOLGB1* gene expression levels increased but *ABI2* gene expression level decreased in tumour kidney tissues when compared to normal ones. *ATXN3*, *ABI2* and *GOLGB1* gene expression levels were significantly higher in Fuhrman grade 4 than other grades ($P < .001$). *ABI2* gene expression levels were significantly associated with higher platelet/lymphocyte ratio of the patients with RCC ($P < .05$). *ATXN3*, *ABI2* and *GOLGB1* may predict higher RCC grades. Also, *ABI2* may regulate platelet/lymphocyte ratio which may be an independent predictor of RCC and other forms of cancer.

KEYWORDS

biomarkers, cancer, gene deletion, micro RNAs

1 | INTRODUCTION

Renal cell carcinoma (RCC) denotes cancer originated from the renal epithelium and accounts for >90% of cancers in the kidney. The term RCC includes several heterogeneous cancers originating from renal tubular cells. Among the urological cancers, RCC, the third most common cause of death after prostate and bladder cancer, accounts for approximately 2% of all adult cancer cases. In addition, among urological cancers, its clinical course is the most lethal one. Like other cancers, RCC is caused by the accumulation of many genetic and epigenetic changes.[1]

Over the last two decades, genetic and clinical studies have shown that RCC is not only heterogeneous in its histology and clinical course, but also heterogeneous in its genetic alterations.[2] The identification of different histological subtypes of RCC provides a better understanding of the molecular mechanism of these different subtypes of carcinogenesis, and one or more important mutations were identified for each subtype. Compared with hereditary RCC, there are more mutations identified for sporadic RCC.[3] It is also known that sporadic cancers arise from multiple (epi)genetic changes. For this reason, promoter hypermethylation of genes is thought to be involved in sporadic and/or hereditary forms of RCC. The epigenetic changes that regulate the formation and progression of RCC are still in the initial stages of reconnaissance.[4] More detailed characterization of epigenetically altered genes and pathways in RCC may lead to the development of new and minimally invasive diagnostic and prognostic tools for RCC. In the long term, epigenetic treatments may

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offer an additional treatment option for advanced RCC that does not respond to standard treatment.[4] Parameters potentially relevant to the pathophysiology of RCC and potentially usable as biomarkers in the progression of the disease need to be explored.

Deletions that lead to the loss of certain genes specific for RCC are observed in some loci of the short arm (p) of chromosome 3. The genes located in the short arm of chromosome 3, which are found to be deleted in RCC cases, are as follows: von Hippel-Lindau (VHL), inositol 1,4,5-trisphosphate receptor type 1 (ITPR1), peroxisome proliferator-activated receptor gamma (PPARG), glycerol-3-phosphate dehydrogenase 1 like (GPD1L), abhydrolase domain 5 (ABHD5), inosine 5′-monophosphate dehydrogenase 2 (IMPDH2), choline dehydrogenase (CHDH), downregulated renal cell carcinoma 1 (DRR1), pyruvate dehydrogenase beta (PDHB) and fragile histidine triad (FHIT). In this region, it was envisaged that microRNAs (miRNAs) that bind to the transcripts of these deleted genes would look for other target messenger RNAs (mRNAs) due to the loss of their targets. These mRNA transcripts have the potential for competing endogenous RNA (ceRNA).[5-8]

CeRNAs are ribonucleic acid (RNA) transcripts that carry common miRNA target regions and can communicate with each other by pulling these miRNAs on themselves.[9] Deletions or decrease of transcription levels of genes carrying a common miRNA target region would cause miRNAs targeting these regions to be released and to seek new targets. These miRNAs will suppress their transcriptional activity by selecting ceRNAs bearing the same miRNA binding region as their new target. Conversely, the increase in transcription levels of these mRNAs, which exhibit ceRNA activity, will also reduce the effect of miRNAs on previous targets by attracting common miRNAs on themselves.[10] In this context, it is thought that ceRNAs may be new actors in elucidating the pathogenesis of many cancer pathogenesis. With this mechanism in mind, specific databases can be used to detect genes that may exhibit possible ceRNA activity and their activities can be tested experimentally.[8]

The prognostic significance of inflammation is a very topical issue in the study of RCC pathophysiology. The rationale for the use of immunomodulating agents derived from the observation that RCC usually shows a diffuse immune-cell infiltrate.[11] Also, the prognostic significance of platelet/lymphocyte ratio (PLR) in different types of cancers had been analysed in past studies. In a study conducted to correlate PLR with RCC, it was found that a high preoperative PLR is correlated with poor prognosis in RCC patients.[12] So, defining all elements associated with PLR in RCC cases has been recognised as of importance recently.

In the study, we first aimed to find genes that could demonstrate the predicted ceRNA activity and could be involved in the mechanism of RCC formation by in silico analysis. We then aimed to comparatively analyse the expression levels of these genes in renal biopsy specimens from tumours and neighbouring healthy kidney tissues of patients with RCC and correlate them with clinical data of patients, especially PLR which may be an independent predictor of prognosis in patients with RCC and other types of cancers.

2 | SUBJECTS AND METHODS

2.1 | Patient selection

A total of 19 patients who attended the Urology Clinic, Faculty of Medicine, Ondokuz Mayıs University (OMU), between June 2016 and June 2017 for the first time and of whom clear cell RCC was diagnosed were included in the study. All the participants gave informed consent. The number of subjects to be included in the study was determined with 95% confidence interval and 80% test power. The statistically significant number of patients was calculated as at least 19. This study is a pilot study due to the relatively low number of patients that were recruited for analysis.

Cancer tissue and some of the healthy kidney tissue around this tumour resected by radical nephrectomy were taken for histopathologic examination. Collected tissues were fixed in 10% formalin and then embedded in paraffin (FFPE) as archival tissue and kept at room temperature in the Department of Pathology. The diagnosis of RCC was made as the result of radiological and histopathological examinations. The histological classification of renal tumours was based on the Vancouver Renal Neoplasia Classification (2012) of the International Society of Urological Pathology (ISUP).[13] Blood samples were collected just before radical nephrectomy operation, and complete blood count and biochemistry were carried out. PLR was calculated as the ratio of platelet count to lymphocyte cell count, and cut-off value was set at 157.8 for PLR (area under the ROC curve [AUC]: 0.921, sensitivity: 86%, specificity: 84%). The ability of PLR to predict pathological responses was analysed using receiver operating characteristic (ROC) curve analysis. Optimal cut-off values were determined by using the control group consisting of 19 healthy individuals. ROC curve analysis proposed the optimal cut-off value of PLR together with AUC.

2.2 | Ethical approval

The study was approved by Clinical Investigation Ethics Committee of OMU (approval no: 2016/139) (Supplementary S1).

2.3 | In silico ceRNA analysis

Previous studies have shown that VHL (96%), ITPR1 (1%), PPARG (1%), GPD1L (80%), ABHD (85%), IMPDH2 (81%), CHDH (78%), DRR1 (68%), PDHB (74%) and FHIT (96%) genes located in chromosome 3p12 have been deleted at the frequency indicated in parentheses.[5-7],[14-16] Eleven miRNAs targeting at least five of these deleted genes were identified using miRWalk database. In order to obtain more realistic results, the validated module was used instead of the predictive module when using the miRWalk database. In the validated mode, the resulting miRNA target list was based on proven data at least at one experimental step-up. In the next step, four genes which are targeted by all of these miRNAs and could potentially show the most probable ceRNA activity were identified using ComiR database. As a result of analysis, ComiR database gave a list of genes targeted by these 11 miRNAs at the same potency. This list was sorted from the highest to the lowest targeting potential. At the top of this list, with the highest targeting coefficient, four genes were selected for the expression analysis.[8,17,18]

2.4 | RNA isolation from FFPE tissues

Excess paraffin over the tumour and surrounding healthy kidney FFPE tissue specimens of each subject in the archive was removed. Subsequently, 3-4 sections of 5 μm thickness were taken using Microtome (Leica Microsystems) and transferred to sterile 1.5 mL microcentrifuge tubes. miRNeasy FFPE Kit (Qiagen GmbH) was used for total RNA isolation from FFPE tissue sections.[19]

2.5 | cDNA synthesis and measurement of cDNA concentration

RNA samples obtained were converted to cDNA by reverse transcription using Ipsogen RT Kit (Qiagen GmbH). 10 μL of each RNA sample was incubated at 65°C for 5 minutes before cDNA conversion reaction. Premix was carefully mixed, briefly centrifuged, and 15 μL of the premix was added to 10 μL of each RNA sample. The resulting mixtures were subjected to reverse transcription on a GeneAmp PCR system 9700 (Applied Biosystems) for 10 minutes at 25°C, 60 minutes at 50°C, 5 minutes at 85°C and 5 minutes at 4°C.[20] cDNAs were stored at −20°C until real-time PCR (RT-PCR) was performed.

A spectrophotometric method was used to determine quality and concentration of cDNA samples obtained before RT-PCR experiments. This analysis was performed using a Microplate Spectrophotometer (Multiscan GO, Thermo Scientific).

2.6 | Gene expression analysis

RT-PCR method was used for gene expression analysis, and Rotor Gene Q (Qiagen GmbH) was used for this purpose.

Primer pairs specific to four genes (*ATXN3*, *ABI2*, *GOLGB1* and *SMAD2*) were used in the expression analysis: Hs_*ATXN3*_1_SG QuantiTect Primer Assay, Hs_*ABI2*_1_SG QuantiTect Primer Assay, Hs_*GOLGB1*_1_SG QuantiTect Primer Assay and Hs_*SMAD2*_1_SG QuantiTect Primer Assay (Qiagen GmbH) respectively. As an internal control, the housekeeping GAPDH gene and the primer pair specific to it (Hs_GAPDH_1_SG QuantiTect Primer Assay) (Qiagen GmbH) were used.[21] RT2 SYBR® Green qPCR Mastermix (Qiagen GmbH) was used as premix for gene expression analysis. RT-PCR reaction mixture was prepared according to the kit protocol. Mixtures were transferred to 25 μL tubes, and the reaction conditions specified by the kit were applied.

2.7 | Statistical analysis

Tumour samples from patients with RCC were compared to healthy tissue on the periphery of cancer tissue of the same patient. Using the Ct values obtained from these tissues, the expression levels of the respective genes were compared statistically. In the method based on partial quantities, the measured values of the expression measurement genes were normalized by GAPDH transcription. For the comparison, Ct values obtained and $2^{-\Delta\Delta Ct}$ formula were used (Formula 1).

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

$$2^{-\Delta\Delta Ct} = 2^{-[\text{Tumour}\,\Delta Ct\,(\text{Gene}-\text{Reference}) - \text{Control}\,\Delta Ct\,(\text{Gene}-\text{Reference})]} \quad (1)$$

$2^{-\Delta Ct}$ formula was used to calculate the gene expression levels separately for the tumour and surrounding healthy kidney tissue (Formula 2). Using this formula, the fold changes in the expression of the genes in tumour and surrounding healthy kidney tissue relative to the reference gene were calculated separately. Then, a statistical analysis of significance of the fold changes in gene expression levels obtained from this formula was performed.

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

$$\begin{aligned} 2^{-\Delta Ct} &= 2^{-[\text{Tumour}\,\Delta Ct\,(\text{Gene}-\text{Reference})]} \\ 2^{-\Delta Ct} &= 2^{-[\text{Control}\,\Delta Ct\,(\text{Gene}-\text{Reference})]} \end{aligned} \quad (2)$$

For the fold change analysis, RT2 Profiler PCR Array Data Analysis Version 3.5 online software (http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php?target=upload) was used. The statistical significance analysis of differences in *ATXN3*, *ABI2*, *GOLGB1* and *SMAD2* genes' expression ratios between tumour and normal samples was performed and statistically correlated with the clinical parameters of

patients. All statistical analyses were performed using SPSS 21 program (IBM software, Pointe Claire, Quebec, Canada). 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 ($P < .05$) 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. Spearman's bivariate correlation was used to identify correlations. $P < .05$ was accepted as a statistically significant value, and the evaluation was made at 0.95 confidence interval.

3 | RESULTS

In our study, four genes with potential ceRNA activity (*ATXN3*, *ABI2*, *GOLGB1* and *SMAD2*) were identified from a RCC-specific deletion in the p-arm of chromosome 3 by a computational approach. Expression levels of these genes in cancer tissues and the healthy kidney tissues surrounding the tumour of 19 RCC patients were compared, and the possible association between the clinical parameters of the patients and the gene expression levels was investigated.

Demographic and clinicopathological characteristics of patients enrolled in this study are presented in Table 1.

11 miRNAs (hsa-miR-335-3p, hsa-miR-4447, hsa-miR-4778-3p, hsa-miR-5693, hsa-miR-587, hsa-miR-6515-3p, hsa-miR-6749-3p, hsa-miR-4679, hsa-miR-4709-3p, hsa-miR-5193 and hsa-miR-6792-3p) targeting at least 5 of the genes (VHL, ITPR1, PPARG, GPD1L, ABHD5, IMPDH2, CHDH, DRR1, PDHB and FHIT) known to be deleted in association with RCC in the short arm of chromosome 3 were determined by using miRWalk database. Four genes (*ATXN3*, *ABI2*, *GOLGB1* and *SMAD2*), which are commonly targeted by all of these miRNAs and may potentially exhibit the highest possible ceRNA activity, were identified using ComiR database (Figure 1).

Tumour samples of patients with RCC were compared to healthy kidney tissues around the tumour of the same patient with respect to the expression levels of *ATXN3*, *ABI2* and *GOLGB1* genes (Figure 2). The expression of *ATXN3* and *GOLGB1* genes in kidney cancer tissues is increased compared to normal kidney tissue, but the expression level of *ABI2* gene is decreased. As shown in Figure 2, the changes in the expression levels of *ATXN3*, *ABI2* and *GOLGB1* genes between tumour and normal kidney tissue were statistically insignificant ($P > .05$).

Patients' *ATXN3*, *ABI2* and *GOLGB1* gene expression level changes were associated with clinical characteristics, and their significance levels were tested. As a result, changes in *ATXN3*, *ABI2* and *GOLGB1* gene expression levels were not significantly associated with TNM stages ($P > .05$) (Figure 3). However, *ATXN3*, *ABI2* and *GOLGB1* gene expression levels were significantly higher in Fuhrman grade 4 than other grades ($P < .001$) (Figure 4). The changes in *ATXN3*, *ABI2* and *GOLGB1*

TABLE 1 Demographic and clinicopathological characteristics of patients

Characteristics	Groups	Patients (n = 19)	*P* value		
			ATXN3	*ABI2*	*GOLGB1*
Gender	Male	9 (47.4%)	>.05		
	Female	10 (52.6%)			
Age	Male	55.3 ± 10.4	>.05		
	Female	58.9 ± 13.2			
Tumour orientation	Right kidney	9 (47.4%)	>.05		
	Left kidney	10 (52.6%)			
TNM staging	Stage I	13 (68.4%)	>.05		
	Stage II	3 (15.8%)			
	Stage III	1 (5.3%)			
	Stage IV	2 (10.5%)			
Fuhrman nuclear grade	Grade 2	13 (68.4%)	<.001 (between grade 4 and others)		
	Grade 3	2 (10.5%)			
	Grade 4	4 (21.1%)			
Histology	Clear cell	19 (100%)	>.05		
Platelet/lymphocyte ratio	Normal	7 (36.8%)	>.05	<.001	>.05
	High	12 (63.2%)			

Abbreviation: TNM, Tumour node metastasis.

FIGURE 1 In silico detection of genes showing potential ceRNA activity, by taking into account genes deleted in short arm of chromosome 3 in RCC

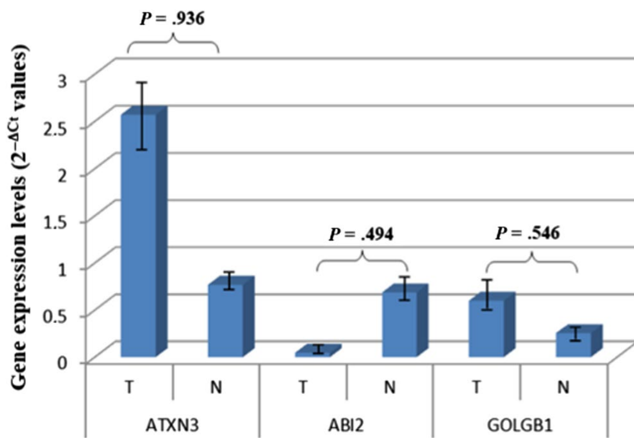

FIGURE 2 Comparison of *ATXN3*, *ABI2* and *GOLGB1* gene expression levels in tumour and normal kidney tissues

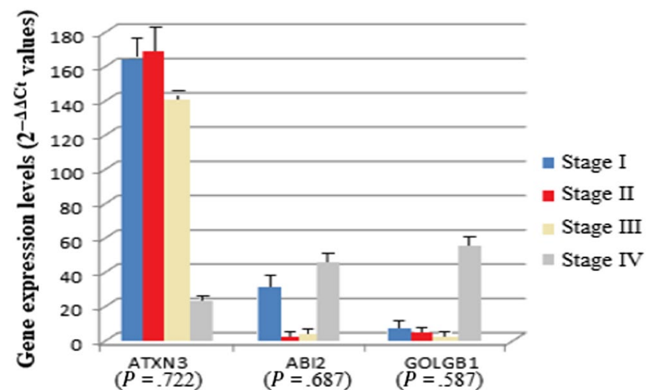

FIGURE 3 Comparison of *ATXN3*, *ABI2* and *GOLGB1* gene expression levels with respect to TNM staging

gene expression levels were not significantly associated with age and gender parameters ($P > .05$).

In addition to routine haemogram and biochemistry parameters, some of the clinical parameters (neutrophil/lymphocyte ratio, platelet/lymphocyte ratio) associated with RCC were also examined in relation to changes in *ATXN3*, *ABI2* and *GOLGB1* gene expression levels. Changes in *ABI2* gene expression levels were correlated significantly with platelet/lymphocyte ratio ($P < .05$).

4 | DISCUSSION

RCC is a type of cancer that occurs only in cells that extend into the kidney bed, as opposed to kidney cancer,

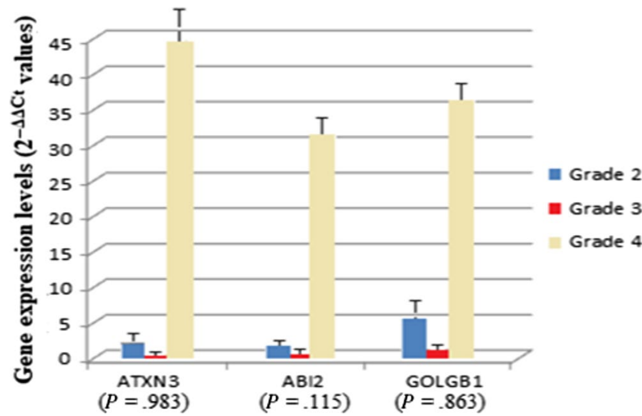

FIGURE 4 Comparison of *ATXN3*, *ABI2* and *GOLGB1* gene expression levels with respect to Fuhrman grades

which includes the renal pelvis or renal medulla. RCC includes several heterogeneous cancers originating from renal tubular cells. It that develops insidiously and usually has metastasized when diagnosed. The subtypes have different prognoses and different responses to treatment. The prognosis cannot be determined only by histopathologic examination. In recent years, although a large number of renal cell biomarkers have been studied and a large number of data has been accumulated, the pathophysiology of the disease is still not well understood.[14]

In this study, the aim was to identify the genes that could play a role in the pathogenesis of RCC. Four genes with the highest potential for ceRNA (*ATXN3*, *ABI2*, *GOLGB1* and *SMAD2*) were identified by in silico analysis, by taking into account the deleted loci in RCC. Expression levels of these genes were then correlated with clinical parameters of RCC patients. In this regard, possible new candidate genes that could play a role in the pathology of the disease were analysed. For this in silico analysis approach, the method of Arancio et al[22] was used as a model in which genes having potential to be a ceRNA and biomarker for RCC were defined by starting from deleted loci of chromosome 5 in the myelodysplastic syndrome patients. Arancio and colleagues foresee that the method they propose in their work represents a new approach to study the effects of genetic deletions in order to identify unexpectedly contributing factors to genomic deletion phenotypes and deserves experimental validity. It is suggested that the same approach can be used to analyse complex syndromes and phenotypes that are not fully understood at present, such as in our work.

In our study, cancer tissues of the patients with RCC were compared with healthy kidney tissues around the cancer tissues of the same patients in terms of expression levels of *ATXN3*, *ABI2*, *GOLGB1* and *SMAD2* genes. Expression of *ATXN3* and *GOLGB1* genes in kidney cancer tissues increased compared to normal renal tissue, but the expression level of *ABI2* gene decreased. The difference between expression levels of *ATXN3*, *ABI2* and *GOLGB1* genes in tumour and normal kidney tissues was found to be statistically insignificant ($P > .05$).

In our study, increased expression level of *ATXN3* in tumour samples supports the literature.[23] It is known that tumour suppressor PTEN gene expression is frequently decreased in cancer types associated with the endocrine system. This decrease is associated with an increase in cell proliferation. Thus this PTEN inhibition is presumably important for suppression of the development of RCC. Furthermore a recent study reported that transcription of PTEN was suppressed by increased activity of *ATXN3*.[24,25] In another approach, exome sequencing has screened genes that may have an oncogenic role at the highest potential in RCC and showed *ATXN3* was among these genes.[26] In addition, *ATXN3* is a deubiquitinase enzyme. *ATXN3* is also likely to have an increased expression in RCC like other deubiquitinase enzymes, USP9x, CYLD and AMSH2, that are overexpressed in RCC.[27]

Decreased expression of *ABI2* gene detected in RCC tumour samples in our study is supported by the literature. *ABI2* is a major c-Abl regulator and a functional homologue of ABI1, a recurrent MLL-translocation partner on chromosome 10p11.2. *ABI2* has been shown to function as a tumour suppressor in chronic myeloid leukaemia with inhibitory function of c-Abl signalling.[28] In another study, the characteristics of the Abi-2 protein were consistent with a dual role as a regulator and potential effector of the c-Abl protein. This suggests that Abi-2 may function as a tumour suppressor in mammalian cells.[29] Although these studies in the literature are not directly based on RCC, the tumour suppressor role predicted for *ABI2* in other cancer types is consistent with the reduced *ABI2* expression in RCC tumour samples of our study.

We predict that *GOLGB1*, which we found to have high expression levels relative to healthy kidney tissue, may have potential oncogene activity for RCC. Although our study was the first study of RCC on *GOLGB1*, the oncogenic role of *GOLGB1* in other types of cancer is supported by the literature. Structural activation of protein tyrosine kinases, resulting from structural chromosome anomalies such as translocation, inversion/ insertion and fusion, is frequently observed in eosinophil-associated myeloproliferative neoplasms. In some patients with myeloproliferative neoplasms associated with eosinophilia, the fusion of PDGFRB to MPRIP, CPSF6 and *GOLGB1*, which supports the oncogenic character of *GOLGB1*, is seen.[30] It has also been found that increased expression level of *GOLGB1* and E2721V mutation in this gene is directly related to the recurrence of liver cancer. In addition, a patent has been issued that *GOLGB1* inhibitors could be used therapeutically to indicate liver cancer.[31] This suggests that *GOLGB1* is an important oncogenic marker.

Upon *SMAD2* gene expression analysis, Ct values were not obtained. The tumour and its surrounding healthy kidney tissue could express too little *SMAD2* gene to be detected. One study found that *SMAD2* expression levels were very low in RCC patients, and another study detected that *SMAD2* expression levels could not be associated with RCC patients' parameters.[32,33] This explains why we cannot obtain information about *SMAD2* expression level in our study.

Lately, numerous molecular markers have been defined to be correlated with the prognosis of RCC.[12,34] Bui et al[35] detected that low CA IX staining was an independent prognostic indicator of poor survival in patients with nonmetastatic RCC and metastatic RCC ($P < .001$ and $P = .085$), using immunohistochemical analysis in 321 cases of RCC patients, which was further confirmed by SandLund's research (n = 288, $P = .001$). Moreover, Gilbert et al[36] stated that CA IX expression in the peripheral blood was correlated with recurrence in patients with renal cortical tumours. The tumour suppressor gene p53, commonly mutant in many types of cancer, has been observed up-regulated in metastatic specimens of RCC and has been suggested as an independent prognostic marker for disease progression for clear cell RCC (n = 240, $P < .0001$).[37] Different studies further confirmed that augmented staining for p53 was associated with poorer survival in patients with clear cell RCC.[38,39] Klatte and colleagues showed that CXCR3 was an independent prognostic marker in patients with localized clear cell RCC, and patients with low CXCR3 expression had a significantly worse prognosis than patients with high CXCR3 expression (n = 154, $P = .009$).[40] In the current study, *ATXN3*, *ABI2* and *GOLGB1* gene expression levels were significantly higher in Fuhrman grade 4 than other grades ($P < .001$) (Figure 4). According to this result, it is possible that *ATXN3*, *ABI2* and *GOLGB1* gene expression levels could be used to predict higher Fuhrman nuclear grades of RCC cases. So, higher *ATXN3*, *ABI2* and *GOLGB1* gene expression levels could be evaluated as a novel and useful prognostic markers for RCC. The correlations of *ATXN3*, *ABI2* and *GOLGB1* gene expression levels with the abovementioned molecular markers needs to be investigated further.

In addition to these parameters, some of the clinical parameters (neutrophil/lymphocyte and platelet/lymphocyte ratios) associated with RCC in the literature have been examined in relation to changes in *ATXN3*, *ABI2* and *GOLGB1* gene expression levels tissues. Only changes in *ABI2* gene expression levels were significantly associated with platelet/lymphocyte ratio. In a study involving 100 RCC patients, it was found that platelet/lymphocyte ratio was a prognostic factor for RCC patients prior to tyrosine kinase inhibitor treatment.[41] Also, in another study performed on metastatic RCC patients, the authors did not find PLR as a predictive factor of response to nivolumab, but SII was an independent predictive factor. This difference may be caused by metastatic status of the patients in the latter study.[42] The association of this ratio with *ABI2* in our study showed that *ABI2* may become an important marker for RCC. More extensive studies, might show that this is a valuable prognostic parameter.

When the limitations of our study were analysed, the starting point of our study design was the genes deleted in RCC-specific manner, but we do not know whether all of the patients with RCC in our study have gene deletions located in chromosome 3 or not. Nevertheless, as we know from previous studies, these deletions are detected at high rates (VHL [96%], ITPR1 [1%], PPARG [1%], GPD1L [80%], ABHD [85%], IMPDH2 [81%], CHDH [78%], DRR1 [68%], PDHB [74%] and FHIT [96%]) in cases of RCC.[5-7,13-15] So, our patients are very likely to carry these deletions. This is again a finding that supports our study design. In addition our study includes a method that has not been applied previously, in the sense that an in silico approach has been used to find new target genes that could play a role in RCC.

5 | CONCLUSION

In our study, we first identified four genes that we predicted could play a role in the mechanism of the formation of RCC by in silico analysis. We aimed to analyse the expression levels of these genes in cancer tissues and the healthy kidney tissues surrounding the tumour of RCC patients. As a result of our study, the expression of *ATXN3* and *GOLGB1* genes in kidney cancer tissues increased compared to normal renal tissue, but the expression level of *ABI2* gene decreased. However, these changes were statistically not significant. Furthermore, it was observed that *SMAD2* gene was expressed too low to be detectable in both cancer tissue and the surrounding normal kidney tissue. Statistically insignificant difference was found when the expression levels of *ATXN3*, *ABI2* and *GOLGB1* genes were correlated with TNM stages, Fuhrman grade, age and gender parameters of the patients.

However, the combination of in silico analysis and gene expression analysis has not been used previously to determine the association between genes and RCCs. In addition, it may be a rational and economic approach to the identification of new candidate genes by in silico database analysis approach, which is the first step of the study, starting from the genes of which the association with RCC has been proven previously. Using the in silico approach to identify genes that are most likely to be markers can provide more economical and faster results than examining many genes that may be potential markers of RCC development separately.

As a result, *ATXN3*, *ABI2* and *GOLGB1* may predict higher RCC grades. Also, *ABI2* may regulate platelet/lymphocyte ratio which may be an independent predictor of RCC and

other cancer types. For this, the alterations in the expression levels of the genes analysed and RCC-specific parameters are needed to be confirmed in the larger study groups. If verified, the expression changes of *ATXN3*, *ABI2* and *GOLGB1* genes may be possible to be used as biomarkers for the prognosis of RCC. The results of this study may suggest that cellular therapies and molecular applications can be designed to alter the level of the expression of ceRNAs in future projects.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

AUTHORS' CONTRIBUTIONS

SE realized in silico ceRNA analysis and involved in RNA isolation from FFPE tissues, cDNA synthesis and measurement of cDNA concentration and gene expression analysis. SE, SG, RB and OA selected patients, planned the study, realized the statistical analysis, drafted the manuscript, revised the manuscript and consented to the final manuscript as submitted.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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