The comprehensive bioinformatic analysis of the hsa-miR-3613-5p in kidney renal clear cell carcinoma

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Abstract

microRNA-3613 (hsa-miR-3613-5p), a biomarker with a dual role, oncogenic or tumor suppressor, is associated with different types of cancers. This study aimed to assess the correlation between the hsa-miR-3613-5p gene expression and Kidney renal clear cell carcinoma (KIRC). Using several bioinformatics tools, we examined the expression level and clinicopathological value of hsa-miR-3613-5p in patients with KIRC compared to normal tissues. Other metrics include survival analysis, diagnostic merit of hsa-miR-3613-5p, downstream target prediction, potential upstream lncRNAs, network construction, and functional enrichment analysis hsa-miR-3613-5p, were performed. We observed that overexpression of hsa-miR-3613-5p in KIRC tissues had valuable diagnostic merit and significantly was correlated with the poor overall survival of KIRC patients. We also realized a correlation between abnormal expression hsa-miR-3613-5p and several clinical parameters such as pathological stage, race, age, and histological grades of patients with KIRC. Moreover, we identified the most potential regulatory of hsa-miR-3613-5p in KIRC with 17 different axes, including four pseudogenes, two lncRNAs, and three mRNAs. Besides, we discovered six variants in mature miRNA of hsa-miR-3613-5p. Finally, pathway enrichment analysis uncovered that top-ranked pathways for hsa-miR-3613-5p are cell cycle, cell adhesion molecules (CAMs), and hepatocellular carcinoma pathways. The present report demonstrated that the higher expression of hsa-miR-3613-5p is associated with the progression of KIRC, therefore. It may be considered a valuable indicator for the early detection, risk stratification, and targeted treatment of patients with KIRC.

1. Backgrounds

Renal cell carcinoma (RCC) is the third most deadly urogenital cancer after prostate and bladder cancers (Liu and Yang, 2021). Kidney renal clear cell carcinoma (KIRC) is the most prevalent kind of kidney malignancy, responsible for 70–75% of all RCC cases, and has a high mortality rate among kidney cancers (Gao, Yan, Zhang, Fan, Jiao and Shao, 2021), Guan, Liu and Ping, 2020). Most KIRC patients face the challenge of chemotherapy and radiotherapy resistance. The primary therapeutic approach for these patients is surgery; however, due to high metastatic potential, poor clinical outcomes, and enhanced risk of recurrence, early detection is crucial in managing and treating KIRC (Liu and Yang, 2021), Yin, Li, Wang, Shi, Wang, Yang and Peng, 2019). It has been documented that dysregulation of many non-coding RNAs, especially microRNAs and IncRNAs, is involved in the progression of various diseases (Wang, Yang, Ma, Wang, Xue, Zhu, Yang, Chen, Chen and Ye, 2020). Some of them are used as diagnostic, prognostic, or predictive biomarkers. So far, few reliable biomarkers have been discovered for KIRC (Zhang, Li, Wu, Li, Wang, Hu, Fang, Yuan and He, 2021). So, identifying a sensitive biomarker would help in the timely diagnosis of KIRC patients and metastasis prevention.

miR-3613 is one of the biomarkers associated with different cancers and may play an oncogenic or tumor suppressor role. hsa-miR-3613-5p is introduced as a tumor suppressor in Pancreatic Cancer (PC). This miRNA inhibits the invasion and migration of PC cells by targeting the CDK6 gene. Hence, the downregulation of miR-3613-5p in pancreatic tumor samples leads to enhanced CDK 6 level, elevated metastasis, and poor prognosis in PC patients (Cao, Wang, Long, Guo, Sheng, Zhan, Yang, Wang and
Yang, 2020). As a result, miR-3613-5p could be considered a potential prognostic marker for pancreatic cancer (Ma, Sun, Song, Li, Li, Xu, Yang, Lan and Li, 2020). Another study found that miR-3613-5p as an oncogene is up-regulated in Lung Adenocarcinoma (LUAD) and promotes cell proliferation through a positive feedback loop RELA/JUN/miR-3613-5p/NR5A2/AKT1/MAPK3/1 (He, Shen, Wang, Wang, He, Zhu, Du, Wang, Li and Zhong, 2020). Others have suggested that miR-3613-3p as a biomarker of liquid biopsy is remarkably up-regulated in dedifferentiated liposarcoma patients and could be used to distinguish them from lipoma cases and healthy people (Fricke, Cimniak, Ullrich, Becherer, Bickert, Pfeifer, Heinz, Stark, Bannasch and Braig, 2018). It has been revealed that the expression level of miR-3613 is increased in drug-resistant breast cancer and decreased in recurrent epithelial ovarian cancer (EOC) and could be applied as a biomarker for predicting the risk of EOC recurrence (Sohn, 2020), Li, Leng, Meng, Li, Li and Zhao, 2021).

The competing endogenous RNA (ceRNA) network is one of the main regulatory mechanisms of gene expression at the post-transcriptional level. Non-coding RNAs (including pseudogenes, lncRNAs, and miRNAs) are critical components of the ceRNA network, and some of them can be utilized as disease-specific biomarkers and therapeutic targets. miRNAs bind to the 3UTR region of the target mRNA and lead to direct destruction or inhibition of translation (Chen, Tan, Yang, Fang and Xu, 2022). Pseudogenes are considered defunct relatives of functional genes (Vanin, 1984), Ding, He, Zhang, Dong and Wu, 2021). Several pieces of evidence have demonstrated that aberrant pseudogenes take part in the occurrence of human diseases. In recent years, pseudogenes have been introduced as critical players in the pathogenesis of multiple human cancer, including lung, liver, gastrointestinal tract, breast, and renal (Shang, Wang, Chen, Yang, Zheng, Wang and Li, 2019), Song, Yang, Zhang, Zhou, Li and Hao, 2019), Lynn, Sun, Ayshiev, Siegler, Rizzo, Karnes, Gonzales Garay, Wang, Casanova and Camp, 2018), Yndestad, Austreid, Skaftnesmo, Lønning and Eikesdal, 2018), Yu, Yao, Gumireddy, Li, Wang, Xiao, Chen, Xiao, Li and Tang, 2014), Nuerzhati, Dong, Song and Zheng, 2019). lncRNAs positively affect the stability and translation of target mRNA by sponging miRNAs (Gao, Zhao, Ma and Zhang, 2021). Jiao et al. have indicated that LncRNA DARS antisense RNA 1 (DARS-AS1) is up-regulated in KIRC samples (Jiao, Guo, Chen, Li and Zhang, 2020). LncRNAs are upstream of miRNAs which can modify several mechanisms in normal cells through binding and targeting miRNAs. The involvement of LncRNA-miRNA in crucial pathways leading to cancer development has been verified by numerous studies (Cao, Jiang, Tang and Liang, 2017), Paraskevopoulou and Hatzigeorgiou, 2016). In the ceRNA network, pseudogenes and IncRNA competes with target mRNA to trap common miRNAs (pseudogenes/IncRNA–miRNA–mRNA axis) due to the similarity of complementary binding sites in the 3UTR region (Ye, Yang, Zhao, Song, Wang and Zheng, 2014), Zhou, Zhang, Sun, Ye, Liu, Zhou and Tang, 2019). A systematic study demonstrated four IncRNAs involved in the IncRNA–miRNA–mRNA ceRNA network, including ADAMTS9-AS1, LINC00536, AL391421.1, and LINC00491, could act as prognostic biomarkers to predict the survival of breast cancer patients (Fan, Ma and Liu, 2018). Another report constructed a Pseudogene-miRNA-mRNA ceRNA network in hepatocellular carcinoma containing four pseudogenes, eight miRNAs, and 30 mRNAs (Yan, Yue, Xu, Jiang, Zhang and Wu, 2020). Overall, dysregulated ceRNA network has been reported in many human cancers (Wang, Yang, Ma, Wang, Xue, Zhu, Yang, Chen, Chen and Ye, 2020), Yu,
Yao, Gumireddy, Li, Wang, Xiao, Chen, Xiao, Li and Tang, 2014), Song, Ye, Jiang, Yin, Chen, Bai, Zhou and Liu, 2018), Tang, Lu, Wang, Li, Wu, Duan and He, 2019).. However, to our knowledge, the regulatory network of hsa-miR-3613-5p in KIRC is unknown.

This study conducted comprehensive bioinformatic assessments to analyze the diagnostic and prognostic significance of abnormal expression of the has-miR-3613-5p, its related ceRNA network, variants in its sequence, and its roles in tumor progression and development of KIRC.

2. Material And Methods

2.1. The Expression analysis

We detected the expression profile of hsa-miR-3613-5p in KIRC by the CancerMIRNome and the ENCORI databases. The CancerMIRNome (http://bioinfo.jialab-ucr.org/CancerMIRNome/) is a user-friendly tool for the interactive analysis and visualization of expression, diagnostic and prognostic significance, and function of miRNAs among various cancers based on the data from The Cancer Genome Atlas (TCGA), and 40 public cancer circulating miRNome profiling datasets from NCBI Gene Expression Omnibus (GEO) and ArrayExpress (Li, Qu, Wang, Wang, Cui, Yu, Chater, Zhou, Jia, Traband, Yuan, Zhu, Zhong and Jia, 2020).. ENCORI (http://starbase.sysu.edu.cn/) is an online resource for studying the RNA interactomes and can be used for survival and differential expression analysis of miRNAs, IncRNAs, pseudogenes, and mRNAs (Li, Liu, Zhou, Qu and Yang, 2014).. The association of expression levels of hsa-miR-3613-5p with clinicopathological parameters in KIRC was assessed via the UALCAN database (http://ualcan.path.uab.edu), which is open access and user-friendly tool for analyzing cancer transcriptome data. UALCAN allows researchers to demonstrate biomarkers or perform in silico validation for interested genes (Chandrashekar, Bashel, Balasubramanya, Creighton, Ponce-Rodriguez, Chakravarthi and Varambally, 2017).. P-value < 0.05 and fold change > 1 were considered statistically significant.

2.2. The Receiver Operating characteristic (ROC) curve analysis

We evaluated the diagnostic merit of hsa-miR-3613-5p in KIRC employing the CancerMIRNome database.

2.3. The survival analysis

We explored the correlation of hsa-miR-3613-5p expression with overall survival (OS) outcome of patients with KIRC utilizing the CancerMIRNome, ENCORI, and Kaplan–Meier plotter databases. The Kaplan–Meier plotter (http://kmplot.com/analysis) is a web tool for investigating the impact of 54,675 genes on survival in 21 cancer types (Gyorffy, Lancerzyk, Eklund, Denkert, Budczies, Li and Szallasi, 2010), Gyorffy, Surowiak, Budczies and Lanczky, 2013).. A log-rank P-value < 0.05 was considered statistically significant.

2.4. mRNA predication
To acquire the putative target genes of hsa-miR-3613-5p, we used the miRTarBase 2020 database, which presents data about experimentally confirmed miRNA-target interactions (Huang, Lin, Li, Huang, Shrestha, Hong, Tang, Chen, Jin, Yu, Xu, Li, Cai, Zhou, Chen, Pei, Hu, Su, Cui, Wang, Xie, Ding, Luo, Chou, Chang, Chen, Cheng, Wan, Hsu, Lee, Wei and Huang, 2020). Considering the ceRNA hypothesis, these potential target mRNAs should be downregulated in KIRC tumor tissues compared to normal controls and had a strong negative correlation with hsa-miR-3613-5p (rho value < -0.1 and p-value < 0.05).

### 2.5. IncRNA prediction

Then we predicted the potential upstream IncRNA of hsa-miR-3613-5p via the miRNet v.2 database (https://www.mirnet.ca/) (Fan and Xia, 2018), Fan, Habib and Xia, 2018), Fan, Siklenka, Arora, Ribeiro, Kimmins and Xia, 2016). Based on the ceRNA theory, the expression of these IncRNAs should be decreased in KIRC tumor samples and negatively correlated with hsa-miR-3613-5p in KIRC (rho value < -0.1 and p-value < 0.05).

### 2.6. Pseudogene prediction

Several reports have proposed that pseudogenes affect the expression of their desired miRNAs. To examine this, we investigated the possible upstream pseudogenes of hsa-miR-3613-5p using the ENCORI database. According to CeRNA theory, we evaluated the expression level of obtained pseudogenes in KIRC samples by the ENCORI database. Only pseudogenes with negative expression profiles and significant relation with hsa-miR-3613-5p in KIRC (rho value < -0.1 and p-value < 0.05) were kept for subsequent assessments.

### 2.7. Construction of a regulatory network of hsa-miR-3613-5p in KIRC

We integrated the hsa-miR-3613-5p–mRNAs, IncRNAs–hsa-miR-3613-5p, and pseudogenes–hsa-miR-3613-5p pairs to construct a potential regulatory network of hsa-miR-3613-5p in KIRC and visualized it by the Cytoscape 3.9.1 (Shannon, Markiel, Ozier, Baliga, Wang, Ramage, Amin, Schwikowski and Ideker, 2003).

### 2.8. Construction of the most potential regulatory axes of hsa-miR-3613-5p in KIRC

To indicate the most potential regulatory axes of hsa-miR-3613-5p in KIRC, we utilized two steps: first, since our data disclosed that hsa-miR-3613-5p is significantly related to OS of patients with KIRC, we speculated that its upstream regulators (pseudogenes and LncRNAs) and downstream targets (mRNA) might have an impact on the survival outcome of KIRC patients too. Therefore, we assessed their association with OS of patients with KIRC through ENCORI database. Those with significant correlations were included for the second step. We then evaluated the association of pseudogenes and LncRNAs with mRNAs. Only pseudogenes-mRNAs and IncRNAs-mRNAs pairs with significant positive correlation (rho value > 0.1 and p-value < 0.05) were kept for axis construction.
2.9. SNPs prediction

The miRNASNP-v3 database is a valuable platform for homo sapiens miRNA-related single nucleotide polymorphisms (SNPs) and their influence on target gain and loss (Liu, Fu, Xia, Zhang, Gu and Guo, 2020). We assessed the impact of nucleotide variations on hsa-miR-3613-5p expression through this database.

2.10. Functional enrichment analysis

Finally, we elucidated the underlying mechanisms of hsa-miR-3613-5p in KIRC by performing the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis through uploading the obtained potential downstream genes to the Enrichr (http://amp.pharm.mssm.edu/Enrichr) database (Kuleshov, Jones, Rouillard, Fernandez, Duan, Wang, Koplev, Jenkins, Jagodnik, Lachmann, McDermott, Monteiro, Gundersen and Ma'ayan, 2016). P-value < 0.05 was considered statistically significant.

3. Results

3.1. The association of hsa-miR-3613-5p expression with clinicopathological features in KIRC

The expression analysis using the CancerMIRNome database revealed that hsa-miR-3613-5p was up-regulated in 516 KIRC samples compared to 71 normal controls (Fig. 1A; P-value = 7.46e-37). The ENCORI database also showed the overexpression of hsa-miR-3613-5p in 517 KIRC tissues comparing their 71 normal counterparts (Fig. 1B; P-value = 5.3e-59). Besides, the expression analysis by the UALCAN database indicated that the expression of hsa-miR-3613-5p in 239 primary KIRC samples was significantly higher than in 69 healthy tissues (Fig. 1C; P-value = 1.62e-12).

When we evaluated the expression profile of hsa-miR-3613 based on the clinicopathological parameters, we observed that the expression level of hsa-miR-3613-5p was up-regulated in Stage 1, Stage 2, Stage 3, and Stage 4 cancer tissues compared to normal samples (Fig. 2A). When the expression analysis was performed according to the patient’s race, the expression level of hsa-miR-3613-5p was increased in Caucasian, African-American, and Asian tumor samples than normal tissues (Fig. 2B). Interestingly, the expression level of hsa-miR-3613-5p in males’ and females’ tumor tissues was higher than in normal samples. However, there was no statistical difference in the expression pattern of hsa-miR-3613 between males’ and females’ cancer tissues (Fig. 2C). Moreover, we detected a significant difference in hsa-miR-3613 expression among KIRC’s different histological grades (Fig. 2D). The expression of hsa-miR-3613 based on the patient’s age and nodal metastasis status were depicted in Fig. 2E and Fig. 2F, respectively. The Table S1 – S7 provide each comparison’s statistical significance mentioned above. These findings suggested that hsa-miR-3613-5p was up-regulated in tumor tissues and could act as an oncogene in KIRC.
3.2. Diagnostic value of hsa-miR-3613-5p in KIRC

The ROC curve analysis via CancerMIRNome database unveiled that miR-99a-5p might exhibit significant diagnostic merit for patients with KIRC with 90.12% sensitivity and 94.37% specificity (Area Under the Curve = 0.964, P-value < 0.0001, 95% Confidence Interval = 0.945–0.977; Fig. 3).

3.3. Prognostic significance of hsa-miR-3613-5p in KIRC

As shown in Fig. 4, the survival analysis by the CancerMIRNome, ENCORI, and Kaplan–Meier plotter databases indicated that overexpression of hsa-miR-3613-5p was associated with unfavorable OS and KIRC patients with lower hsa-miR-3613-5p experienced a better OS outcome than those with high expression hsa-miR-3613-5p.

3.4. Identification of regulatory network of hsa-miR-3613-5p in KIRC

The miRTarBase database was initially involved in predicting the putative mRNA of hsa-miR-3613-5p. We found that hsa-miR-3613-5p probably regulates the expression of 47 mRNAs (Table S8). As noted in Figure S1-S2, only 19 mRNAs (ARID1A, CDK6, COG6, F11R, H3F3C, LORI, LIMA1, MT1E, MTX3, MYO10, NHSL1, STRBP, VEZF1, CUL3, NTNG1, XKR4, SOGA3, PRKAA1, and SMC3) was lower expressed in KIRC tissues and reversely correlated with hsa-miR-3613-5p. To ensure hsa-miR-3613-5p regulates the expression of these mRNAs, we evaluated the expression level of these genes at protein levels by the CPTAC tab of the UALCAN database. We observed that the F11R, MTX3, MYO10, NHSL1, STRBP, and VEZF1 proteins were downregulated in KIRC samples (Figure S3). We also investigated the upstream IncRNAs potentially bind to hsa-miR-3613-5p utilizing the miRNet database. The results identified that 17 IncRNAs might control the expression level of the hsa-miR-3613-5p (Table S9). Additional assessments by the ENCORI database uncovered that the expressions of FGD5-AS1, HCG18, LINC00885, NORAD, and PAXIP1-AS2 were reduced in KIRC samples and had a negative association with hsa-miR-3613-5p (Figure S4-S5). Moreover, we explored the interaction between pseudogenes and hsa-miR-3613-5p. We found that among 82 pseudogenes-hsa-miR-3613-5p pairs predicted by the ENCORI database (Table S10), HS6ST1P1, IL6STP1, EIF4EP2, UBE2D3P1, and AC006210.2 were downregulated in KIRC tissues and negatively related to hsa-miR-3613-5p (Figure S6-S7). Merging the acquired pairs enabled the construction of a potential regulatory network of hsa-miR-3613-5p in KIRC, consisting of five pseudogenes, five IncRNAs, and six mRNAs.

3.5. Demonstration of the most potential regulatory axes of hsa-miR-3613-5p in KIRC

Using the data retrieved from ENCORI database, we realized that among genes in constructed network, the aberrant expression of four pseudogenes (IL6STP1, EIF4EP2, AC006210.2, and UBE2D3P1), two
IncRNAs (NORAD and PAXIP1-AS2), and three mRNAs (NHSL1, STRBP, and VEZF1) were significantly associated with OS of patients with KIRC (Figure S8). Co-expression analysis also revealed the positive correlation of IL6STP1, EIF4EP2, and AC006210.2 with three mRNAs (NHSL1, STRBP, and VEZF1) and UBE2D3P1 with two (STRBP and VEZF1) (Figure S9). Moreover, NORAD and PAXIP1-AS2 were significantly associated with NHSL1, STRBP, and VEZF1 (Figure S10). Table 1 lists the most potential regulatory axes of hsa-miR-3613-5p in KIRC with four pseudogenes, three IncRNAs, and three mRNAs.

Table 1
The most potential regulatory axes of hsa-miR-3613-5p in KIRC.

<table>
<thead>
<tr>
<th>Upstream Regulator</th>
<th>Interaction</th>
<th>miRNA</th>
<th>Interaction</th>
<th>Downstream Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL6STP1</td>
<td>Suppress</td>
<td>hsa-miR-3613-5p</td>
<td>Suppress</td>
<td>NHSL1</td>
</tr>
<tr>
<td>EIF4EP2</td>
<td></td>
<td></td>
<td>Suppress</td>
<td>STRBP</td>
</tr>
<tr>
<td>AC006210.2</td>
<td></td>
<td></td>
<td></td>
<td>VEZF1</td>
</tr>
<tr>
<td>UBE2D3P1</td>
<td>Suppress</td>
<td></td>
<td>Suppress</td>
<td>STRBP</td>
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<td></td>
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<td>Suppress</td>
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<tr>
<td>NORAD</td>
<td>Suppress</td>
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<td>NHSL1</td>
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<tr>
<td>PAXIP1-AS2</td>
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<td>Suppress</td>
<td>STRBP</td>
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<td></td>
<td></td>
<td></td>
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<td>VEZF1</td>
</tr>
</tbody>
</table>

KIRC: Kidney renal clear cell carcinoma.

3.6. The hsa-miR-3613-5p’s variants

The miRNASNP-v3 database demonstrated the no SNP or Disease Related Variations (DRV; from GWAS, ClinVar and COSMIC) in the seed region of hsa-miR-3613-5p and six variations, including five SNPs in mature miRNA and one DRV in mature miRNA. As indicated in Fig. 6A, it is probable that rs1279899144, rs201796222, rs201796222, rs1438135163, rs1327340301, and COSN28863682 decrease and rs1363404935 mildly changes the expression of hsa-miR-3613-5p.

3.7. Gene ontology and KEGG pathway enrichment analysis

The Enrichr database conducted the functional enrichment analysis to obtain further insight into the function of the achieved network. As illustrated in the Fig. 7A-7C, the target genes of hsa-miR-3613-5p remarkably were enriched in GO terms, comprising the cell cycle G1/S phase transition (GO:0044843), regulation of Rho protein signal transduction (GO:0035023), and actomyosin structure organization (GO:0031032) in biological process (BP); npBAF complex (GO:0071564), MIB complex (GO:0140275), and SAM complex (GO:0001401) in cellular component (CC); and cAMP-dependent protein kinase activity (GO:0004691), histone serine kinase activity (GO:0035174), and cyclic nucleotide-dependent protein kinase activity (GO:0004690) in molecular function (MF). Additionally, the KEGG pathway enrichment
analysis uncovered that cell cycle, cell adhesion molecules (CAMs), and hepatocellular carcinoma pathways were the top-ranked pathways for hsa-miR-3613-5p (Fig. 7D).

4. Discussion

Kidney renal clear cell carcinoma (KIRC) is known to have poor treatment outcomes and the worst overall survival (OS) among different kidney cancer subtypes (Hu, Zeng and Liu, 2019). Hence, detecting prognostic gene expression profiles for KIRC is a priority. In the present study, we first analyzed the expression hsa-miR-3613-5p and then evaluated its diagnostic and prognostic merit for patients with KIRC. Then using the data retrieved from various databases, we identified the most potential regulatory axes of hsa-miR-3613-5p in KIRC. We also investigated the variation of hsa-miR-3613-5p and its impact on expression profile. Besides, we performed functional enrichment analysis to shed light on oncogenic signaling pathways induced by hsa-miR-3613-5p, which probably are involved in KIRC development.

The expression data revealed that the upregulation of hsa-miR-3613-5p in KIRC tissues compared to normal samples had a high diagnostic value (AUC = 0.964) and significantly was correlated with the poor OS of KIRC patients. Therefore, its abnormal expression in KIRC could be a promising diagnostic and prognostic indicator for these patients. Abnormal expression of miR-3613-5p has been reported in the serum of patients with endometriosis (Cosar, Mamillapalli, Ersoy, Cho, Seifer and Taylor, 2016). It has been shown that overexpression of microRNA-3613-5p in clinical samples is related to poor prognosis outcomes in LUAD patients (He, Shen, Wang, Wang, He, Zhu, Du, Wang, Li, Zhong, Huang and Yang, 2020). In contrast, Rong Cao et al. have documented a significant association between reduced expression of miR-3613-5p in tumor tissues and decreased cumulative survival rate among patients with pancreatic cancer (Cao, Wang, Long, Guo, Sheng, Zhan, Yang, Wang and Yang, 2020). Moreover, Li Qin et al. have suggested that miR-3613-5p is a good indicator for patients with hepatocellular carcinoma (Qin, Huang, Wang, Wu, Li, Yi, Qin and Huang, 2019). In a recent study, Zhan et al. have proposed seven miRNAs as significant predictive indicators for KIRC, consisting of hsa-miR-3613-5p, which has the highest expression level in KIRC tissue (Zhan, Zhang, Li, Xu, Zhu, Yang, Zheng and Guo, 2021).

The prediction of downstream gene target of miR-3613-5p with several databases uncovered that hsa-miR-3613-5p targets 19 mRNAs and probably reduces the expression of six mRNAs (F11R, MTX3, MYO10, NHSL1, STRBP, and VEZF1) at protein levels in KIRC tissue compared with normal samples. Interestingly, the reduced expression level of NHSL1, STRBP, and VEZF1 were correlated with OS of patients with KIRC, introducing them as the most potential downstream targets of miR-3613-5p in KIRC. A group of related genes for hsa-miR-3613-5p (CDK6, H3F3C, LCOR, STRBP, VEZF1, MT1E, XKR4, PRKAA1, and SMC3) are related to the cell cycle pathway. CDK6 is a well-known tumor suppressor gene that blocks the G1 to S phase transition via Rb phosphorylation and induces the expression of p16, which creates an autoregulatory feedback loop to restrain cell proliferation (Tigan, Bellutti, Kollmann, Tebb and Sexl, 2016). Previous studies have shown that downregulation of CDK6 by miRNAs is a prognostic factor for renal cell carcinoma (RCC) and Clear cell renal cell carcinoma (ccRCC) (Guo, Wang and Zhang, 2018), Guo, Lv and Jia, 2020). H3F3C encodes for the histone H3.5 component, which influences the
nucleosome stability, and several missense mutations have been found in different tumors (Urahama, Harada, Maehara, Horikoshi, Sato, Sato, Shiraishi, Sugino, Osakabe, Tachiwana and chromatin, 2016). LCOR is a transcriptional inhibitor of ligand-activated estrogen receptors (ERs) (Jalaguier, Teyssier, Achour, Lucas, Bonnet, Rodriguez, Elarouci, Lapierre and Cavaillès, 2017). ER involves in the cell cycle process through its interaction with Cyclin D1 and consequently arresting G1 to S phase (Moghadam, Hanks and Keyomarsi, 2011). Flindris et al. have demonstrated that endometrioid endometrial cancer patients with a lower expression level of LCOR have worse survival against patients expressing high levels (Flindris, Katsoulas, Goussia, Lazaris, Navrozoglou, Paschopoulos and Thymara, 2021). XKR4 involves the facilitation of Phosphatidylycerine apoptosis, which impedes cell cycle machinery (Suzuki, Imanishi and Nagata, 2014). PRKAA1 is a subunit of AMP-activated protein kinase (AMPK), and it has been proposed that AMPK could cause G1 arrest by increasing the expression of G1-S transition inhibitors (Fogarty, Ross, Ciruelos, Gray, Gowans and Hardie, 2016). SMC3 involves in the segregation of sister chromatids (Bessat and Ersfeld, 2009). Previous studies have shown that a low level of SMC3 is associated with centrosome abnormalities which can activate the p53-dependent mitotic checkpoint (Ghiselli, 2006). STRBP is a double-stranded RNA binding protein widely expressed in various tissues (Zhang, Dai, Li, Yin, Lang, Yang, Xiao, Zhu, Liu and Liu, 2020). It is also associated with microtubules, which pull chromosomes to opposite poles of cells during mitosis progress (Zhang, Dai, Li, Yin, Lang, Yang, Xiao, Zhu, Liu and Liu, 2020), Vicente and Wordeman, 2015). In addition, two transcript fusions of STRBP have been identified in lung adenocarcinoma and breast cancer (Zhang, Dai, Li, Yin, Lang, Yang, Xiao, Zhu, Liu and Liu, 2020). VEZF1 is a transcriptional activator of MT1, which contributes to cell cycle arrest at the G1 phase, especially in endothelial cells (Miyashita and Sato, 2005). It is important to note that, Kidney organ is enriched with endothelial cell populations (Jourde-Chiche, Fakhouri, Dou, Bellien, Burtey, Frimat, Jarrot, Kaplanski, Le Quintrec and Pernin, 2019). The other group of hub-genes for hsa-miR-3613-5p suggested in this study (F11R, LIMA1, MYO10, NHSL1, CUL3, COG6, and NTNG1) is considered to be associated with cell adhesion molecules-related pathways. F11R is one of the immunoglobulin superfamilies, shaping tight junctions between the epithelium and endothelium cells (Blaskewicz, Pudney and Anderson, 2011). The role of F11R carcinogenesis still is a controversial issue. While the higher expression of F11R has been reported in many human cancers, including breast, ovarian, cervical, glioblastoma, and head and neck squamous cell carcinoma, its downregulation have documented in multiple tumors such as pancreatic, endometrial, renal, and gastric cancers (Czubak-Prowizor, Babinska and Swiatkowska, 2022). LIMA1 acts as an adaptor at integrin adhesion sites and is an actin regulator in cancer metastasis (Collins, Jiang, Hargest, Mason, Sanders and Reviews, 2015). MYO10 contributes to integrin in cell adhesion (Zhang, Berg, Li, Wang, Lång, Sousa, Bhaskar, Cheney and Strömblad, 2004). It has been revealed that the MYO10 mRNA expression ratio is an independent prognostic factor for the overall survival of patients with lung squamous cell carcinoma (Dvornikov, Schneider, Ohse, Szczygiel, Titkova, Rosenblatt, Muley, Warth, Herth and Dienemann, 2018). It has been reported that MYO10 enhances tumor progression by triggering genomic instability (Pozo, Geng, Tamagno, Jackson, Heimsath, Hammer, Cheney and Zhang, 2021). A recent study has proposed that higher expression of MYO10 is associated with poor prognosis in cervical cancer and could promote proliferation and migration capacity of tumor cells through mediating the PI3K/Akt Signaling pathway.
(He, Chen, Zhang, Chen, You, Hu, Xu and Chen, 2020). NHSL1 contributes to F-actin assembly and inhibits cell migration and metastasis (Law, Jalal, Pallett, Mosis, Guni, Brayford, Yolland, Marcotti, Levitt and Poland, 2021). In the study of Álvarez, it has been shown that antibodies against NHSL1 are related to lower survival and clinical prognosis of colorectal cancer patients (Álvarez, Manchado and Babel, 2013). Integrative genomic analysis by Youngwook Kim et al. demonstrated a novel fusion gene MYB-NHSL1 in salivary duct carcinoma (Kim, Song, Lee, Swatloski, Kang, Ko, Park, Jeong and Park, 2020). CUL3 regulates cytoskeletal and adhesion protein abundance (Morandell, Schwarz, Basilico, Tasciyan, Dimchev, Nicolas, Sommer, Kreuzinger, Dotter and Knaus, 2021). and is a prognostic biomarker for lung adenocarcinoma (Zhou, Zhang, Xu, Ye, Li, Chen, He and metastasis, 2020). NTNG1 encodes for netrin-G1, which is a membrane adhesion protein (Bruikman, Zhang, Kemper and van Gils, 2019). In a recent study by Hao et al., it has been suggested that NTNG1 is a potential biomarker and therapeutic target for various cancers (Hao, Yu, Lin, Liu, Xing, Yang, Sun, Chen, Jiang and Tang, 2020). Three recommended target genes for hsa-miR-3613-5p (COG6, MTX3, and SOGA3) are related to other mechanisms leading to tumorigenesis. COG6 is a subunit of the Golgi transport complex, which mediates glycosylation reactions (Ungar, Oka, Vasile, Krieger and Hughson, 2005). Glycosylation is a post-translational modification. It has been confirmed that abnormal glycans could trigger determinant events leading to cancer progression (Ho, Hsu, Huang, Kadomatsu, Nakagawara and oncology, 2016). MTX3 encodes a protein located on the outer surface of mitochondria, which is necessary for necrotic cell death and inhibits uncontrolled cell proliferation (Wang, Ono, Kim, Kravchenko, Lin and Han, 2001). SOGA3 involves regulating autophagy and decreasing glucose production (Cowerd, Asmar, Alderman, Alderman, Garland, Busby, Bodnar, Rusyn, Medoff and Tisch, 2010). Oncologic research revealed that tumor cells need glucose to generate the required ATP (Jang, Kim, Lee and medicine, 2013). Hence, the downregulation of SOGA3 in KIRC tissue cells may prevent the reduction of glucose production. Thus, cancerous cells may uptake the essential glucose required for proliferation and tumorigenesis. Decreased mRNA expression of ARIDA1A is observed in HCC tissues, which is associated with metastasis and poor prognosis in patients (Peng, Gao, Zhou, Chen, Xie, Huang and Li, 2020).

Bioinformatics prediction of lncRNA-hsa-miR-3613-5p pairs demonstrated five lncRNAs (FGD5-AS1, HCG18, LIN00885, NORAD, and PAXIP1-AS2) have a negative association with hsa-miR-3613-5p and significantly decreased in KIRC tissues compared with normal tissues. Downregulation of NORAD, and PAXIP1-AS2 was positively correlated with lower expression of NHSL1, STRBP, and VEZF1 and was related to OS of KIRC patients. Most of the obtained lncRNAs are related to the cell cycle process, which the KEGG database uncovered in this study as the top-ranked pathway for hsa-miR-3613-5p. IncRNA FGD5-AS1 has been reported as an independent prognostic predictor for melanoma (Gao, Zhu and Mao, 2020). IncRNA FGD5-AS1 can regulate the cell cycle process by activating the wnt/β-catenin pathway (Wu, Zhu, Song, Guo, Liang and Yan, 2020). Additionally, FGD5-AS1 significantly increased in patients with ccRCC (clear cell renal cell carcinoma), and survival analysis results showed that these patients showed shorter OS (Yang, Zhou, Zhang, Li, Xu, Ma, Xie, Cai, Gong and Gong, 2021). IncRNA HCG18 retarded cell proliferation by impeding the S phase of the cell cycle and was confirmed to be linked to the adverse prognosis of patients with gastric cancer (Xi, Jiang, Wang, Yu, Wang, Wu and He, 2017). IncRNA
LINC00885 modulates bioprocesses related to the TP53 signaling pathway, and it has been documented that oncogenic LncRNA LINC00885 is associated with the early stage of breast cancer development (Abba, Canzoneri, Gurruchaga, Lee, Tatineni, Kil, Lacunza and Aldaz, 2020). IncRNA NORAD is activated by DNA damage and is necessary for genome stability and possibly cell cycle arrest (Chaudhary and Lal, 2017). Recently, the function of this biomarker in carcinogenesis pathways consisting of cell proliferation, apoptosis, and metastasis has been clarified (Han, Wu, Hu, Chen, Jia, He, Bian, Wang, Guo, Kang and disease, 2020). IncRNA PAXIP1-AS2 is known for its contribution to DNA damage repair using the reduction of translesion DNA synthesis (TLS) (Swain, Friedlander, Sehrawat, Sarusi-Portuguez, Rotkopf, Ebert, Paz-Elizur, Dikstein, Carell and Geacintov, 2021). TLS is a process of direct replication of DNA damage that exclusively happens in the S phase of the cell cycle (Swain, Friedlander, Sehrawat, Sarusi-Portuguez, Rotkopf, Ebert, Paz-Elizur, Dikstein, Carell and Geacintov, 2021).

The acquired data from the ENCORI database indicated the negative correlation between downregulation of HS6ST1P1, IL6STP1, EIF4EP2, UBE2D3P1, and AC006210.2 and overexpression of hsa-miR-3613-5p in KIRC tissues. Among these, only lower expressed IL6STP1, EIF4EP2, UBE2D3P1, and AC006210.2 was associated with OS of KIRC patients and had a positive relation with reduced expression of NHSL1, STRBP, and VEZF1. As far as we know, there is no report about these pseudogenes and their role in cancer. Therefore, the current effort proposes four novel pseudogenes that may participate in KIRC tumorigenesis and progression and act as a helpful biomarker for these patients.

A growing body of studies demonstrated that the CeRNA network plays a crucial role in tumorigenesis, and its components could be a potential biomarker for different types of cancers (Fan, Ma and Liu, 2018), Yin, Zeng, Ai, Yu and Li, 2020). For example, Jing Chen et al. introduced the DUXAP8/DUXAP9-miR-29c-3p-COL1A1/COL1A2 as a vital signaling pathway in tumorigenesis and progression KIRC (Chen, Lou, Ding and Wang, 2019). A recent study also has shown that IncRNA MSC antisense RNA 1 (MSC-AS1) is highly up-regulated in KIRC tissues and directly reduces the expression of miR-3924. This miRNA also downregulates the WNT5A gene. IncRNA MSC-AS1 increases the proliferation and migration of KIRC cells through the MSC-AS1/miR-3924/WNT5A/β-catenin axis via augmenting WNT5A expression, and stimulating the WNT5A/β-catenin signaling pathway, While knockdown of MSC-AS1 has the opposite effect (Hu, Li, Cheng, Liu, Zheng, Peng and Zhang, 2020). In this research, instead of producing a network encompassing numerous nodes and edges without any clinical significance, we defined restricted criteria, applied consecutive steps, and identified the most potential regulatory of hsa-miR-3613-5p in KIRC with 17 different axes consisted four pseudogenes, two IncRNAs, and three mRNAs.

The miRNASNP-v3 database indicated five SNPs in mature miRNA and one DRV in mature miRNA of hsa-miR-3613-5p. The data suggested that rs1279899144, rs201796222, rs201796222, rs1438135163, rs1327340301, and COSN28863682 may decrease and rs1363404935 mildly changes the expression of hsa-miR-3613-5p. These findings have important implications because lower expression of hsa-miR-3613-5p in KIRC may reverse all mentioned phenomena and provide a better survival outcome for patients with KIRC. Therefore, studying these variants and their impact on the expression hsa-miR-3613-5p in KIRC seems inevitable.
This study’s KEGG pathway enrichment analysis revealed that the top-ranked pathways for hsa-miR-3613-5p are cell cycle, cell adhesion molecules (CAMs), and hepatocellular carcinoma pathways. Preliminary studies have confirmed that the alternative pattern of cell adhesion molecules (CAMs) have a determinant role in various tumor metastasis, especially in renal cell carcinoma (RCC) (Allory, Matsuoka, Bazille, Christensen, Ronco and Debiec, 2005). This data can show that hsa-miR-3613-5p regulates these pathways via increasing oncogenic activity and decreasing the tumor-suppressive activity of its downstream target genes involved in these processes.

Taken together, integrated bioinformatics analysis of the hsa-miR-3613-5p and its potential regulatory network in this study revealed that hsa-miR-3613-5p might mediate KIRC development through crucial proliferative pathways. As far as we know, this is the first study that suggests there is a potential network related to hsa-miR-3613-5p in KIRC cancer. Noteworthy, laboratory analysis is required to validate the results of this study, which is limited to in-silico experimentations.

**Declarations**

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**Competing Interest**

The authors have no conflicts of interest.

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Figures

Figure 1

The expression analysis of the hsa-miR-3613-5p in KIRC tissues and corresponding normal tissues using the CancerMIRNome database (A), the ENCORI database (B), and UALCAN database (C). P-value < 0.05 was considered statistically significant. KIRC: Kidney renal clear cell carcinoma, N: Sample count
Figure 2

The expression level of hsa-miR-3613 in KIRC based on the clinicopathological parameters: individual cancer stages (A), patient’s race (B), patient gender (C), tumor grade (D), patient age (E), nodal metastatic status (F). P-value < 0.05 was considered statistically significant. KIRC: Kidney renal clear cell carcinoma, N: Sample count
Figure 3

The diagnostic value of the hsa-miR-3613-5p expression in KIRC using ROC curves analysis (Tumor vs. Normal) via the CancerMIRNome database. P-value < 0.05 was considered statistically significant. ROC: Receiver Operating characteristic, KIRC: Kidney renal clear cell carcinoma, AUC: Area Under the Curve, CI: Confidence Interval.
Figure 4

The overall survival curves of patients with KIRC based on hsa-miR-3613-5p expression through CancerMIRNome (A), the ENCORI (B), and the Kaplan–Meier plotter (C) databases. KIRC: Kidney renal clear cell carcinoma. Log-Rank P-value < 0.05 was considered statistically significant. KIRC: Kidney renal clear cell carcinoma, N: Sample count

Figure 5

The regulatory network of hsa-miR-3613-5p in KIRC. V shape indicates pseudogenes, triangle determines IncRNAs, rectangle demonstrates miRNA, and Eclipse shows mRNAs. Green and red colors represent downregulation and upregulation in KIRC, respectively. KIRC: Kidney renal clear cell carcinoma
The hsa-miR-3613-5p’s variants were retrieved from the miRNASNP-v3 database. The characteristics of hsa-miR-3613-5p’s variants (A), the mature sequence of hsa-miR-3613-5p, and the position of variants in its mature sequence (B).

Figure 6

The hsa-miR-3613-5p’s variants were retrieved from the miRNASNP-v3 database. The characteristics of hsa-miR-3613-5p’s variants (A), the mature sequence of hsa-miR-3613-5p, and the position of variants in its mature sequence (B).

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