Review

MicroRNAs in Renal Cell Carcinoma: Implications for Pathogenesis, Diagnosis, Prognosis and Therapy

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Abstract. *Renal cell carcinoma (RCC) is a potentially curable disease, especially if the tumor is limited to the kidneys and no systemic metastatic spread has occurred by the time of diagnosis. Despite the potential for successful surgical removal of the tumor-bearing organ in localized stages and the likelihood of treatment success, the complications and long-term morbidity and mortality of RCC are difficult to accurately predict. The currently used drugs were developed based on the understanding of the molecular details of pathogenesis at the time, which has improved over the past several decades. However, more efforts should be made to improve early diagnosis, the surveillance of patients who undergo resection and treatment for metastatic RCC. Recently, small non-coding RNAs (microRNAs) were found to play pivotal roles in the metastatic dissemination of tumor cells in different types of cancer. The aim of this review is to provide an overview of the role of microRNAs in the pathogenesis of RCC and to discuss their potential as diagnostic, prognostic and, ultimately, therapeutic biomolecules.*

Renal cell carcinoma (RCC) accounts for approximately 3% of all adult malignancies, and its incidence has increased over the past two decades (1). Although the reasons for the observed epidemiological changes remain enigmatic, there is a significant trend towards smaller tumor size, a phenomenon called stage migration (2). The treatment of choice for localized, kidney-confined tumors is surgery. The risk of tumor dissemination and disease recurrence differs depending on the tumor stage and other clinical and pathological prognostic factors (3-5). Several antitumor agents are currently available for the treatment of metastatic RCC, primarily targeting the angiogenesis and the mammalian target of rapamycin (mTOR) signaling pathways (6). All of these agents have been proven to improve clinical endpoints, such as progression-free or overall survival, but their efficacy is limited, and the duration of response is often short. Therefore, there is a clear need for novel molecular biomarkers that enable the early diagnosis of RCC, for better risk assessment, to select patients for more aggressive treatment modalities, and for molecules that serve as novel drug targets.

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MicroRNAs

MicroRNAs (miRNAs) are involved in many physiological and pathological cellular processes (7). They are short RNAs of approximately 22 nucleotides (hence the micro-terminology) that are never translated into a protein and are therefore categorized as non-coding RNAs. miRNAs are post-transcriptional regulators that control the translation of large messenger RNAs (mRNAs). After a complex biogenesis process, in which the miRNAs are first generated as primary (pri-)miRNAs, then transferred from the nucleus to the cytoplasm by the exportin 5 pore protein, they are cleaved into a pre-miRNA and finally associate with complex protein machinery in the cytoplasm (8). There, the miRNAs bind to complementary sequences in the 3'-untranslated region (UTR) or other regions of their target mRNAs, usually resulting in gene silencing through the induction of mRNA degradation or the inhibition of protein translation. miRNAs are thought to be involved in many biological processes, and some miRNAs have been linked to kidney cancer (8). The aim of this review is to discuss the involvement of miRNAs in the pathogenesis of kidney cancer, describing their potential as novel diagnostic and prognostic markers, and to outline how these small molecules might eventually serve as therapeutic targets.

MicroRNAs and the Pathophysiology of RCC

Hypoxia and other mechanisms. The up- and down-regulation of various miRNAs in RCC were described in several recently published studies. The cellular effects of miRNA dysregulation are diverse and often lead to typical features of cancer, which are described in more detail in the following sections (for an overview see also Figure 1). Because the same miRNA can target multiple mRNAs and multiple miRNAs can control the same molecule, it is often difficult to precisely analyze the effects of a particular miRNA (9). For instance, miR-17-5p and miR-224 both target hypoxia-inducible factor 1α (HIF1α) and Von Hippel-Landau protein (VHL), whose expression is lost in approximately 70% of all RCCs (10). In addition to VHL and HIF1α, miR-17-5p expression is also negatively-correlated with the expression of its two predicted targets, vascular endothelial growth factor A (VEGFA) and egl nine homolog 3 (EGLN3). The expression of miR-224 is negatively-correlated with that of its targets, Sma- and Mad-related protein 4 (SMAD4) and SMAD5. These findings underline the fact that in RCC specimens, dysregulated miRNAs were found to modulate the function of VHL-HIF-1α by targeting downstream signaling pathways (10). Hypoxic conditions are often observed in rapidly growing tumors, including RCC. The *VHL* and *HIF1α* genes play an important role in hypoxia and pathogenesis of RCC, and some related oncogenic miRNAs were found to interfere with the VHL HIF1α signaling cascade. For instance, *miR-92* and *VHL* mRNA expression were prominently inversely correlated (11). In clear cell RCC, miR-138 targets HIF1α and suppresses its expression, which, in turn, can affect apoptosis and migration of RCC cancer cells (12). In addition, HIF1α accumulation is associated with miR-210 up-regulation, and miR-210 overexpression induces centrosome amplification and aneuploidy *via* the down-regulation of E2F transcription factor 3 (E2F3). This mechanism may contribute to tumorigenesis and tumor progression (13). In another study, carbamylated albumin, a metabolite with unknown biological functions, was found to be potentially involved in the pathogenesis of RCC by stimulating miR-146a/b expression. Although miR-146a/b are often up-regulated in RCC and the oncogenic effects of miR-146a/b were reported in breast cancer, their specific role in RCC has not yet been studied (14).

Cell proliferation. One of the hallmarks of cancer is uncontrolled cell growth, and miRNAs have been described to alter cell proliferation in RCC. For instance, the tumor suppressor miR-1285, which is down-regulated in RCC, normally targets the oncogene transglutaminase 2 (*TGM2*), which affects proliferation and wound healing (15). miR-199a is frequently down-regulated in RCC (in 59% of RCC cases), and the restoration of miR-199a expression down-regulates glycogen synthase kinase 3b (GSK3b). GSK3b was described as a growth promoter in RCC, and targeting GSK3b through the restoration of miR-199a expression inhibits RCC cell growth (16). Another miRNA involved in cell proliferation is miR-205, which is significantly down-regulated in many RCC cases. The overexpression of miR-205 inhibits cell proliferation by targeting the oncogenic phospho-sarcoma (Src) tyrosine kinase-regulated extracellular signal-regulated kinase 1 and 2 (ERK1/2) pathway (17). The inhibition of miR-21, an miRNA that was widely identified as being oncogenic for many types of cancer, has a significant antiproliferative effect in RCC cell lines (18). Insulin-like growth factor 1 (IGF1) is targeted by several miRNAs, and VEGFA is targeted by miR-126. Both factors are positive regulators of cellular proliferation, thus a disturbance of the miRNA networks in RCC may influence this biological feature (19).

Cell migration and invasion. miR-21 was shown to promote tumor invasion (18). The oncogene *TGM2*, which is widely overexpressed in RCC tissues and is one target of the frequently down-regulated miR-1285, is linked to cell migration and invasion (19). miR-34a inhibits the oncogene myelocytomatosis viral oncogene homolog (*MYC*) by binding to its 3'UTR, thereby suppressing the function of the MYC-S-phase kinase-associated protein 2 (Skp2)- Myc-associated zinc-finger protein 1 (Miz1) complex, which normally activates the Ras homolog gene family, member A (RhoA) and the c-Myc- positive transcription elongation factor (P-TEFb) complex that extends transcription through RNA polymerase II, which, in turn, activates several genes in the RCC tumor cells (20). One study reported a typical metastatic signature in clear cell RCC that gradually develops over the course of tumor progression. Interestingly, 57% of the differentially expressed miRNAs found are organized in clusters that target the same molecule and thus could have a greater synergetic effect. VEGF, HIF1α, platelet-derived growth factor-beta (PDGFB), PDGFC, matrix metalloproteinase 2 (MMP2), mouse double minute 2 homolog (MDM2) and thymidylate synthase (TYMS) are key molecules that are targeted by multiple miRNAs which are dysregulated in metastatic clear cell RCC (19). The overexpression of miR-215 induces cell migration and invasion *in vivo* through the direct targeting of zinc finger E box-binding homeobox 2 (ZEB2) and the subsequent promotion of the epithelial-to-mesenchymal transition (EMT), thus affecting the metastatic potential of RCC cells (21). miR-708 expression is widely attenuated in RCC tissue. miR-708 targets ZEB2 and polycomb ring finger oncogene 1 (BMI1), proteins that are often up-regulated in cancer tissues. These proteins affect EMT, a key embryonic program that is reactivated inappropriately during tumorigenesis. The tumor

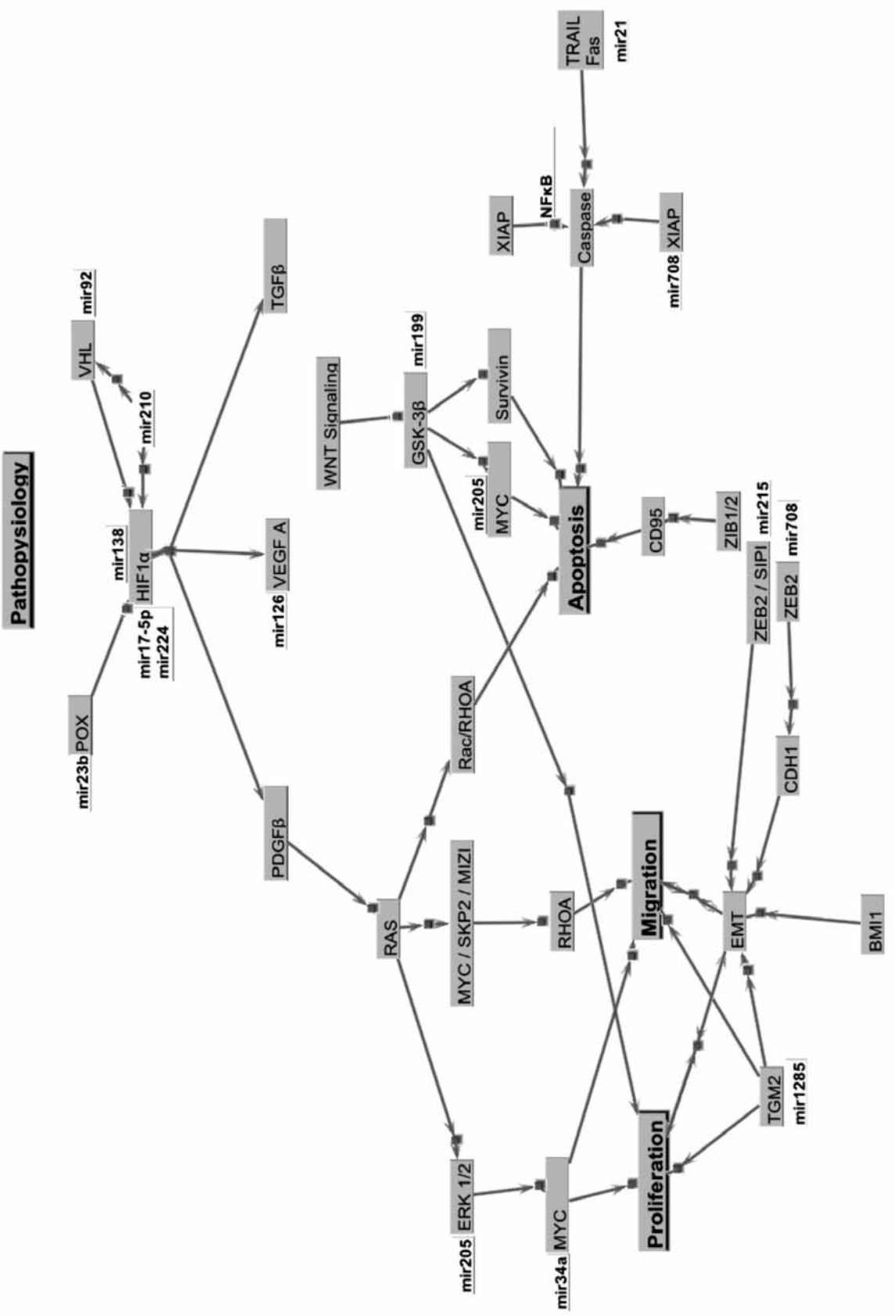

Figure 1. *Overview of the complex interaction signaling networks of renal cell carcinoma.*

suppressor miR-708 directly affects cell migration and invasion mediated by the reduced expression of the EMT regulators ZEB2 and BMI1, in parallel with the induction of E-cadherin expression and the suppression of fibronectin (22).

Apoptosis and cell cycle. The down-regulation of GSK3b caused by miR-199 overexpression leads to a down-regulation of X-linked inhibitor of apoptosis (XIAP) and the anti-apoptotic B-cell chronic lymphatic leukemia/lymphoma 2 (BCL-2), mediated by nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) (16). The restoration of miR-708 expression strongly induces apoptosis through the cleavage of caspase-7 and caspase-3 in RCC cell lines; this induction is mediated by the TNF-related apoptosis-inducing ligand (TRAIL) receptor. The pro-apoptotic function of miR-708 may be mediated primarily through survivin, a cellular homeostasis regulator that modulates cell death, cell cycle and cell survival (22). G_0/G_1 cell cycle arrest and apoptosis, indicated by lower levels of cyclin D1 and MYC, are induced by the stable overexpression of miR-205 in RCC cells (17). As mentioned above, miR-21 targets several genes in RCC and plays a key role in the processes regulating cancer progression, including apoptosis. The inhibition of miR-21 leads to an increase in the proportion of cells in the G_0/G_1 phase *in vitro* through cyclin-dependent kinase inhibitor 1S (cDKN1S or p21) and mitogen-activated protein kinase (MAPK) up-regulation and a reduction in cyclin E2. Furthermore, a reduction of miR-21 inhibits the expression of the TNF receptor superfamily member 6 (FAS) ligand and tissue inhibitor of metalloproteinase 3 (TIMP3), thus overriding the antiapoptotic function of miR-21 (18, 23). Proline oxidase (POX) is a mitochondrial tumor suppressor that induces apoptosis by generating reactive oxygen species (ROS) and repressing HIF signaling. POX is not detectable in RCC because it is targeted by the oncogene *miR-23b*, which is frequently up-regulated in RCC tissue (24).

Diagnosis

Today, an increasing number of RCC cases are detected at earlier stages due to the widespread use of imaging techniques (2). However, many early RCCs are not accurately distinguishable from other non-malignant renal lesions, which can lead to incorrect diagnoses and a high rate of unnecessary nephrectomies. In addition, after the successful resection of a histologically-confirmed RCC, patient surveillance relies mainly on imaging techniques and the evaluation of clinical symptoms. Therefore, novel biomarkers that can be detected in the blood or urine could enable early diagnosis with higher specificity and sensitivity and could provide tumor markers for the early detection of disease recurrence or metastatic lesions. miRNAs have great potential as novel biomarkers because they are stable in several body fluids, and they are easy and relatively inexpensive to detect by standard quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) assays (25). Several studies have discussed the diagnostic implications of serum miRNA levels in the diagnosis of RCC. For instance, circulating miR-1233 was described as a potential biomarker for RCC, although that study was limited by its lack of correlation of clinical and pathological parameters with the observed levels of miR-1233 expression (26). The characterization of RCC subtypes is of major clinical importance because each subtype has a different prognosis and future therapeutic approaches might include histology as a selection criterion (27). In this context, it should be noted that a molecular classification of RCCs based on miRNA expression and virtual karyotyping was able to differentiate amongst the four most common types of adult renal tumor using a small number of miRNAs (28). From a more clinical perspective with the aim of supporting diagnoses, a stepwise decision tree was created to differentiate between kidney cancer subtypes (clear cell, papillary and chromophobe) and oncocytoma, depending on the miRNA signatures in a maximum of four steps. This method is valuable in small biopsy samples, and in cases where the morphological assessment is not sufficient for diagnosis (29). This metastatic signature in RCCs, which includes several key molecules influencing metastasis development, may be a good biomarker for kidney cancer metastasis and may improve treatment selection in the future by enabling the identification of tumors with a high potential for metastasis (19). As a routine component of diagnosis and treatment, subtype- or stage-specific miRNA expression profiling may offer more personalized therapy and targeted treatments.

Prognosis

Several studies showed that aberrant miRNA expression is related to overall survival, disease stage and the development of metastases and recurrences. For instance, the expression of miR-21 is inversely-correlated with the overall survival of patients with RCC. A very high level of miR-21 expression is generally correlated with advanced stages of RCC, as is low miR-199a expression (16, 18). Meanwhile, miR-9-1 and miR-9-3 are both significantly down-regulated in RCC. The methylation levels of their encoding genes were found to be higher in the primary tumors of patients who developed a recurrence, compared to patients without a recurrence, with an almost 30-month decrease of the recurrence-free survival time (30). Another potential prognostic marker could be miR-708, which targets survivin, an independent predictor of progression and death in RCC. In patients with metastases, significant down-regulation of miR-106b was a predictive marker of early metastasis after nephrectomy (22, 31). Some

studies showed that many miRNAs have the potential to be prognostic biomarkers. Large independent external validation studies have been performed for traditional prognostic models (32, 33), and therefore more validation studies on miRNAs are recommended.

Therapy

Several miRNAs that target key molecules in RCC pathogenesis were identified, and this role seems to make them an attractive option for future therapeutics. However, individual molecules can be targeted by different miRNAs, and a single miRNA can affect multiple targets along the same pathway, which can offer novel therapeutic opportunities but also raises potential complications. In RCC, miR-224 affects at least two molecules of the transforming growth factor-beta (TGFβ) pathway (namely SMAD4 and SMAD5), and miR-17-5p and miR-224 target the tumor suppressor VHL and the oncogenic HIF1α (10). Genistein, a chemopreventative agent, reduced miR-21 expression *in vitro* and *in vivo*, and mice injected with genistein-treated RCC cells (A-498) developed significantly smaller tumors than did control mice (18). miR-155 may function as an oncogene by inhibiting basic leucine zipper transcription factor 1 (BACH1). Therefore, targeting miR-155 using an anti-miRNA might provide a novel therapeutic approach (34). The up-regulation of miR-199 targets GSK3b and suppresses cancer cell growth; therefore, miR-199 may be useful as a new therapy (16). Another option for intervention may involve miR-205, which inhibits the proto-oncogenic Src family tyrosine kinases (*SFKs*) (17).

Conclusion

MicroRNAs are novel molecules that offer interesting insights into the pathogenesis of RCC. Further efforts are needed to decipher their roles in RCC, which might provide the basis for the use of these small molecules as "theranostics" in patients with RCC.

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