MicroRNAs in renal cell carcinoma:
A systematic review of clinical implications (Review)

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Abstract. Despite recent advances in the understanding of the biology of renal cell carcinoma (RCC), successful surgical treatment and implementation of novel-targeted therapies, the prognosis for RCC patients remains poor. Late presentation, tumor heterogeneity and in particular the lack of molecular biomarkers for early detection, classification and the surveillance of RCC treatments are major obstacles. The increasing knowledge regarding the functional role of microRNAs (miRNAs) in pathophysiological processes may provide an important link to the identification of suitable therapeutic targets and diagnostic/prognostic biomarkers for RCC. The aim of this review was to provide new insight into the function of miRNAs in the pathogenesis of RCC and to emphasize their potential as diagnostic and prognostic markers, as well as therapeutic targets.

Contents

1. Introduction

Renal cell carcinoma (RCC) is the most common malignant solid tumor in adults. A total of 63,920 new cancer cases and 13,860 deaths from kidney and renal pelvis cancer were estimated to occur in the United States in 2014 (1). There are four major histologic subtypes of RCC, the clear cell RCC (ccRCC) comprise the main histological category that accounts for 75% of cases, followed by the papillary RCC (pRCC) (12%), the chromophobic RCC (chRCC) and oncocytomas (4% each) and rare subtypes (5%) (2). Although surgical resection remains the best curative therapy approach for RCC, ~20-30% of these patients experience local and/or distant disease recurrence (3). Moreover, up to 30% of patients have metastases at the time of the initial diagnosis (4). However, RCC has a highly resistant phenotype to conventional therapeutic modalities, including chemotherapy and radiation, thus it remains an extremely lethal disease (5,6). Another issue concerning RCC is the absence of biomarkers for the early detection and follow-up of the disease, which complicates the early diagnosis and makes it a challenge for the field of oncology (7). Additionally, besides the clinicopathological parameters, there are no molecular markers for the prognosis of RCC.

MicroRNAs (miRNAs) are a class of ~22 nucleotide non-coding RNA molecules that negatively regulate the expression of a wide variety of genes mainly through direct interaction with the 3'-untranslated regions (3'-UTR) of corresponding mRNA targets (8). Since being identified in 1993 by Lee *et al* (9), miRNAs have unraveled new mechanisms for the regulation of gene expression and have provided new directions for cancer research. miRNAs are pivotal regulators of all hallmarks of cancer, which include cell growth and cell cycle control, evasion of apoptosis, tissue invasion and metastasis, angiogenesis and unlimited replicative potential (10). Investigations conducted on miRNAs in RCC have increased at an exponential rate. In the present review, we systematically describe the profiling of miRNAs in RCC and their roles in renal carcinogenesis, diagnosis, prognosis and the potential roles in RCC therapy.

2. Biogenesis of microRNA

Most miRNAs are produced from either intergenic or intronic regions of coding or non-coding genes (11,12). They are transcribed primarily by RNA polymerase II (pol II) as part of longer primary miRNA (pri-miRNA) transcripts that are capped, spliced, and polyadenylated (13,14). The first step in

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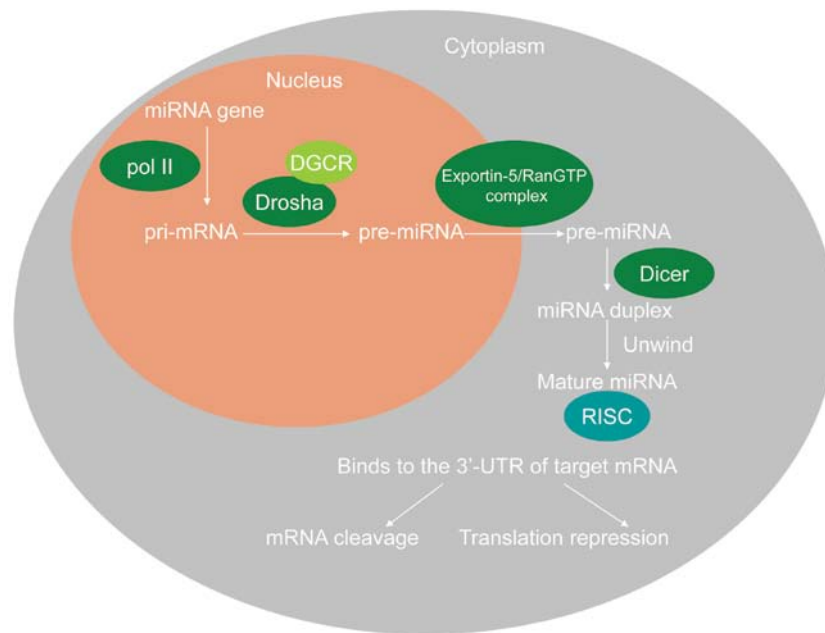E-mail: wubin_cmu@163.com

Figure 1. microRNA biogenesis and processing mechanism.

pri-miRNA maturation is carried out in the nucleus by the RNase III enzyme Drosha and its cofactor DGCR8. This step produces the precursor miRNA hairpin (pre-miRNA) (15,16). The pre-miRNA was then exported to the cytoplasm by the Exportin-5/RanGTP complex where it is cleaved by Dicer to generate the double-trand miRNA (17,18). A helicase then unwinds the duplex into mature miRNAs (19). Mature miRNAs are incorporated into the RNA-induced silencing complex (RISC) and bind to the complementary 3'-UTR of their specific target mRNAs. This process results in the inhibiton of mRNA translation or promotes its degradation and leads to post-transcriptional gene silencing (20-22) (Fig. 1).

3. microRNAs in renal cell carcinoma

A number of approaches have been developed to quantify miRNA levels and numerous studies on miRNA expression profiles and the determination of their mRNA targets and the functional analysis have been carried out in RCC. The deregulated miRNAs in RCC are presented in Tables I and II (23-64). The microarray-based experiments identified 13 overexpressed and 20 downregulated miRNAs in RCC samples. Expression in ccRCC tissue samples compared with matched non-malignant samples measured by RT-PCR was increased on average by 2.7- to 23-fold for the miR-16, -452*, -224, -155 and -210, but decreased by 4.8- to 138-fold for miR-200b, -363, -429, -200c, -514 and -141 (65). Gottardo *et al,* using miRNA microarray hybridization analysis found that miR-28, -185, -27, and let-7f-2 were significantly upregulated in RCC compared to normal kidney (66). Results of another study showed that miR-34a, -224 and -21 were upregulated, whereas miR-141, -149 and -429 were downregulated in the ccRCC tissues (67). Faragalla *et al* also found the expression of miR-21 was significantly upregulated in RCC compared with healthy kidney. A significant difference was found in the expression levels between RCC subtypes, with the highest levels of expression in ccRCC and pRCC subtypes. Significantly higher miR-21 levels were associated with higher

stage and grade (68). However, Silva-Santos *et al* reported that RCC exhibited significantly lower expression levels of miR-21, -141 and -200b compared with that of normal tissues, and expression levels of all miRNAs differed significantly between malignant and benign renal cell tumors (69).

Recent findings have shown that miR-10b/-19a/-19b/-20a/-29a/-29b/-29c/-100/-101/-126/-127/-130/-141/-143/-145/-148a/-192/-194/-200c/-210/-215/-370/-514 were downregulated in metastatic tissue samples compared with normal tissue (70). In addition, a miRNA signature that distinguishes between metastatic and non-metastatic ccRCC was detected, including miR-451, -221, -30a, -10b and -29a, as well as a group of 12 miRNAs, including let-7 family, miR-30c, -26a, which were decreased in highly aggressive primary metastatic tumors (71).

Circulating and urinary miRNAs have also been found to be deregulated in RCC. Redova *et al* identified 30 miRNAs that were differentially expressed between the serum of RCC patients and healthy controls: 19 miRNAs were upregulated and 11 miRNAs were downregulated in RCC patients. Levels of miR-378 were increased, while those of miR-451 were decreased in the serum of RCC patients (72). In another study, miR-34a, -21 and -224 were upregulated, miR-141 was downregulated in the sera of patients with ccRCC, and the serum miR-21 expression levels were significantly correlated with the clinical staging of the patients with ccRCC (67). It was found that RCC patients presented higher circulating expression levels of miR-221 and -222 than healthy individuals. The RCC patients with metastasis at diagnosis also presented higher circulating expression levels of miR-221 than patients without metastasis (73). However, whether these changes are the cause of RCC or as a consequence of RCC remain to be determined.

4. Pathophysiology of renal cell carcinoma

VHL/HIF signaling pathway. RCC is frequently associated with inactivation of the von Hippel-Lindau (VHL) gene, resulting in elevated levels of hypoxia-inducible transcription

Table I. Downregulated microRNAs in renal cell carcinoma.

MicroRNAs	Function	Target genes	Specimen	Pathways/mechanisms involved	Refs.
miR-1/133a	Tumor suppressor	TAGLN2	RCC tissues, cell lines	Proliferation, invasion, apoptosis, cell cycle	23
miR-30c	Tumor suppressor	Slug	RCC tissues, cell lines	Hypoxia, EMT	24
miR-30d	Tumor suppressor	Cyclin E2	RCC tissues	Proliferation, colony formation, cell cycle	25
miR-34a	Tumor suppressor	c-Met, c-MYC, Notch1	RCC tissues	Cell growth, cell cycle	26,27
miR-99a	Tumor suppressor	mTOR	RCC tissues	Cells growth, clonality, migration, invasion, cell cycle	28
miR-133b	Tumor suppressor	MMP-9	Cell lines	Proliferation, migration, invasion	29
miR-135a	Tumor suppressor	c-MYC	RCC tissues	Cell proliferation, cell cycle	30
miR-138	Tumor suppressor	VIM, HIF-1α, EZH2	Cell lines	Migration, invasion, senescence	31-33
miR-141		CDC25B	RCC tissues	Cell growth, metastasis, EphA2/p-FAK/p-AKT/MMPs signaling	34,35
miR-143/145 cluster		Hexokinase-2 (HK2)	RCC tissues	Cell proliferation, invasion	36
miR-145	Tumor suppressor	ADAM17, ANGPT2, NEDD9	RCC tissues	Proliferation, migration	37,38
miR-182-5p	Tumor suppressor	FLOT1	RCC tissues	AKT/FOXO3a signaling	39
miR-187	Tumor suppressor	B7 homolog 3 (B7-H3)	Tumor tissue, plasma	Proliferation, tumor growth, motility	40
miR-192/194/215		ZEB2, MDM2, TYMS	Metastatic tumors	Migration, invasion, proliferation	41
miR-199a-3p		c-Met	RCC tissues, cell lines	HGF/c-Met signaling	42
miR-200c		ZEB1	RCC tissues	EMT, p-Akt, Akt	43
miR-205		SFKs	RCC tissue, cell line	Ras/Raf/ERK1/2 signaling, cell cycle, apoptosis, proliferation, colony formation, migration, invasion	44
miR-217	Tumor suppressor		ccRCC tissues	Proliferation, motility	45
miR-218	Tumor suppressor	CAV2	RCC tissues	Migration, invasion, focal adhesion	46
miR-508-3p/509-3p	Tumor suppressor		RCC tissues, plasma	Proliferation, apoptosis, migration	47
miR-509-5p			RCC tissues, plasma	Proliferation, migration, apoptosis	48
miR-584	Tumor suppressor	ROCK-1	Cell lines	Cell motility	49
miR-708	Tumor suppressor	Survivin, ZEB2, BMI1	RCC tissues	Cell growth, clonality, invasion, migration, apoptosis	50
miR-1285	Tumor suppressor	TGM2	RCC tissues	Proliferation, invasion, migration	51
miR-1291	Tumor suppressor	SLC2A1/GLUT1	RCC tissues	Cell proliferation, migration, invasion	52
miR-1826	Tumor suppressor	β-catenin, MEK1	RCC tissues	Proliferation, invasion, migration, apoptosis, cell cycle	53

EMT, epithelial to mesenchymal transition.

Table II. Upregulated microRNAs in renal cell carcinoma.

MicroRNAs	Specimen	Function	Target genes	Pathways/mechanisms involved	Refs.
miR-7	RCC tissues	Oncogene		Migration, proliferation, apoptosis	54
miR-21	RCC tissues, metastatic RCC, cell lines	Tumor marker	PDCD4, TPM1, PTEN, FASL, TIMP3	Growth, apoptosis, cell cycle, invasion, migration	55-58
miR-23b-3p	RCC tissues, cell lines		PTEN	Cell cycle, apoptosis, invasion, migration	59
miR-23b	Cell lines, RCC tissues	Oncogene	POX	HIF, apoptosis	60
miR-155	RCC tissues, cell lines	Oncogene	BACH1	Proliferation, migration, apoptosis	61
miR-210	Cell line		E2F3	Cell cycle, migration, invasion	62
miR-224/383	ccRCC tissues		DIO1	Tissue hypothyroidism	63
miR-590-5p	ACHN, 786-O cells	Oncomir	PBRM1	Proliferation, invasion, cell cycle	64

factors (HIF). Increasing evidence supports the involvement of alternative mechanisms in the regulation of VHL/HIF expression, including suppression by miRNAs. For example, both the VHL and hypoxia-inducible factor 1-α (HIF1α) gene were direct targets of miR-17-5p and miR-224 in RCC (74). In addition, miR-138 targeted HIF1α and suppressed its expression, and affected the apoptosis and migration of ccRCC cells (32).

By contrast, miRNAs can also be regulated by VHL in an HIF-dependent or -independent manner in RCC (75), thereby affecting downstream signaling. miR-210 has been shown to be expressed at significantly higher levels in tumors with either VHL mutations or methylation of the VHL promoter and to be correlated with the expression of CA9, a known transcriptional target of HIF transcription factors (75). In another study, due to HIF1α accumulation, miR-210 upregulation induced aneuploidy via E2F3 downregulation at least in part, and played a role in tumorigenesis and/or progression of RCC (62). In addition, miR-31, -21, and let-7i were upregulated in RCC cells with functional VHL, whereas miR-155, -193b, -17, -18a, -20a, and -210 were downregulated. The knockdown of HIF1A or HIF1B also reduced miR-210 and miR-155 expression levels (75).

PI3K/Akt signaling pathway. The upregulation of miR-122 was shown to play an important role in the progression of RCC by activating the PI3K/Akt signaling pathway and is a potential molecular target for anti-cancer therapeutics (76). Findings of another study showed a molecular order of a phosphatase-kinase couple involving PTEN/Akt/IKKβ and NFκB-dependent cyclin D1 expression for renal carcinoma cell proliferation by increased miR-21 levels (77). Furthermore, miR-21 was shown to directly downregulate the proapoptotic protein PDCD4 to increase the migration and invasion of ACHN and 786-O RCC cells as a result of the phosphorylation/activation of Akt and IKKβ, which activated NFκB-dependent transcription. Thus, miR-21 promoted cancer cell hyperplasia and contributed to tumor cell transformation and metastasis (78). In addition, miR-200c decreased the metastatic ability of RCC cells by upregulating E-cadherin through ZEB1. Additionally, modulation of the expression of miR-200c influenced Akt protein levels, suggesting the presence of an Akt-miR-200c-E-cadherin axis in the epithelial-to-mesenchymal transition (EMT) process in RCC (43).

Methylation and miRNA. miRNAs have been shown to play a role as targets and effectors in gene hypermethylation and silencing in cancer cells. Two genes encoding for miR-9 were demonstrated to be significantly hypermethylated in ccRCC tumors compared with adjacent normal tissues resulting in decreased expression. Additionally, the methylation of these genes was more significant in DNA obtained from the primary tumor for patients who developed a recurrence than in tumors from non-recurrent patients. Furthermore, methylation of miR-9-3 was significantly associated with an increased risk of recurrence, and high methylation levels of miR-9-1 or -9-3 resulted in a significant, almost 30-month decrease in recurrence-free survival time (79). Another study showed that hypermethylation of miR-124-3 in samples of RCCs was identified compared with adjacent normal tissues. miR-124-3 methylation was significantly increased in tumors with state of advanced disease. Higher relative methylation

was associated with worse recurrence-free survival. Moreover, miR-124-3 CpG island (CGI) methylation was identified as a relevant epigenetic marker for ccRCC, while methylation of miR-124-3 was suggested as an independent prognosticator for ccRCC (80). In addition, the frequency of methylation of miR-124a-2, -124a-3, -9-1, -9-3, -34b/c and -129-2 was significantly higher in tumor samples than in normal tissues (81). On the other hand, DNA promoter methylation levels were found to be inversely correlated with the expression of miR-21, -10b and -30a in ccRCC (82).

Other novel mechanisms. The fundamental role of miRNAs on the pathophysiology of RCC involve the downregulation of their target genes by recognizing the 3'-UTR, through which, they act as oncogenes or tumor suppressors and affect the biology of cell processes such as proliferation, migration and invasion (Tables I and II). However, other mechanisms are also involved.

Single-nucleotide polymorphisms (SNPs) in miRNAs genes are currently being identified for contributing to cancer risk, prognosis and survival. miR-196a2 SNP rs11614913 was associated with RCC susceptibility in a recessive model and with survival of RCC in a dominant model (83).

Chromosomal instability enables tumor development, in part by aberrant expression of the mitotic checkpoint protein Mad2. In VHL-positive RCC cells, enhanced expression of miR-28-5p decreased Mad2 levels and promoted checkpoint weakness and chromosomal instability. Conversely, in checkpoint-deficient VHL-negative RCC cells, inhibition of miR-28-5p function restored Mad2 levels, mitotic checkpoint proficiency, and chromosomal stability (84).

Of note, Prior *et al* identified a novel paracrine mechanism through which high miR-942 levels in metastatic renal cell carcinoma (mRCC) cells upregulate MMP-9 and VEGF secretion to enhance endothelial migration and sunitinib resistance (85).

5. Biomarkers and diagnosis

Recent studies identified a number of novel deregulated miRNAs specific for each subtype of RCC and these miRNAs were able to discriminate ccRCC from the normal kidney (86,87). miR-141 was demonstrated as a potential biomarker for discriminating ccRCC from normal tissues and a crucial suppressor of ccRCC cell proliferation and metastasis by modulating the EphA2/p-FAK/p-AKT/MMPs signaling cascade (35). Another study showed that malignant and non-malignant tissue were clearly differentiated by their miRNA profile, and a combination of miR-141 and -155 resulted in a 97% overall correct classification of samples (65). Moreover, the miR-141 or -200b panel accurately distinguished RCC from normal kidney, oncocytoma from RCC and chRCC from oncocytoma (69). In addition, Faragalla *et al* demonstrated that miR-21 expression distinguished ccRCC and pRCC from chRCC and oncocytoma with 90% specificity and 83% sensitivity (68). Youssef *et al* developed a classification system that can distinguish the different RCC subtypes using unique miRNA signatures in a maximum of four steps. The system has a sensitivity of 97% in distinguishing normal from RCC, 100% for ccRCC, 97% for pRCC, and 100% accuracy in distinguishing oncocytoma from chRCC (88).

As extracellular miRNAs such as serum or urine miRNAs were also deregulated in RCC patients, they are considered promising candidates as biomarkers for the diagnosis and prognosis of RCC. The levels of miR-378 were increased, while those of miR-451 were decreased in the sera of RCC patients, and they were shown to be able to distinguish RCC from healthy controls. The combination of the two miRNAs improved stratification power with a sensitivity of 81% and a specificity of 83% and AUC =0.86 (72). Serum miR-1233 levels were increased in RCC patients with a sensitivity of 77.4% and a specificity of 37.6%. The circulating miR-1233 was identified as a potential biomarker for RCC patients (89). In addition, serum miR-210 upregulation occurred in the early stage of ccRCC (90), and serum miR-210 levels were significantly higher in ccRCC patients than in normal controls with a sensitivity of 81.0% and specificity of 79.4% in discriminating diagnosis (91). In urine, upregulated miR-15a was measured from patients with RCC but was almost undetectable in oncocytoma, other tumors, and urinary tract inflammation (92).

The high degree of diagnostic accuracy suggests that miRNA in RCC patients may serve as next-generation biomarkers for detection of the disease. However, large-scale investigations and additional improvements are required to confirm the results and verify the feasibility of routine clinical utilization (93).

6. Prognosis

Increasing studies showed that aberrant miRNA expression is associated with 5-year survival, overall survival, disease grade and stage, recurrence and metastasis. miRNAs with low expression associated with poor prognosis (shorter survival or early recurrence) included miR-187 (40), -215 (41), -217 (45), 155 (94) and -1826 (53). In addition, the expression levels of miR-143, -26a, -145, -10b, -195 and -126 were lower in the tumors of RCC patients who developed tumor relapse, while the lowest levels of these miRNAs were identified in primary metastatic tumors. By using Kaplan-Meier analysis, miR-127-3p, -145 and -126 were significantly correlated with relapse-free survival of non-metastatic RCC patients (95). On the other hand, a high or positive expression of miR-21 (68), -23b-3p (59), -100 (96) and -630 (97) was associated with shorter survival or early recurrence.

Metastasis is extremely common in RCC and increasing studies can pave the way to the clinical use of miRNAs as prognostic markers for metastasis. miR-10b, -139-5p, -130b and -199b-5p were associated with ccRCC metastasis and prognosis (98). In addition, the expression levels of miR-106b were significantly lower in tumors of patients who developed metastasis, with miR-106b being a potential predictive marker of early metastasis after nephrectomy in RCC patients (99).

In plasma, higher circulating expression levels of miR-221 were associated with poor overall survival in RCC patients (73). miR-187 was downregulated in the plasma and tumor tissue of ccRCC patients. Decreased miR-187 expression levels were associated with increased tumor grade and stage. Patients with high miR-187 expression survived 5 years, while of those with low miR-187 expression, only 42% survived (40).

In addition, miRNA-related SNPs may influence the recurrence and survival in RCC patients. Future investigations in

larger populations and functional characterizations are necessary to validate these results (83).

Targeting therapy is one of the most effective approaches for the treatment of mRCC patients, however, the important issue is prediction of the response. One study showed that miR-141 was significantly downregulated in tumors of poor responders to sunitinib compared to good responders (100). Another study showed that miR-942 was the most accurate predictor of sunitinib efficacy for mRCC patients. A high expression of miR-942, -628-5p, -133a and -484 was significantly associated with decreased time to progression and overall survival in mRCC patients, and these miRNAs were also overexpressed in the sunitinib-resistant Caki-2 cell line in comparison with the sensitive cell line (85).

7. Therapy

With the significant roles that miRNAs play in the pathogenesis, increasing efforts are dedicated to the development of miRNA-based therapies. There is great interest in the potential application of the restoring functions of tumor suppressive miRNAs and the inhibiting oncogenic miRNAs.

For example, reintroducing miR-199a-3p in 769-P and Caki-1 RCC cell lines inhibited cell proliferation and caused G_1-phase arrest (42). Restoration of miR-138 in RCC cells changed the EMT-like morphology and suppressed cell migration and invasion (31). Simultaneously expressed miR-424 and -381 synergistically inhibited proliferation, abrogated G2/M arrest, and induced apoptosis. The combination led to Cdc2 activation through WEE1 inhibition, which was more effective when cells were treated with the two miRNAs compared with either miRNA alone, indicating synergy between these miRNAs (101). miR-138 induced SN-12 cell senescence by downregulating EZH2 expression and upregulating P16 expression in ccRCC (33). In addition, the transient and stable overexpression of miR-205 in A498 cells resulted in the induction of G_0/G_1 cell-cycle arrest and apoptosis, decreased levels of cyclin D1 and c-Myc, suppressed cell proliferation, colony formation, migration, and invasion in RCC cells. miR-205 also inhibited tumor cell growth *in vivo* (44). Furthermore, miR-34a suppressed RCC cell growth, tube formation and metastasis *in vitro* and *in vivo* by targeting CD44 (102).

On the other hand, silencing of miR-210 expression decreased the viability of ACHN and Caki-2 cells and accumulation of Caki-2 in G_2 phase of the cell cycle. Downregulation of miR-210 also reduced the migratory and invasive potential of ACHN metastatic RCC cells (103). The knockdown of miRNA-23b-3p expression in RCC cell lines caused an induction of apoptosis and reduced invasive abilities by inducing PTEN gene expression with a concomitant reduction in PI3-kinase, total Akt and IL-32 (59). In addition, the suppression of miR-155 inhibited cell proliferation and migratory activity and induced apoptosis in RCC cells by inhibiting BACH1 protein (61). Moreover, the downregulation of miR-7 with synthesized inhibitor suppressed cell migration *in vitro* as well as cell proliferation, and induced RCC cell apoptosis (54).

The miRNA expression can be controlled by epigenetic silencing, which is a regulatory mechanism of miRNA. Therefore, epigenetic modulation of the gene expression may be useful for modulating miRNA expression. In ccRCC cell lines, treatment with inhibitors of the DNA methyltransferase and histone deacetylase causes re-expression of silenced miRNAs with putative tumor suppressive function (104).

miRNA-based therapies may also be used together with other therapeutic strategies in pre-clinical studies. For instance, miR-381 increased sensitivity of 786-O cells to 5-FU by inhibiting WEE1 and increasing Cdc2 activity (105). In another study, miR-185 enhanced radiation-induced apoptosis and inhibition of proliferation by repressing the ATR pathway (106). Notably, the reintroduction of miR-141 *in vitro* led to EMT reversal and increased sensibility to a hypoxic environment (100).

8. Conclusion

Emerging evidence suggests that miRNAs have a significant impact on our understanding of the pathogenesis of RCC. More studies are required to accurately identify the mechanisms by which miRNAs affect RCC. Moreover, miRNAs present new potential tumor biomarkers that may improve our diagnostic, prognostic and predictive abilities and, consequently, cancer patient treatment strategy. Since the finding that miRNAs can have direct biological effects on cancer, there has been much interest in developing novel miRNA-based cancer therapies. The development of a useful miRNA therapy has the capability to revolutionize personalized cancer therapy.

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