Epigenomics of clear cell renal cell carcinoma: mechanisms and potential use in molecular pathology

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Abstract: Clear cell renal cell carcinoma (ccRCC) is one frequent form of urologic malignancy with numerous genetic and epigenetic alterations. This review summarizes the recent major findings of epigenetic alterations including DNA methylation, histone modifications, microRNAs and recently identified long noncoding RNAs in the development and progression of ccRCC. These epigenetic profilings can provide a promising means of prognostication and early diagnosis for patients with ccRCCs. With the developed high-throughput technologies nowadays, the epigenetic analyses will have possible clinical applications in the molecular pathology of ccRCC.

Keywords: DNA methylation; histone modification; microRNA; long noncoding RNA; clear cell renal cell carcinoma (ccRCC)

Kidney cancer, now being the 7th leading cancer in men in US, contributing to the estimated 61,560 newly diagnosed and 14,080 deaths in 2015 (1). Renal cell carcinoma (RCC) is the most common form of kidney cancer of which 70–80% of cases are defined as clear cell RCC (ccRCC), which is the RCC subtype we discuss below. The incidence of RCC has been steadily rising by 2–4% each year in recent decades worldwide. Even though the incidence of RCC in Asia is lower than in US and Europe, the mortality-to-morbidity ratio is much higher in Asia than in Western countries (2). In China, there is obvious increment of the RCC incidence in recent years with the early diagnosed cases increasing.

Epigenetics is a phenomenon that involves heritable changes in gene expression that does not affect the nucleotide sequence but impacts the conformation of the DNA (3). Epigenetics crucially determine which genes are expressed by which cell type, and when (4). Epigenetic alterations are considered as a hallmark of cancer (5). At least three types of epigenetic modifications regulate chromatin: DNA methylation, histone modifications, and non-coding RNAs. The most well documented epigenetic alterations are DNA methylation and histone modifications. Unlike genetic alteration, such epigenetic changes can potentially be reversed, making enzymes involved in such processes promising therapeutic targets. RCC is characterized by numerous genetic and epigenetic alterations. Here we reviewed the important epigenetic alterations in DNA methylation, histone modifications and non-coding RNAs including microRNAs and recently identified long noncoding RNAs during RCC tumorigenesis and progression, especially their potential uses in molecular diagnosis, prognostic and possible therapeutic prediction.

DNA methylation in ccRCC

DNA methylation and tumorigenesis of ccRCC

DNA methylation includes hypermethylation of area rich in cytosine and guanine dinucleotides (CpG islands, CpGIs) within the promoter that results in tumor suppressor gene

(TSG) inactivation and affects noncoding RNA expression or promoter CpGIs hypomethylation resulting in proto-oncogene activation. Recent data have shown that the non-CpGIs loci are also very important in gene regulation. Furthermore, newer high-resolution assays reveal that gene body methylation may be even more important in gene regulation than promoter methylation.

It is well known that the familial and nearly 70% of sporadic ccRCC cases are characterized by the alterations of von Hippel–Lindau (*VHL*) gene resulting from either somatic mutation in majority status, loss of heterozygosity and promoter hypermethylation (6). The dysfunction of *VHL* leads to the accumulation of hypoxia-inducible factors (HIFs) and increased effects of angiogenesis and pathogenesis of ccRCC (7,8). Although the *VHL* inactivation by promoter methylation occurs in ~15% of sporadic ccRCC, inactivation of the TSG in RCC most commonly results from promoter methylation, whilst intragenic mutations are rare.

By comparing DNA methylation profiles (1,505 CpGIs analyzed) between familial and sporadic *VHL* wild type ccRCC cases, several loci, including *RASSF1*, *PITX2*, *CDH13*, *HS3ST2*, *TWIST1*, *TAL1*, *TUSC3*, and *DCC* were found to be more frequently methylated in sporadic *VHL* wild-type ccRCC than in familial ccRCCs (9). This finding might suggest that DNA methylation complicated the ccRCC tumorigenesis pathway(s) and give possible explanation of low effectiveness in cancer therapy if only blocking the downstream effects of *VHL* inactivation.

Numerous genes have been found to be frequently methylated in ccRCC by candidate-gene approaches (50% of cases), including *CDH1*, *APAF1*, *COL1A1*, DKK2, *DKK3*, *SFRP1*, *SFRP4*, *SFRP5*, *WIF*, *PCDH17* and *TCF21*, which are not methylated or are rarely methylated in matched normal renal tissues (<10%) (10-12). These genes are involved in different aspects of tumorigenesis, such as signal transduction, apoptosis, angiogenesis, adhesion and tumor invasion. Another recent comparative study of CpG methylation status in 38 ccRCCs and 9 matched normal kidney tissues including 27,500 CpGs at >14,000 genes showed that 55 genes were identified as methylated in ccRCC but not in normal controls. Further detailed functional study revealed eight novel ccRCC TSG candidates, including *OVOL1*, *DLEC1*, *BMP4*, *SST*, *TMPRSS2*, *TM6SF1*, *SLC34A2*, and *COL1A2*. Among them, *OVOL1* epigenetic silencing was identified to be capable of increasing c-Myc expression and may lead to c-Myc pathway activation commonly seen in ccRCC

formation (13).

Several functional epigenetic strategies were applied for ccRCC TSGs identification. One strategy is RCC cell lines treated with 5-aza-2-deoxycytidine were subjected to high-density gene expression microarray analysis. The re-expressed genes after demethylation were further validated in primary tumor samples. Five genes including *BNC1*, *COL14A1*, *CST6*, *PDLIM4*, and *SFRP1* were revealed to show frequent (>30%) promoter region methylation associated with transcriptional silencing. Further the cell growth assay *in vitro* by over expression or RNAi knock down of these genes suggested their tumor suppressor activities (12). The other strategy is methylated DNA immunoprecipitation (MeDIP) in combination with high-density whole-genome expression microarray for direct analysis of genomic methylation patterns in primary ccRCC. Nine genes, including *ATP5G2*, *PCDH8*, *CORO6*, *KLHL35*, *QPCT*, *SCUBE3*, *ZSCAN18*, *CCDC8*, and *FBN2* were found frequently methylated in primary ccRCC and promoter hypermethylation of these genes resulted in significant reduction of their expression level (14).

Using the HELP (HpaII tiny fragment enrichment by ligation-mediated PCR) assay, a very recent study determined the CpG methylation status of 1.3 million loci across the genome in RCC and found that the aberrant methylation was particularly enriched in kidney-specific enhancer regions. These aberrantly hypermethylated regions revealed enrichment for binding sites of *AP2a*, *AHR*, *HAIRY*, *ARNT*, and *HIF1* transcription factors, contributing to the dysregulated hypoxia signaling pathways in RCC (15).

DNA methylation and prognosis of ccRCC

Genome-scale methylation analysis is a powerful tool to identify multi-gene profiles reflecting tumor behavior, supported by a comprehensive methylome analysis using single-CpG resolution infinium array. This study has revealed ccRCCs with positive CpGI methylator phenotype on the *FAM150A*, *GRM6*, *ZNF540*, *ZFP42*, *ZNF154*, *RIMS4*, *PCDHAC1*, *KHDRBS2*, *ASCL2*, *KCNQ1*, *PRAC*, *WNT3A*, *TRH*, *FAM78A*, *ZNF671*, *SLC13A5* and *NKX6-2* genes were characterized by more aggressive tumor phenotypes and poorer patient outcomes (16).

The very recent high-resolution methylome analysis of RCC demonstrated that only methylation at enhancers was highly prognostic for survival in even multivariate analysis (15).

DNA methylation and molecular diagnosis of ccRCC

Some promoter hypermethylation have been detected in serum, peripheral blood and urine, suggesting the blood and body fluid-based DNA methylation candidate marker's potential value in early non-invasive detection of ccRCC (10,17). For example, the levels of promoter hypermethylation of *RASSF1A* (Ras association domain family member 1A) and *VHL* detected in serum of ccRCC patients (18), as well as that of *KILLIN* (a novel p53-regulated TSG proximal to *PTEN*) and long interspersed nuclear elements (*LINE*-1) detected in peripheral blood (19,20), were significantly higher than that in patients with benign tumors and healthy controls, respectively. Certainly the practical significance of these methylation candidate markers deserves validation in large, independent cohorts.

So far, there is no data available for DNA methylation markers in prediction of chemotherapeutic response in RCC patients, even though several differentially methylated genes were proposed as candidate markers of drug response in human cancer (21). 'Epigenetic treatment', with DNA methyltransferases inhibition and silenced gene activity restoration, resulting in enhanced apoptosis and decreased cancer cell growth, has become a promising therapeutic strategy for ccRCC. This novel strategy may reprogram the gene expression profiles in cancer cells thereby making ccRCC more susceptible to standard therapy (22).

Histone modifications in ccRCC

DNA, histones and nonhistone proteins are condensed into a highly complex nucleoprotein structure known as chromatin, either in a compact heterochromatin form or a more open euchromatin form, which is related to transcriptionally active genes. Histones on the N-terminus modifications include methylation, acetylation, phosphorylation, ubiquitylation and sumoylation of specific residues, of which histone acetylation and methylation are the most studied (23). Generally, acetylation causes active transcription and is associated with a more open chromatin conformation, whereas the transcriptional effects of methylation depend on the residue affected as well as the degree of methylation (mono-, di- or trimethylation). For example, methylation of H3K4, H3K26 and H3K79 is associated with active marks, whereas methylation of H3K9, H3K27 and H4K20 is associated with repressive marks (24).

Histone deacetylase (HDACs), histone acetyltransferases (HATs), histone methyltransferases (HMTs) and recently discovered histone demethylases (HDMTs) are critical enzymes in regulating various biological and cellular processes including cell proliferation, angiogenesis, hypoxia-related effects and cell cycle regulation. Increasing studies have found the deregulation of these enzymes in ccRCC. Targeting these enzymes, reversing their epigenetic modifications has the potential of reactivating TSGs or suppressing oncogenes, thus, affecting tumor growth or progression of ccRCC.

Histone modification, especially histone-modifying enzymes in ccRCC initiation and progression

HIF isoforms are shown to be correlated with histone-modifying enzymes. The interaction between HDAC4 and HIF-1α could protect HIF-α from proteasomal degradation (25). *JARID1C*, which encodes a HIF-targeted histone H3K4 demethylase and has truncating mutations in RCC (3%), is believed to be a TSG in *VHL* null tumors (26). JMJD1A, one HMT transcriptionally induced by HIF2α under hypoxic conditions, is consistent overexpressed in RCC. Several studies have reported that *SETD2*, which encodes a histone H3K36 methyltransferase located (3p21.31) in close proximity to *VHL* gene within a commonly lost (approximately 90%) 3p locus, as a novel TSG in ccRCC (27). The somatic truncating mutations in *SETD2* (3–8%) were associated with *VHL* mutations (28), hypoxic phenotype and decline in chromatin remodeling complex protein PBRM1 in RCC tumor samples, as well as advanced stage (29). These results indicate that histone-modifying enzymes are especially important in RCC with *VHL* mutation or deletions.

Epithelial-to-mesenchymal transition (EMT) is believed to play an important role in ccRCC progression, as well as in many other cancers. HDAC inhibition could suppress TGF-β-induced EMT of renal proximal tubular epithelial cells (30), thereby may indicate a potential role for HDAC inhibitor in reversing EMT during RCC progression, which deserve further exploration.

HDACs are also essential in the regulation of the cell cycle and apoptosis through their effects on pRB and p53. When combined with other agents, such as rapamycin (an inhibitor of mTORC1), HDACs inhibitors have been shown to have strong anti-tumor activity in xenografts of certain RCC cell types (31).

Enhancer of zeste homolog 2 (EZH2), the core catalytic subunit of polycomb repressive complex (PRC2), acts as a HMT by adding 3 methylgroups to lysine 27 of histone 3

(H3K27). The trimethylation of H3K27 leads to chromatin condensation and mediates epigenetic silencing of a broad range of TSG involved in cancer progression. EZH2 could promote migration and invasion of RCC cells via E-cadherin repression (32). MiR-101 act as a negative regulator of EZH2 expression (33).

Histone modification and prognosis of ccRCC

The level of histone H4 acetylation (H4Ac) was inversely correlated with the pathological stage and nuclear grade of ccRCC, whereas lower H3Ac levels were associated with systemic metastatic spread and tumor progression. Furthermore, the low H3K18Ac level (lysines 18 in histone H3) was significantly correlated with RCC progression in univariate analysis and it could be used as an independent predictor of cancer progression and poor survival following surgery in localized RCC (34).

A series of reports have showed that the lower methylation levels of histone 3 in different lysine loci were of worse prognostic value. The lower levels of global H3K4 methylation (mono-, di- and trimethylation), especially H3K4me2, in RCC tumor samples were reported to be correlated with the adverse clinicopathological parameters such as more advanced pathological stage and higher Fuhrman grade as well as worse progression-free survival or cancer-specific survival in univariate analysis; suggesting they could be used as a predictor of poor prognosis of RCC (35,36). The methylation H3K9 level was also found to be of prognostic significance in RCC patients. The lower H3K9me2 level was shown to be a significant predictor of worse outcome in RCC even in multivariate analysis including nuclear grade, tumor location and the putative biomarkers (Ki67 index and p53) (34). The lower level of global methylation levels of lysine 27 of histone H3 (H3K27me1, H3K27me2 and H3K27me3) were shown to be associated with advanced pathological stage, higher Fuhrman grade and vascular invasion. In addition, lower H3K27me3 level was observed in patients with distant metastasis. Furthermore progression-free survival was shorter in patients with lower H3K27me1 and H3K27me3 levels, although only in a univariate analysis (37).

Numerous reports have showed that EZH2 overexpression was an adverse prognostic biomarker in different types of carcinomas. So far limited studies have suggested that the higher intratumoral expression of EZH2 was correlated with the advanced stages and poor survival of ccRCC patients both in primary and metastatic cases (38,39), however the conflicting results exist.

miRNA and ccRCC

MiRNAs are a group of small (around 22 nucleotides) non-coding RNAs regulating post-transcriptional gene expression through the epigenetic mechanism of RNA interference (40). The 6 to 8 nucleotides at 5' end of a miRNA known as seed region, can determine the specificity of its interaction with the target mRNA on its 3' untranslated region, resulting in the inhibition of translation and/or degradation of mRNA (41,42). The abnormal expression of miRNAs is related to cancer development, progression and metastasis. Overexpressed miRNAs in cancer may act like oncogenes by down-regulating TSG. Vice versa, down-regulated tumor suppressor-like miRNAs could result in the up-regulation of oncogenes. In general the oncogenic miRNAs are upregulated and tumor suppressor miRNAs are downregulated in malignancy. Accumulating evidences show that miRNAs play important roles in the tumorigenesis and progression of RCC (43).

miRNA and initiation of ccRCC

The role of miRNA in the VHL-HIF pathway and their relationship have been of particular interest and explored in many reports. MiR-17-5p, miR-224, miR-200 family, miR106a/b, miR-21, miR-221, miR-199a, and miR-214 have been identified to have prominent roles in ccRCC formation. They are predicted to target the VHL-HIF1 axis along with the targets of HIF/HIF transcription factors: vascular epithelial growth factor (VEGF), platelet-derived growth factor, transforming growth factor, the downstream PI3K/AKT and RAS/RAF/MEK/ERK pathways, and mTOR pathway. The same miRNA can control multiple targets along the same pathway, and multiple miRNAs can target the same molecule. This implies that even subtle alterations in these miRNAs may cause significant influences on the outcome of the regulated pathway and the tumorigenesis of ccRCC. This also indicates that miRNAs can control the hypoxia-related VHL-HIF1 axis and the common cancer-related pathways in a tight regulatory network.

MiR-17-92 cluster are oncogenic miRNAs in many cancers (44). MiR-92a, being a member of miR-17-92 cluster, is found to be overexpressed in ccRCC and correlated negatively with VHL mRNA levels (45). Meanwhile, the presence of direct interactions between miR-17-5p (also a member of miR-17-92 cluster), miR-224 with VHL and HIF1-α was validated

Table 1 Well-defined miRNAs and targeted molecules in ccRCC

Function in CCRCC	mi-RNA	Targeted molecules/pathways
Initiation		
HIF dependent mechanism	miR-17-5p	VEGFA, ENLN3
	miR-224	SMAD4, SMAD5
	miR-210	ICSU, E2F3
HIF independent mechanism	miR-1826	β-catenin
	miR-28-5p	Mad2
Progression		
EMT	miR-200 family	ZEB1, ZEB2
	miR-141	EphA2/p-FAK/p-AKT/MMPs
	miR-30c	Slug, E-cadeherin
Migration and invasion	miR-21	PDCD4, AKT, IKKβ, NF-κB, mTOR
	miR-138	Vimentin
	miR-218	CAV2

by luciferase assay and Western blot analysis, as well as in ccRCC specimens. In addition, miR-17-5p can regulate multiple key components of hypoxia-related signaling pathways, including VEGFA and ENLN3. MiR-224 can inhibit the TGF-β pathway members SMAD4 and SMAD5 (46). MiR-210 is the most ubiquitously upregulated miRNA in multiple cancer cell lines under hypoxia. As a result of HIF1 or HIF2 over expression in ccRCC cells, miR-210 is found to be upregulated and target ISCU (iron sulphur cluster homologue), which acts as a scaffold protein for the formation of Fe-S clusters which may contribute to anaerobic respiration in tumors (47). Moreover, the accumulation of miR-210 may induce aneuploidy via E2F3 down regulation and contribute to the initiation of ccRCC (48). Besides the HIF pathway, dysfunction of VHL also works through other ways. One of major VHL functions is inhibiting beta-catenin. In VHL inactivated RCC, beta-catenin is the direct target of miR-1826 and they show an inverse correlation (49). In another report, increased expression of miR-28-5p could promote chromosome instability by inhibiting mitotic checkpoint protein Mad2 in VHL inactivated tumors (50). However, so far few studies report how miRNAs function in VHL independent ccRCCs.

miRNA and progression of ccRCC

The invasion and metastasis of cancer start with the

enhanced ability of tumor cell to destruct extracellular matrix (ECM) proteins and invade ECM, which can be assisted by metastasis promoting miRNAs (51). MiR-21 is overexpressed in ccRCC and contributes to ccRCC invasion and metastasis. Studies revealed miR-21 could inhibit the tumor suppressor PDCD4. Overexpressed miR-21 results in the phosphorylation and activation of AKT and IKKβ, further activation of NF-kB dependent signal pathway transcription. Together with its blocking PDCD4 mediated the inhibitory effects; miR-21 overexpression could restore the invasion and migration of ccRCC cells. Moreover, IKKβ activates mTOR1, which further restores cell invasion and migration (52). EMT is a crucial process involved in tumor motility and invasion. Down regulation of several miRNAs has been shown to cause EMT in ccRCC (53). Tumor suppressors miR-200 family (miR-200a/b/c, miR-141, miR-429) are significantly downregulated in RCC cells, which repress the expression of E-cadherin through direct targeting their transcriptional inhibitor ZEB1 and ZEB2 (54,55). For miR-141, its decrease can activate the EphA2/p-FAK/p-AKT/MMPs signaling cascade to facilitate the invasion of RCC cells (56). The process of EMT can also be triggered by HIF dependent down-regulation of miR-30C, which leads to subsequent increase of slug and decrease of E-cadherin (57). Furthermore, tumor suppressive miR-138 is significantly reduced in ccRCC. Through its targeting of vimentin, a significantly overexpressed intermediate filament protein in ccRCC tissues, miR-138 could contribute to cell migration and invasion (58). CAV2, a member of caveolin family, function as a signal transduction regulator in the invasion and migration of ccRCC cells. Tumor suppressor miR-218 is reported to directly regulate CAV2. Downregulation of miR-218 and up-regulation of CAV2 might be frequent events in ccRCC (59).

Some well-defined miRNAs and their targets in ccRCC are summarized in *Table 1*. The regulatory functions of these important miRNAs in the major signaling pathways of ccRCC initiation and progression are illustrated in *Figure 1*.

miRNA and molecular diagnosis of ccRCC

MicroRNAs are highly stable and abundant in plasma, serum and other body fluids. They can passively leak from damaged or apoptotic cells into circulation. Some miRNAs are contained within the extracellular vesicles, thus resistant to RNase and persistent in blood. Detection and characterization of circulating miRNAs can be a promising non-invasive way to detect cancer (60,61). Recent studies
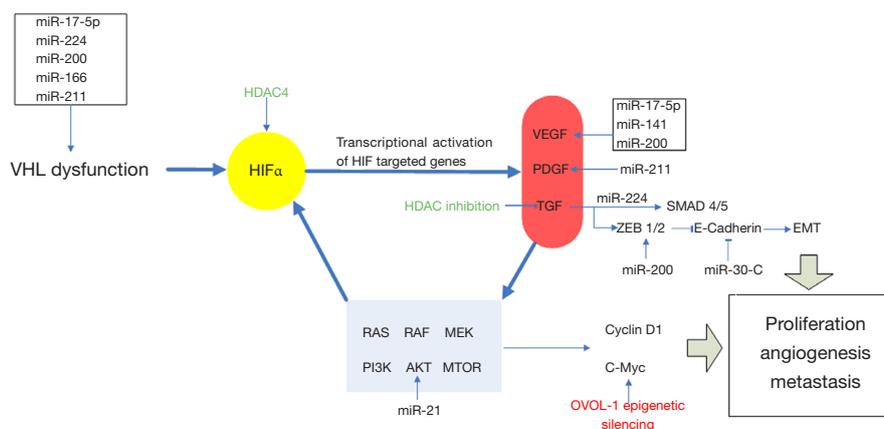
Figure 1 Epigenetic regulation in the formation and progression of ccRCC. Schematic outline of some of the epigenetic alterations, especially some important miRNAs, regulate the signaling pathways that are proposed to contribute to the proliferation, angiogenesis and metastasis of ccRCC.

Table 2 Well-defined miRNAs and prognosis of ccRCC patients

Expression level	miRNAs	Prognosis
Up-regulation	miR-21	Poor
	miR-21/10b ratio	Poor
	miR-221	Poor
	miR-501	Poor
	miR-126	Good
Down-regulation	miR-497	Poor
	miR-23b-27b	Poor
	miR-514	Poor
	miR-501	Good
	miR-126	Poor

ccRCC, clear cell renal cell carcinoma.

suggested miR-210 as a potential biomarker for early diagnosis of ccRCC. As we mentioned before, the elevation of miR-210 could be resulted from the over activation of VHL-HIF pathway. As in cancer tissues, miR-210 level in serum was found to be increased significantly in RCC patients compared to non-cancer controls (62). Another study found that the level of miR-378 was increased and miR-451 level was decreased in the serum of RCC patients. These two miRNAs combination enable the identification of RCC in serum with the sensitivity of 81% and specificity of 83% (63). A panel of 5 serum miRNAs, including three significantly increased miRNAs (miR-193a-3p, miR-362 and miR-572) and two markedly decreased miRNAs (miR-28-5p and miR-378), can differentiate stage I RCC from non-cancer controls, suggesting it may have the potential to be used clinically as an auxiliary

tool for the early detection of RCC (64).

Analysis of urinary miRNAs is another promising approach for early diagnosis of urological cancer (65), although there are limited studies available for RCC. Same as in blood, the miRNA level in urine also shows stability (66). Different from the usual down-regulation of miR-15a in other cancers, miR-15a is upregulated in ccRCC biopsies and urine samples, which is not seen in other urinary and nonurinary tumors, and inflammatory conditions of the urinary tract. The finding suggests miR-15a can be a potential RCC biomarker (67).

By integrating the miRNAs, mRNAs, and pathways, some miRNAs are suggested to be the novel ccRCC biomarkers, such as miR-425, miR-136, miR-335, miR-340 and miR-320d (68). So far none of these markers are identified to be specific for RCC. For instance, miR-210 and miR-425 aberrations are also detected in carcinomas of digestive system.

miRNA and prognosis of ccRCC

Many studies have investigated the relationship between the alteration of miRNAs and prognosis of ccRCC (*Table 2*). The aberrant expression of miRNAs is frequently related to poorer prognosis, because tumor suppressor miRNAs are usually downregulated and pro-oncogenic miRNAs are upregulated. Up-regulation of miR-21 is a marker for poor prognosis in several cancers including RCC (69). Furthermore, the ratio of miR-21/10b was shown to be an independent prognostic marker for metastasis-free ccRCC (70). For the tumor suppressing miRNAs, studies have showed that the decreased expression of miR-497, miR-23b-27b

cluster are associated with the advanced stage and shorter overall survival of RCC patients (71,72). As for miR-210, its overexpression was demonstrated as a poor prognosis marker for RCC in one study (73), while a conflict result relating to good prognosis was shown in another study (47).

MiR-501 is differently expressed in RCC, regulating the balance of cell apoptosis and proliferation. Its high expression level predicts a worse outcome and vice versa (74). Similarly, miR-126 can also be differently expressed, however its down-regulation is related to the poor prognosis and its up-regulation is associated with significantly prolonged disease-free survival of RCC patients (75).

By comparing the miRNA expression in primary ccRCC and metastatic ccRCC, miR-122 was found to be upregulated in primary tumor and reduced in metastatic tumor. MiR-514 was shown to be downregulated in primary tumor and decreased further in metastatic tumor. These miRNA alterations are significantly related to recurrence risk and miR-514 could provide independent prognostic information for ccRCC patients (76).

Patients with higher plasma miR-221 levels had a significantly lower survival rate than those with lower expression levels, revealing miR-221 as an independent prognosis factor for ccRCC.

LncRNAs and ccRCC

Long non-coding RNAs (lncRNAs) are operationally defined as non-protein coding RNAs that are over 200 nucleotides in length, which corresponds to a convenient cutoff in biochemical fractionation and excludes all known small RNAs (77). LncRNAs are divided into two main categories, the intergenic lncRNA and the intragenic lncRNA. They are differentially expressed in tissue specific manner. Many lncRNAs undergo 5' end capping and 3' end polyadenylation similar to mRNAs. They were once considered as transcriptional noise while accumulated evidences show that lncRNAs have a regulatory role in cellular development, proliferation, differentiation by cis-regulation and trans-regulation (78). And their misregulation could cause various human diseases including cancer (79). Studies have revealed the cancer-involved functions of lncRNAs as oncogenes, tumor suppressor, or both.

LncRNAs and progression of ccRCC

LncRNA MALAT1, located on chromosome 11q13, is expressed ubiquitously in normal tissues (80). Several studies found that the expression of MALAT1 was elevated in multiple cancers such as non-small cell lung carcinoma, hepatocellular carcinoma, and pancreatic carcinoma, suggesting MALAT1 acting as an oncogene. It could enhance cell growth, tumorigenesis and metastasis by activating E2F1 transcriptional factor or ERK/MAPK pathway (81,82). MALAT1 was also upregulated significantly in ccRCC compared to the adjacent normal tissue. Further, the up-regulation of MALAT1 was related to tumor size, stage and lymph node metastasis but not histological grade and distant metastasis, which prompted MALAT1 overexpression was correlated with ccRCC progression and development (83). HOTAIR is another oncogenic lncRNA that interacts with the PRC and suppresses its target genes. HOTAIR is upregulated in several types of cancers and stimulates cancer development (84-86). The knockdown of HOTAIR could inhibit the proliferation, migration, and invasive ability of RCC cells. In addition, inhibition of this lncRNA by siRNA suppressed tumor formation *in vivo* (87). One recent study showed the expression and function of HOTAIR could be regulated by tumor suppressor miR-141. MiR-141 binds to HOTAIR in a sequence specific manner and induces the cleavage mediated by Ago2 complex (88). LncRNA SPRY4-IT1 was found to be elevated significantly in several types of cancers including ccRCC. Moreover its high expression was shown to be associated with advanced histological grade, tumor stage and lymph node or distant metastasis. Knocking down its expression could inhibit the proliferation, migration and invasion of ccRCC cells. All these findings indicate the oncogenic role of lncRNA SPRY4-IT1 in ccRCC (89). For the tumor suppressor lncRNAs, lncRNA GAS5 was found to be decreased significantly in ccRCC both *in vitro* and *in vivo*. Its normal expression suppressed the invasion and migration of ccRCC cell. However, GAS5 expression did not show any correlation with the clinicopathological factors (90). Another studied tumor suppressing lncRNA is CADM1-AS1, which is located at the antisense direction of a coding exon of CADM1. CADM1 is a tumor suppressor that encodes a cellular adhesion molecule and regulates cell cycle, apoptosis and differentiation (91). The study revealed that lncRNA CADM1-AS1 expression was significantly associated with the CADM1 mRNA expression and reversely correlated with the RCC stage (92).

LncRNAs and molecular classification of ccRCC

By next-generation deep RNA-sequencing of the 475

Table 3 The newly identified lncRNAs, their targets and prognosis of ccRCC patients

Expression level	LncRNA	Targets	Prognosis
Up-regulation	MALAT1	E2F1, ERK/MAPK	Poor
	HOTAIR	PRC, Ago2	Poor
	SPRY4-IT1	–	Poor
Down-regulation	GAS5	–	Poor
	CADM1-AS1	–	Poor

ccRCC, clear cell renal cell carcinoma.

primary ccRCC samples from The Cancer Genome Atlas (TCGA), lncRNA expressions were assessed and the first lncRNA based RCC classification was proposed in a recent study. Totally 1,934 expressed lncRNA were identified. Unsupervised clustering analysis unraveled four lncRNA subclasses in ccRCC associated with distinct clinicopathological and genomic features. The cluster C2 is enriched for 9p deletion, chr8q24.22 gain and chromatin remodeler BAP1 somatic mutations. Bearing frequent 1p deletion, 17q gain, and MiTF/TFE translocations, the cluster C4 (7.8%) is related to tumor subtypes arising from the distal tubules of the nephron, which is proved to be chromophobe RCC, clear cell papillary RCC and translocation associated RCC (92).

LncRNAs and prognosis of ccRCC

In the lncRNA subtype classification of ccRCC we mentioned above, cluster C2 (23.4%) defines the most aggressive tumors, with the highest Fuhrman grade, stage and the worst overall survival (92). The oncogenic lncRNAs are usually upregulated and related to poor prognosis in RCC. MALAT1 and SPRY4-IT1 showed the potential to become the powerful independent prognostic factor for RCC (83,91). Down-regulation of tumor suppressor lncRNA CADM1-AS1 is related to worse prognosis compared to patients with higher expression level of CADM1-AS1 (92). The above limited findings are listed in *Table 3*.

Epigenomics and target therapy for ccRCC

The recent advances in the clarification of molecular mechanisms involved in ccRCC have led to the development and approval of multiple agents targeting VEGF, such as bevacizumab plus interferon, sorafenib,

sunitinib, pazopanib and axitinib, or mTOR pathways, such as temsirolimus and everolimus for treatment of advanced ccRCC (93). The epigenomic studies, especially inhibiting HDACs and reversing promoter methylation of those important ccRCC TSG, are providing promising targets for novel therapies. Several agents targeting Class I, II and IV HDACs have been tested in preclinical studies, functioning as a single agent or in combination with proteasome inhibitor bortezomib, temsirolimus, interferon or IL-2 etc. Furthermore, demethylating agents such as azacitidine and decitabine have been shown to be useful for the treatment of advanced ccRCC besides its successful utility in myelodysplastic syndrome and acute myelogenous leukemia.

In conclusion, epigenetic alterations regulating the formation and progression of RCC are still in the initiating stage of discovery. Further characterization of the genes and pathways that are epigenetically altered in ccRCC may lead to the development of novel minimally-invasive diagnostic and prognostic tools for ccRCC. In the long term, epigenetic therapies might provide an additional treatment option for advanced RCC that is unresponsive to standard management.

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Footnote

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