



# The epigenetics of renal cell tumors: from biology to biomarkers

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Renal cell tumors (RCT) collectively constitute the third most common type of genitourinary neoplasms, only surpassed by prostate and bladder cancer. They comprise a heterogeneous group of neoplasms with distinctive clinical, morphological, and genetic features. Epigenetic alterations are a hallmark of cancer cells and their role in renal tumorigenesis is starting to emerge. Aberrant DNA methylation, altered chromatin remodeling/histone onco-modifications and deregulated microRNA expression not only contribute to the emergence and progression of RCTs, but owing to their ubiquity, they also constitute a promising class of biomarkers tailored for disease detection, diagnosis, assessment of prognosis, and prediction of response to therapy. Moreover, due to their dynamic and reversible properties, those alterations represent a target for epigenetic-directed therapies. In this review, the current knowledge about epigenetic mechanisms and their altered status in RCT is summarized and their envisaged use in a clinical setting is also provided.

**Keywords:** epigenetics, kidney, renal cell tumors, DNA methylation, chromatin remodeling, histone post-translational modifications, microRNAs, epigenetic-based therapeutics

## INTRODUCTION

Renal cell tumors (RCT) are the most common neoplasms of the kidney in adults, representing 2–3% of all non-cutaneous malignant neoplasms, ranking 14th in incidence for both genders, with a mortality rate of 1.6/100,000, worldwide (Ferlay et al., 2010). RCTs are a very heterogeneous group of neoplasms, ranging from benign to malignant behavior, displaying variable clinical aggressiveness and considerable histopathological diversity (Lopez-Beltran et al., 2009). The most prevalent benign RCT is oncocytoma, whereas among malignant RCTs the most common subtypes are clear cell renal cell carcinoma (ccRCC, 70–75% of cases), papillary renal cell carcinoma (pRCC, 10–15% of cases), and chromophobe renal cell carcinoma (chrRCC, 5–10% of cases). Owing to the fact that RCT are mostly asymptomatic at their earliest stages, when curative treatment is more likely to be effective, a significant proportion of cases (up to a third of all RCCs) are diagnosed at a late stage, when the neoplasm has already spread locally or systemically (Lam et al., 2005). On the other hand, an increasing number of RCT is being identified through routine ultrasonography, posing new diagnostic challenges at a pre-operative stage, such as the discrimination between benign and malignant tumors.

Presently, there are no effective screening or early diagnostic tools for RCT that might be used in at-risk groups or in a wider population setting (Rini et al., 2009). However, the development of such tools might have a relevant clinical impact, mainly if they would be based on non-invasive approaches (Scelo and Brennan, 2007). In this regard, cancer-related genetic and/or epigenetic alterations might be used as

biomarkers enabling the detection of cancerous cells in clinical samples, thus increasing the ability to identify tumors at their earliest stages (Esteller, 2008). Eventually, those alterations might also prove useful for assessing prognosis, response to therapy, and also for patient monitoring (Esteller, 2008). RCTs display characteristic and often discriminative chromosomal alterations (Baldewijns et al., 2008). These, however, are not easily amenable for detection in clinical samples because it requires tissue culture for karyotyping or FISH analysis using tumor biopsies. On the contrary, epigenetic alterations carry an enormous potential as specific cancer biomarkers (Mulero-Navarro and Esteller, 2008). Furthermore, owing to the reversible nature of epigenetic alterations, these might constitute attractive therapeutic targets, as demonstrated for some hemato-lymphoid neoplasms (Rodriguez-Paredes and Esteller, 2011). Thus, in this review, we address not only the role of epigenetic alterations in renal carcinogenesis, but also their clinical potential in RCT management.

## EPIGENETIC MECHANISMS AND THEIR DeregULATION IN CARCINOGENESIS

Epigenetics might be defined as modifications of the DNA or associated proteins, other than DNA sequence variation itself, that carry information content during cell division (Feinberg and Tycko, 2004). Presently, three main epigenetic mechanisms are recognized: DNA methylation, chromatin remodeling/post-translational histone modifications, and microRNA (miRNA) regulation. DNA methylation and chromatin remodeling/post-translational histone modifications cause changes in chromatin

structure that modulate the accessibility of the nuclear transcriptional machinery to specific DNA sequences. On the other hand, miRNAs interact with mRNAs regulating translation to protein.

### DNA METHYLATION

The most extensively studied epigenetic mechanism is DNA methylation which, in mammals, consists on the addition of a methyl group to cytosines preceding guanines (so-called “CpG dinucleotides”), a chemical reaction which is catalyzed by DNA methyltransferases (DNMTs; Goldberg et al., 2007; Lopez-Serra and Esteller, 2008). These CpG dinucleotides are usually clustered in DNA stretches called “CpG Islands,” preferentially found at the regulatory regions of genes, i.e., promoter, untranslated regions, and exon 1. In a normal cell, CpG methylation is present mainly in repetitive sequences, retrotransposons, and parasitic sequences, thus contributing to genetic stability, whereas most of the CpG islands within promoters remain unmethylated during development and even after differentiation, with the notable exception of some imprinted genes, genes on the silenced copy of X chromosome in females, and tissue-specific genes (Vaissiere et al., 2008). Gene silencing associated with methylation of promoter regions containing CpG islands may be due to an obstruction of the access of transcription factors or through the promotion of binding of methylcytosine-binding proteins (MBD), which cooperate with DNMTs and histone deacetylases (HDACs; Fraga et al., 2005; Sharma et al., 2010). Recently, an important role in gene expression regulation has been also credited to lower density CpG regions located in the vicinity of CpG islands, the so-called “CpG island shores” (Doi et al., 2009). These are sequences up to 2 kb distant from CpG islands, which are associated with gene expression (Doi et al., 2009; Irizarry et al., 2009). Remarkably, the methylation pattern at CpG island shores is mostly tissue-specific and cancer-associated alterations in those patterns occur at sites that vary normally in tissue differentiation (Irizarry et al., 2009). These observations are in line with the so-called “epigenetic progenitor model of cancer” (Feinberg et al., 2006).

Aberrant DNA methylation is probably the best characterized cancer-related epigenetic alteration and it is considered by some as one of the earliest events in the process of tumorigenesis (Feinberg et al., 2006). Those aberrations include both global and gene-specific hypomethylation as well as gene-specific CpG island promoter hypermethylation (Mulero-Navarro and Esteller, 2008; Sharma et al., 2010). The impact of gene-specific alterations in DNA methylation depends on the function of the affected gene and the type of alteration. Whereas promoter hypomethylation may cause activation of proto-oncogenes, hypermethylation induces silencing of cancer-related genes with tumor suppressive properties (Feinberg et al., 2006; Sharma et al., 2010). On the other hand, decrease of global genome methylation (genome-wide hypomethylation) may lead to genomic instability in repetitive sequences, especially at pericentromeric regions, predisposing to abnormal recombination, facilitating translocations, deletions, and chromosomal rearrangements (Ehrlich, 2005; Mulero-Navarro and Esteller, 2008; Sharma et al., 2010). It was also demonstrated that aberrant methylation may affect large extensions of DNA in cancer cells, resulting in extensive epigenetic

reprogramming of entire chromosomal regions (Frigola et al., 2006).

### CHROMATIN REMODELING AND HISTONE MODIFICATIONS

Chromatin remodeling refers to covalent and non-covalent modifications of histones, the proteins that form the core of nucleosomes which are the basic unit of chromatin. The N-terminal tails of histones protrude from the nucleosome and may be subjected to a wide range of post-translational covalent changes (methylation, acetylation, ubiquitylation, sumoylation, and phosphorylation) of specific amino acid residues and these are involved in regulation of transcription (Kouzarides, 2007a). This set of histone modifications – the so-called “histone code” – determines the configuration of chromatin, adjusting the accessibility to effector proteins (Kouzarides, 2007a). For instance, whereas lysine acetylation is associated with active transcription, lysine methylation may lead to transcription activation or repression depending on the residue affected and the degree of modification (Kouzarides, 2007a). For example, trimethylation of lysine 4 on histone H3 (H3K4me3) is associated with active transcription, whilst H3K27me3 and H3K9me3 are the two chief repressive marks (Kouzarides, 2007a). There is a large number of enzymes involved in the addition or removal of the covalent modifications, including histone acetyltransferases (HATs), deacetylases (HDACs), methyltransferases (HMTs), and demethylases (HDMs; Kouzarides, 2007a,b; Shi, 2007). Besides their important role in regulation of gene expression in somatic cells, covalent histone modifications are also critical for embryonic stem cell (ESC) development and differentiation (Kouzarides, 2007a,b; Shi, 2007). Indeed, ESCs display bivalent chromatin domains which provide epigenomic plasticity during normal development. Those bivalent chromatin marks consist on the simultaneous occupancy of trimethylated histone H3 lysine 27 (H3K27me3, a repressive mark) and of di- and trimethylated histone 3 lysine 4 (H3K4me2 and H3K4me3, which are both active marks) at the promoters of genes encoding transcription factors that regulate developmentally important genes (Mikkelsen et al., 2007). Thus, neoplastic transformation would be associated with an alteration of these marks, leading to silencing of tumor suppressor genes (TSG; through the loss of H3K4me2 and H3K4me3) with associated promoter hypermethylation and/or activation of proto-oncogenes with concomitant loss of methylation (through loss of H3K27me3; Bloushtain-Qimron et al., 2008; Kondo et al., 2008). On the other hand, non-covalent mechanism involved in chromatin remodeling include alterations in nucleosome positioning and incorporation of histone variants (Henikoff, 2008). It should also be emphasized that DNA methylation and histone modifications work in concert to achieve stable patterns of gene expression (Ballestar and Esteller, 2005; Vaissiere et al., 2008). Indeed, nucleosome remodeling may be involved in inappropriate gene silencing through the cooperation with aberrant DNA methylation and repressive histone covalent modifications (Lin et al., 2007). Concerning histone modifications, global loss of both acetylation of lysine 16 (H4K16ac) and of trimethylation of lysine 20 of histone H4 (H4K20me3) are commonly found in cancer cells, usually in association with DNA hypomethylation at repetitive DNA sequences (Fraga et al., 2005). Loss of H4K16ac correlates with gene silencing (Kapoor-Vazirani

et al., 2008), but H4K20me3 is normally found in constitutive heterochromatin regions and other regions that contain silenced genes (Fullgrabe et al., 2011). Thus, it seems paradoxical that, in cancer development, the presence of H4K20me3 is also associated with local silencing of genes (Fullgrabe et al., 2011). In addition, altered methylation patterns of H3K9 and H3K27 have also been found in cancer cells (Nguyen et al., 2002; Valk-Lingbeek et al., 2004). Those modifications, which result in inappropriate gene silencing, are accomplished by histone-modifying enzymes. The expression of some of those enzymes, most notably of HDAC1 and EZH2, is upregulated in several cancers and this is thought to contribute decisively for cancer initiation and progression (Halkidou et al., 2004; Valk-Lingbeek et al., 2004; Song et al., 2005). In this regard, HDMs, like LSD1 which targets methylated H3K4 and H3K9, seem also to play an important role in some cancer models (Metzger et al., 2005; Schulte et al., 2009).

### MICRORNAs

MicroRNAs constitute a class of small (~18–25 nt in length), non-coding RNAs, which are synthesized (pri-miR) and processed in the nucleus (pre-miR), and subsequently in the cytoplasm (mature miR), to finally incorporate the RISC (RNA-induced silencing complex) protein complex (Garzon et al., 2009; Guil and Esteller, 2009). MicroRNAs interact with the 3'UTR of target mRNAs, inducing translational repression or degradation of the latter (Guil and Esteller, 2009). Interestingly, a specific miRNA may regulate multiple mRNAs in the same manner that a single mRNA may be targeted by multiple miRNAs (Shenouda and Alahari, 2009). Moreover, a large proportion of human gene transcripts (estimated between 30 and 70%) are known to be regulated by miRNAs, and these display temporal and tissue-specific patterns. Thus, miRNAs are one of the main classes of regulatory human genes, involved in multiple important cell processes, including cell proliferation, apoptosis, differentiation, development, and metabolism (Guil and Esteller, 2009).

The altered expression of miRNAs in cancer has been more recently documented (Lu et al., 2005). Owing to their role as regulators of mRNA expression, they may act as oncogenes (overexpressed miRNAs that target TSG) or TSG (downregulated miRNAs targeting proto-oncogenes; Guil and Esteller, 2009). Examples of the former include miR-21, targeting *PTEN* (Chan et al., 2005), whereas miR-15 and miR-16, which target *BCL2*, and let-7, targeting *RAS*, typify the latter (Zhang et al., 2007; Ventura and Jacks, 2009). The mechanisms that lead to altered miRNA expression are similar to those of regular genes and include amplification, deletion, mutation, and promoter hypermethylation (Guil and Esteller, 2009; Dudzic et al., 2011).

### EPIGENETIC ABERRATIONS IN RENAL CELL TUMORS AND CLINICAL APPLICATIONS

As mentioned in the previous section, the delicate epigenetic homeostasis that characterizes normal cells is frequently disturbed in cancer. Indeed, when compared to genetic alterations such as point mutations, deletions, and amplifications, epigenetic events seem to occur much more frequently and at earlier stages (Sharma et al., 2010; Berdasco and Esteller, 2011). These characteristics not

only endow epigenetic alterations a critical role in tumorigenesis, but they also set the basis for their use as cancer biomarkers for early detection, diagnosis, assessment of prognosis, patient monitoring, and prediction of response to therapy, as previously stated (Sharma et al., 2010; Berdasco and Esteller, 2011). In the next sections, the role of each epigenetic mechanism in renal cell tumorigenesis, as well as its potential use as biomarker and therapeutic target, will be discussed.

### DNA METHYLATION

Most studies that have investigated promoter hypermethylation in RCT used either a candidate-gene approach (i.e., cancer-related genes deregulated through this mechanism in other malignancies and/or genes known to be mutated in familial RCC) or a functional epigenomic approach, based on genome-wide CpG methylation analysis platforms (e.g., CpG island arrays). **Table 1** provides an overview of the most commonly methylated gene promoters in RCTs according to the cellular pathway in which they are involved. Studies using the candidate-gene strategy disclosed promoters with a high frequency (>70% of cases) of hypermethylation, which include *APAF1* (Christoph et al., 2006a,b), *MDR1* (Costa et al., 2007), and *PTGS2* (Costa et al., 2007), those with intermediate frequency (70–20%), like *RASSF1A* (Battagli et al., 2003; Dulaimi et al., 2004; Gonzalzo et al., 2004; Hoque et al., 2004; Costa et al., 2007; Peters et al., 2007), *CDH1* (Esteller et al., 2001; Morris et al., 2003; Hoque et al., 2004; Costa et al., 2007), *DAPK1* (Morris et al., 2003; Christoph et al., 2006a), *KRT19* (Paiva et al., 2011), *TIMP3* (Esteller et al., 2001; Battagli et al., 2003; Dulaimi et al., 2004; Hoque et al., 2004; Costa et al., 2007), *UCHL1* (Kagara et al., 2008; Seliger et al., 2009), *PCDH17* (Costa et al., 2011), and *TCF21* (Costa et al., 2011), and infrequently methylated (<20%) such as *APC* (Esteller et al., 2001; Dulaimi et al., 2004; Gonzalzo et al., 2004; Hoque et al., 2004; Costa et al., 2007), *CASP-8* (Morris et al., 2003; Christoph et al., 2006b), *CDH13* (Morris et al., 2003), *GSTP1* (Esteller et al., 2001; Dulaimi et al., 2004; Hoque et al., 2004; Costa et al., 2007), *JUP* (Breault et al., 2005), *MGMT* (Esteller et al., 2001; Morris et al., 2003; Dulaimi et al., 2004; Hoque et al., 2004; Costa et al., 2007), *p14<sup>ARF</sup>* (Esteller et al., 2001; Dulaimi et al., 2004; Hoque et al., 2004; Costa et al., 2007), *p16<sup>INK4a</sup>* (Herman et al., 1995; Esteller et al., 2001; Morris et al., 2003; Dulaimi et al., 2004; Hoque et al., 2004; Costa et al., 2007), *RARβ2* (Battagli et al., 2003; Morris et al., 2003; Dulaimi et al., 2004; Hoque et al., 2004; Costa et al., 2007), and *VHL* (Herman et al., 1994; Clifford et al., 1998; Dulaimi et al., 2004; Banks et al., 2006).

Moreover, because the frequency of point mutations affecting genes involved in familial RCC is relatively low in the sporadic cases, except for *VHL*, the relevance of aberrant promoter methylation in RCC carcinogenesis has been highlighted (McRonald et al., 2009). Indeed, several genes, like *RASSF1A*, *SFRP1*, *DAPK1*, and *SPINT2*, were consistently found to be silenced by promoter hypermethylation but rarely mutated in sporadic RCC (Morris et al., 2011).

In this setting, functional epigenomic approaches emerged as efficient strategies to identify genes whose expression was silenced by promoter methylation in RCC, through the identification of genes re-expressed after treatment with demethylating drugs in RCC cell lines and further validated in primary tumor samples.

**Table 1 | Genes frequently methylated in renal cell tumors, according to their function/pathway.**

Pathway	Gene	Designation	Frequency (%)	Reference
Hormonal response	<i>ESR1</i>	Estrogen receptor 1	69–70	Hoque et al. (2004), Costa et al. (2007)
	<i>ESR2</i>	Estrogen receptor 2	51–53	Hoque et al. (2004), Costa et al. (2007)
	<i>RARB2</i>	Retinoic acid receptor $\beta$ 2	0–53	Battagli et al. (2003), Morris et al. (2003), Dulaimi et al. (2004), Hoque et al. (2004), Costa et al. (2007)
Signal transduction	<i>DKK2</i>	Dickkopf 2	58	Hirata et al. (2009)
	<i>DKK3</i>	Dickkopf 3	50	Urakami et al. (2006)
	<i>RASSF1A</i>	Ras association domain family protein 1 isoform A	21–88	Battagli et al. (2003), Dulaimi et al. (2004), Gonzalgo et al. (2004), Hoque et al. (2004), Costa et al. (2007), Peters et al. (2007)
	<i>SFRP1</i>	Secreted frizzled-related protein 1	34–80	Urakami et al. (2006), Costa et al. (2007), Dahl et al. (2007), Gumz et al. (2007), Morris et al. (2010)
	<i>SFRP2</i>	Secreted frizzled-related protein 2	53	Urakami et al. (2006)
	<i>SFRP4</i>	Secreted frizzled-related protein 4	53	Urakami et al. (2006)
	<i>SFRP5</i>	Secreted frizzled-related protein 5	57	Urakami et al. (2006)
Tumor invasion	<i>WIF</i>	Wnt inhibitory factor	73	Urakami et al. (2006)
	<i>CDH1</i>	E-cadherin	11–59	Esteller et al. (2001), Morris et al. (2003), Dulaimi et al. (2004), Hoque et al. (2004), Costa et al. (2007)
	<i>JUP</i>	Junction plakoglobin	91	Breault et al. (2005)
	<i>PCDH8</i>	Protocadherin 8	58	Morris et al. (2011)
	<i>PCDH17</i>	Protocadherin 17	61	Costa et al. (2011)
	<i>SLIT2</i>	Slit homolog 2 ( <i>Drosophila</i> )	25	Astuti et al. (2004)
	<i>TIMP3</i>	TIMP metalloproteinase inhibitor 3	15–78	Esteller et al. (2001), Battagli et al. (2003), Dulaimi et al. (2004), Hoque et al. (2004), Costa et al. (2007)
	Angiogenesis	<i>GREM</i>	Gremlin1	20–55
<i>COL15A1</i>		Collagen type XV alpha-1	53	Morris et al. (2010)
<i>COL1A1</i>		Collagen type I alpha-1	56	Ibanez de Caceres et al. (2006)
Apoptosis	<i>APAF1</i>	Apoptotic protease activating factor 1	97–100	Christoph et al. (2006a,b)
	<i>DAL1/4.1B</i>	Differentially expressed in adenocarcinoma of the lung	47	Yamada et al. (2006)
Others	<i>DAPK</i>	Death-associated Kinase	33–41	Morris et al. (2003), Christoph et al. (2006a)
	<i>FHIT</i>	Fragile histidine triad	52	Costa et al. (2007)
	<i>MDR1</i>	Multidrug resistance receptor 1	86	Costa et al. (2007)
	<i>PTGS2</i>	Prostaglandin endoperoxidase synthase 2	94	Costa et al. (2007)
	<i>TCF21</i>	Transcription factor 21	61	Costa et al. (2011)

This strategy allowed for the discovery of more than 10 candidate TSG in RCC: *SPINT2* (Morris et al., 2005), *IGFBP1*, *IGFBP3*, *COL1A1* (Ibanez de Caceres et al., 2006), *UCHL1* (Kagara et al., 2008), *CXCL16*, *KTN19* (Morris et al., 2008), *BNC1*, *COL14A1*, *SFRP1*, *CST6*, and *PDLIM4* (Morris et al., 2010). Furthermore, gene promoters found to be methylated in cell lines but not in primary tumors (e.g., *SST*, *PTGS1*, *ISG15*, and *THY1*) might be important for cancer progression, whereas upregulated genes following treatment of cell lines with demethylating drugs but without promoter methylation (e.g., *BAP*, *IGSF4*, *RRM2*, *PMAIP1*, *Claudin1*, and *ICAM*), might be reactivated due to changes in promoter methylation status at upstream regulators (Morris et al., 2008). The identification of new candidate TSG may also be accomplished using high-throughput CpG methylation analysis platforms (McRonald et al., 2009) or by isolating methylated DNA by immunoprecipitation (methylated DNA immunoprecipitation, MeDIP) which is then used to perform whole-genome

microarray analysis (Morris et al., 2011). These strategies enabled the identification of several new candidate TSG in RCC, including *HTR1B*, *CALCA*, *IGFBP2*, *SOX17*, *COL1A2*, *BMP4*, *HS3ST2*, *FRZB*, *TAL1*, *MCM2*, *KCNK4*, *HOXC6*, *PITX2*, *SEPT5*, *IRF7*, *CCNA1*, *HOXA11*, *TERT*, *TMEFF2*, *EPHA3*, *PGF*, *MYOD1*, *MMP2*, *TNFRSF10C*, *PENK*, *EYA4*, *MYLK*, *IRAK3*, *ZNF215*, *SMARCB1*, *TWIST1*, *SCGB3A1*, and *IGFBP7* (McRonald et al., 2009), as well as *ATP5G2*, *PCDH8*, *CORO6*, *KLHL35*, *QPCT*, *SCUBE3*, *ZSCAN18*, *CCDC8*, and *FBN2* (Morris et al., 2011). Deriving from data collected in several studies, a CpG island methylator phenotype (CIMP) for RCC has been suggested (Dulaimi et al., 2004; McRonald et al., 2009), but this topic warrants further investigation in larger series of cases.

Loss of promoter methylation has been seldom reported in RCC, although several gene promoters less frequently methylated in RCC tumor samples compared to normal kidney tissue have been found, including *CARD15* (methylated in 18% of tumors),

*HLA-DRA* (24%), *SPARC* (34%), *IL8* (35%), *SEPT9* (39%), *HLA-DPBI* (45%), *TNFSF10* (47%), *VAMP8* (50%), *PRKCDPB* (55%), *HLA-DPA1* (56%), *HDAC1* (58%), *BTK* (58%), *S100A2* (60%), *MPO* (61%), *CRK* (61%), *CAPG* (61%), *NEU1* (69%), *ELL* (71%; McRonald et al., 2009), and *CA 9* (Cho et al., 2001).

The accumulating data on gene promoter hypermethylation in RCC supports the development of clinically relevant biomarkers. Interestingly, different RCC subtypes seem to display different gene sets deregulated by promoter hypermethylation (Dulaimi et al., 2004), and a gene panel (*CDHI*, *PTGS2*, and *RASSF2*) intended for the discrimination among the most frequent RCT subtypes in tissue samples has been evaluated (Costa et al., 2007). However, the application of this approach in an early detection setting requires testing of clinical samples obtained by minimally invasive or (ideally) non-invasive means, of which urine and serum stand as the most likely candidates. This has been attempted using a three-gene panel (*APC*, *RAR $\beta$ 2*, *RASSF1A*) which detected RCC with high specificity and sensitivity (Hoque et al., 2004). Moreover, *RASSF1A* promoter methylation might also prove useful for tumor surveillance/monitoring of RCC cancer patients (Peters et al., 2007).

Promoter hypermethylation of some genes has been associated with clinical and pathological features of tumor aggressiveness and also to have prognostic relevance. Thus, aberrant promoter methylation of *APAF1* and *DAPK1* (Christoph et al., 2006a), as well as of *GREM1* (van Vlodrop et al., 2010) has been associated with aggressive forms of RCC. Moreover, *APAF1*, *DAPK1* (Christoph et al., 2006a), *JUP* (Breault et al., 2005), *PTEN* (Kim et al., 2005), *UCHL1* (Kagara et al., 2008), *DAL1-4.1B/EPB41L3* (Yamada et al., 2006), *BNC1*, *COL14A1*, and *SFRP1* (Morris et al., 2010) promoter methylation have been associated with poorer survival, and most of them (*JUP*, *APAF1*, *DAPK1*, *PTEN*, *DAL1-4.1B*, *BNC1*, and *COL14A1*) retained independent prognostic value in multivariate analysis (Breault et al., 2005; Christoph et al., 2006a; Morris et al., 2010). In addition, a genome-wide methylation profile of ccRCC using bacterial artificial chromosome array-based methylated CpG island amplification (BAMCA) and unsupervised hierarchical clustering analysis, found that the clusters obtained for ccRCC tumor tissues predicted recurrence and these were clinicopathological valid since tumors with vascular invasion, renal vein neoplastic thrombi and higher pathological TNM stage clustered together (Arai et al., 2009). Interestingly, DNA methylation status of non-cancerous tissues was similar to that of respective RCC samples, and they were also associated with patient outcome, suggesting an association of RCC prognosis with precancerous molecular alterations (Arai et al., 2009). Finally, it has been also reported that *ABCG2/MDR1* gene promoter was methylated in RCC cell lines, and because this gene is associated with resistance of cancer cells to chemotherapeutic agents, this biomarker might become clinically useful for prediction of response to therapy (To et al., 2008).

Due to the association between promoter methylation and gene silencing, inhibition of DNMTs has been proposed as a therapeutic strategy to reactivate dormant genes. The nucleoside analog 5-aza-2'-deoxycytidine (DAC), a DNMTs inhibitor (DNMTi) has been tested in combination with conventional chemotherapeutic agents (e.g., vinblastine or paclitaxel; Takano et al., 2010; Shang

et al., 2011) in RCC cell lines. A synergistic effect was observed in both settings, but clinical studies are required to conclusively demonstrate the therapeutic usefulness of DAC in RCC.

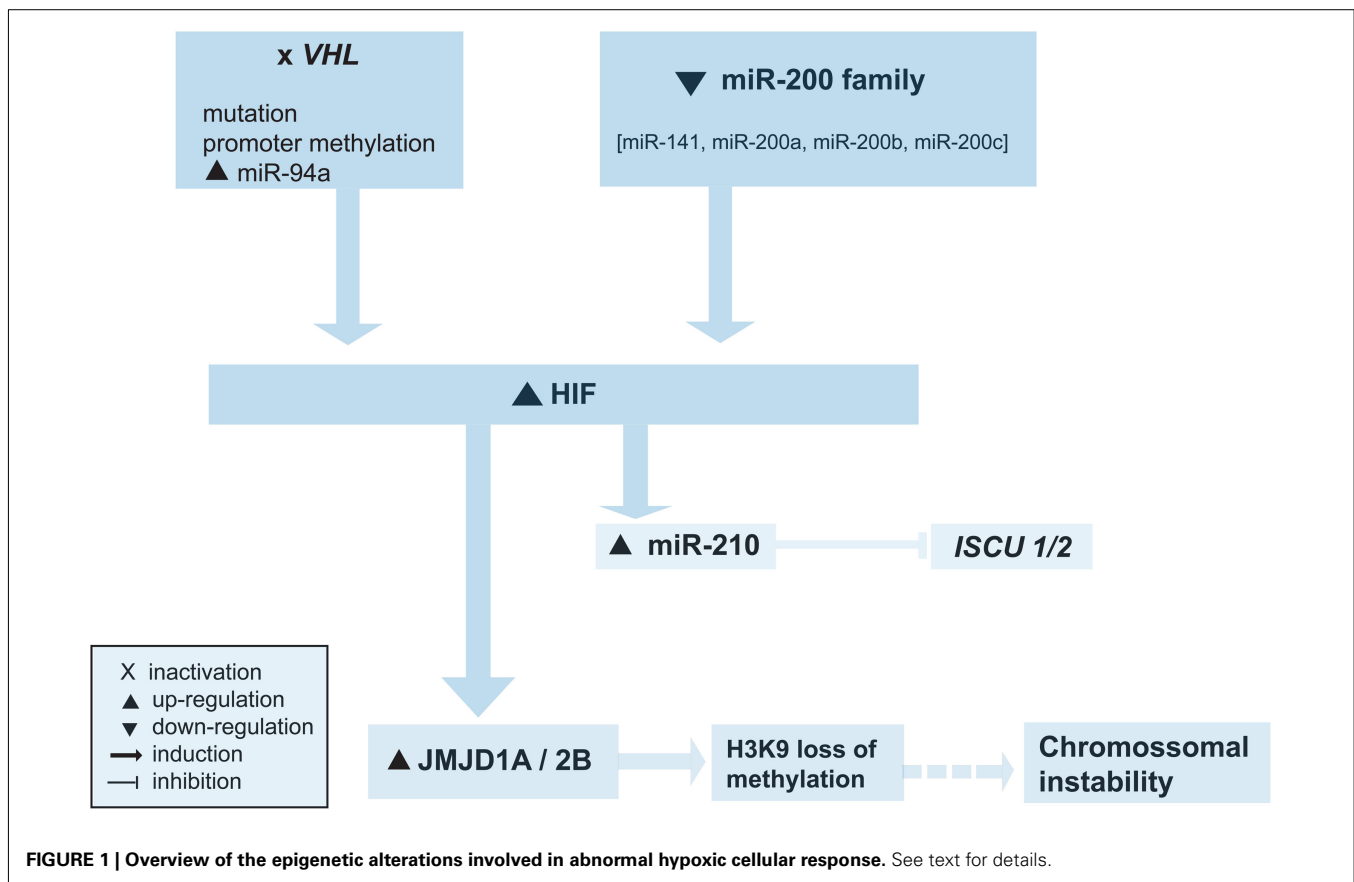
#### CHROMATIN REMODELING AND HISTONE ONCO-MODIFICATIONS

Most studies on chromatin remodeling and histone onco-modifications in RCT have dealt with the relationship with hypoxia, as well as with the prognostic relevance of those alterations and the potential therapeutic use of histone deacetylase inhibitors.

It is widely acknowledged that *VHL* dysfunction is a pivotal event in ccRCC carcinogenesis (Baldewijns et al., 2008), leading to constitutive expression of genes that mediate cellular adaptation to hypoxia. This gene encodes for the VHL protein (pVHL), a substrate recognition component of an E3 ubiquitin ligase complex that targets hypoxia-inducible factors (HIF; e.g., HIF1 $\alpha$  and HIF2 $\alpha$ ) for ubiquitination and proteasome degradation. HIFs are constitutively expressed and under normoxic conditions they are hydroxylated, allowing for their recognition by pVHL and subsequent targeting for degradation. Lack of pVHL leads to increased levels of HIFs and increased expression of genes involved in cellular response to hypoxia, promoting angiogenesis, invasion and metastasis, evasion of cell death, and cellular metabolism (Rathmell and Chen, 2008; Linehan et al., 2010).

Recent evidence unraveled a strong link between hypoxic cellular response and epigenetic regulation, especially histone modifications, albeit in non-RCC cancer cell lines (Figure 1; Johnson et al., 2008). Indeed, hypoxia was reported to be associated with widespread repression of total RNA and mRNA synthesis and to induce global histone modifications typically associated with transcriptional repression (loss of H3K9ac, increase in H3K9me2, H3K27me2, H3K9me3, H3K27me3, H3K4me1), but also unpredictably with gene activation (increase in H3K14ac, H4R3me2 which may facilitate acetylation of histones associated with activation of transcription, H3K4me2, H3K4me3, and H3K7me2; Chen et al., 2006; Johnson et al., 2008). Gene-specific histone modifications included a decrease in H3K9ac and an increase in H3K9/27me2 at hypoxia-repressed genes, an increase in H3K9ac and a decrease in H3K9/27me2 at hypoxia-induced genes, and an increase in H3K4me3 and a decrease in H3K27me3 in all hypoxia-responsive genes (activated and repressed), suggesting that repressed chromatin H3K4me3 enriched and H3K27me3 deprived might be rapidly activated when hypoxia is reversed (Chen et al., 2006; Johnson et al., 2008).

One of the mechanisms that might be involved in altered epigenetic landscape due to hypoxic effects is the regulation of Jumonji domain containing histone demethylases by HIF1 $\alpha$ , a critical mediator of hypoxic response (Beyer et al., 2008; Pollard et al., 2008; Figure 1). Genes encoding Jumonji domain containing 2-oxoglutarate-dependent oxygenases, characterized by a catalytic Jumonji C (JmjC) domain, were defined as a class of HIF-responsive hypoxia-inducible genes (Pollard et al., 2008). Histone demethylases Jumonji domain containing 1A (JMJD1A), Jumonji domain containing 2B (JMJD2B), and Jumonji domain containing 2C (JMJD2C) showed consistent patterns of regulation by hypoxia, especially JMJD1A and JMJD2B, which were found to be induced by HIF1 $\alpha$  but not by HIF2 $\alpha$  (Pollard et al., 2008). Since



HIF upregulation is a feature of ccRCC, the regulation of histone methylation status, and thus of genetic expression, by JmJc histone demethylases might be another mechanism leading to epigenetic changes in RCC carcinogenesis. Indeed, both JMJD1A and JMJD2B were found to be elevated in a RCC cell line with VHL loss of function (Beyer et al., 2008), and the expression of JMJD1A was reported to be higher in RCC cancer tissue than in adjacent normal renal tissue, mainly in cancer cells surrounding blood vessels, suggesting that JMJD1A is involved in tumor angiogenesis (Guo et al., 2011).

In addition, genes encoding for histone-modifying enzymes have been also reported to be mutated in ccRCC (van Haaften et al., 2009; Dalgliesh et al., 2010; **Table 2**). Specifically, inactivating mutations were described for *SETD2* (H3K36 methyltransferase), *JARID1C/KDM5C* (H3K4 demethylase), *UTX/KMD6A* (H3K27 demethylase), and *MLL2* (an H3K4 methyltransferase; van Haaften et al., 2009; Dalgliesh et al., 2010). More recently, mutations in the SWI/SNF chromatin remodeling complex gene *PBRM1* has been identified in 41% of ccRCCs (Varela et al., 2011). Interestingly, the induction of the H3K9 specific demethylases JMJD1A and JMJD2B, which target H3K9me1/me2 and H3K9me2/me3 respectively, might contribute to an increased mutation rate in tumors with upregulation of HIF, as H3K9 loss of methylation promotes chromosomal instability (Beyer et al., 2008).

The diagnostic potential of histone modifications and/or histone modifiers in RCC has not been explored thus far. They have

been, however, proposed as molecular biomarkers of prognosis, easily translated to routine pathology because they may be assessed by immunohistochemistry in formalin-fixed, paraffin-embedded tissue sections. Several histone marks have been associated with poor prognosis in RCC, including low H3K4me2, H3K18ac, and H3K9me2 (Seligson et al., 2009). H3K4me1–3 levels were also found to be inversely correlated with Fuhrman grade, pT stage, lymph node involvement and distant metastases, and an H3K4me score (combining staining levels of H3K4me) was an independent factor for RCC progression-free survival (Ellinger et al., 2010). Similar observations were made for global H3Ac and H4Ac levels, as well as for H3K9Ac levels in RCCs treated with partial nephrectomy (Minardi et al., 2009), whereas H3K18Ac levels were an independent predictor of RCC progression after surgery (Mosashvilli et al., 2010). Concerning histone modifiers, EZH2 was found to be upregulated in ccRCC, but this alteration was unexpectedly associated with a favorable prognosis (Hinze et al., 2009).

Histone onco-modifications might also carry therapeutic implications, as patients with marks of poor prognosis including low levels of H3K4me2, H3K18ac, and H3K9me2 could benefit from innovative treatments with histone deacetylase inhibitors (HDACi). Histone onco-modifications might also carry therapeutic implications, as patients with marks of poor prognosis including low levels of H3K4me2, H3K18ac, and H3K9me2 could benefit from innovative treatments with histone deacetylase inhibitors (HDACi; Seligson et al., 2009). Some preclinical studies on RCC

**Table 2 | Histone onco-modifications and deregulated modifiers in renal cell tumors.**

Chromatin remodeling alterations		Reference
Histone post-translational modifications	H3K18Ac, H3K4me1, H3K4me2, H3K4me3	Seligson et al. (2009), Ellinger et al. (2010), Mosashvili et al. (2010)
Histone modifiers	Inactivating mutations of <i>SETD2</i> , <i>JARID1C</i> , <i>UTX</i> , <i>MLL2</i> EZH2 upregulation	van Haaften et al. (2009), Dalglish et al. (2010) Hinz et al. (2009)

cell lines using the HDACi Vorinostat demonstrated an increase in the anticancer activity of temsirolimus, a mammalian target of rapamycin (mTOR) inhibitor, through survivin downregulation, leading to increased apoptosis, and enhanced inhibition of angiogenesis (Mahalingam et al., 2010). Moreover, the proteasome mediated degradation of Aurora A and Aurora B kinases through inhibition of HDAC3 and HDAC6 by the HDACi LBH589, was found to induce G2-M arrest and apoptosis in RCC, highlighting its potential therapeutic use (Cha et al., 2009). Valproic acid is also an HDACi which causes growth arrest, preventing tumor cell attachment to endothelium and matrix proteins, and blocking integrin-dependent signaling (Jones et al., 2009a). The combination of valproic acid with interferon-alpha enhanced the effects of the former (Jones et al., 2009a,b) and similar synergism was found when combined with AEE788, a multiple receptor tyrosine kinase inhibitor (Juengel et al., 2010). The combination of HDACi and retinoids might also provide an alternative therapeutic strategy because *RARB2* expression is reduced in RCC, in part owing to gene-specific histone hypoacetylation, and its re-expression is associated with anti-neoplastic effects through the abrogation of retinoid-resistance (Touma et al., 2005; Wang et al., 2005). Interestingly, this synergistic activity with retinoids has been demonstrated for both HDACi MS-275 (Wang et al., 2005) and trichostatin A (TSA; Touma et al., 2005) in RCC cell lines.

### MICRORNAs

Deregulation of miRNA expression seems to be pivotal for RCC development and progression (Valera et al., 2011). Indeed, several miRNAs have been found to be deregulated in RCTs, although most studies have focused mainly on ccRCC. Upregulation of miR-16 (Jung et al., 2009; Zhou et al., 2010), miR-18a (Neal et al., 2010), miR-20a (Neal et al., 2010), miR-21 (Liu et al., 2010a; Neal et al., 2010; Zhou et al., 2010), miR-34a (Liu et al., 2010a; Zhou et al., 2010), miR-34b (Liu et al., 2010a; Zhou et al., 2010), miR-92a (Valera et al., 2011), miR-155 (Jung et al., 2009; Liu et al., 2010a; Neal et al., 2010; Zhou et al., 2010), miR-185 (Liu et al., 2010a; Zhou et al., 2010), miR-210 (Jung et al., 2009; Liu et al., 2010a; Neal et al., 2010; Zhou et al., 2010), miR-224 (Jung et al., 2009; Liu et al., 2010a) and let-7 i (Neal et al., 2010; Zhou et al., 2010), and downregulation of miR-125b (Liu et al., 2010a; Zhou et al., 2010), miR-141 (Nakada et al., 2008; Jung et al., 2009; Liu et al., 2010a), miR-133b (Liu et al., 2010a; Zhou et al., 2010), miR-200b (Jung et al., 2009; Liu et al., 2010a), miR-200c (Nakada et al., 2008; Jung et al., 2009; Liu et al., 2010a), miR-429 (Jung et al., 2009; Liu et al., 2010a; Zhou et al., 2010), miR-506 (Zhou et al., 2010; Li et al., 2011), miR-508-3p (Zhou et al., 2010; Li et al., 2011), miR-509-5p (Zhou et al., 2010; Li et al., 2011), miR-509-3-5p (Zhou et al., 2010; Li et al., 2011), miR-510 (Liu et al., 2010a; Zhou et al., 2010;

Li et al., 2011), and miR-514 (Jung et al., 2009; Liu et al., 2010a; Zhou et al., 2010; Li et al., 2011) are the most consistently reported alterations.

Renal cell tumors display distinct cytogenetic alterations and these might cause miRNA deregulation as matching patterns between deregulated miRNAs and chromosomal aberrations have been reported in ccRCC (Chow et al., 2010a,b). On the other hand, miRNA deregulation might serve as an alternative mechanism for gene expression alterations due to chromosomal aberrations. This is well illustrated by the miR-204/211 family. Whereas in pRCC gain of 3q is a common finding, leading to upregulation of several genes including *C3orf58*, *CCDC50*, *DTX3L*, *PLD1*, *TRIM59*, *ECT2*, *RAP2B*, and *SERP1*, targeted by miR-204/211 (Liu et al., 2010a), in ccRCC miR-204/211 downregulation might be the mechanism causing upregulation of the aforementioned genes, since 3q gain is rare (Liu et al., 2010a). Moreover, it has been postulated that most miRNAs are tandemly clustered (Lee et al., 2002; Seitz et al., 2003), and, accordingly, a co-expression pattern for miRNA families miR-8 (or miR-200), miR-199, and miR-506 has been found in ccRCC (Li et al., 2011). Another interesting example is provided by the miR-506 family members miR-506, miR-508-3p, miR-509-5p, miR-509-3-5p, miR-510, and miR-514, which are downregulated in ccRCC (Zhou et al., 2010). The corresponding genes are tandemly clustered in the fragile site Xq27.3 and their predicted targets are upregulated, including some genes involved in key signaling pathways like *LDHA*, *HK1*, *VEGFB*, and *PSMA1* (Zhou et al., 2010).

Bioinformatics, anti-correlation analysis of miRNA/mRNA levels and functional studies in paired tumorous and normal tissues are also revealing interesting data on cell function alterations due to deregulated miRNA in RCTs. Those have showed that deregulated microRNAs target genes are commonly involved in metabolic (71 target genes of 13 deregulated microRNAs), focal adhesion, cell adhesion molecules and ECM receptor interactions (30 target genes of 25 deregulated microRNAs), cell cycle regulation (24 target genes of 22 deregulated microRNAs), and apoptosis (14 target genes of 11 deregulated microRNAs) pathways in ccRCC (Zhou et al., 2010; **Table 3**).

Furthermore, the miR-200 family is also known to be involved in epithelial-to-mesenchymal transition, and its downregulation might contribute to tumor invasion and metastasis (Liu et al., 2010a; Zhou et al., 2010; Li et al., 2011).

Downregulation of TSG in RCC has been also correlated with upregulation of oncogenic miRNAs. Indeed, elevated miR-185 was correlated with downregulation of *PTEN* (with subsequent activation of PI3K-AKT-mTOR signaling pathway), *PTPN13* (a Fas-associated tyrosine phosphatase that can inhibit PI3K/AKT signaling, induce apoptosis and suppress the cell survival effects

**Table 3 | MicroRNA deregulation in renal cell tumors, according to their function/pathway and target genes'**

Pathway	Upregulated	Target gene(s)	Reference	Downregulated	Target gene(s)	Reference
Metabolism	miR-210	<i>ISCU1/2</i>	Liu et al. (2010a), Neal et al. (2010), Zhou et al. (2010)	miR-508-3p	<i>LDHA</i>	Zhou et al. (2010)
Cell Adhesion/Invasion	NA			miR-509-3p miR-141 and miR-200c miR-149	<i>HK1</i> <i>ZEB2/ZFHX1B</i> <i>LOX</i>	Nakada et al. (2008) Liu et al. (2010a)
Apoptosis	miR-23b miR-438-3p	<i>POX</i> <i>BBC3/PUMA</i>	Liu et al. (2010b) Veronese et al. (2010)	NA		
VHL-HIF pathway	miR-92a	<i>VHL</i>	Valera et al. (2011)	NA		
Angiogenesis	miR-29a	<i>TIS11B</i>	Sinha et al. (2009)	miR-200bc and miR-429	<i>VEGF</i>	Zhou et al. (2010)
Signal transduction	miR-34a miR-185 miR-224	<i>SFRP1</i> <i>PTPN13</i> <i>ERBB4</i>	Liu et al. (2010a)	NA		
Other	miR-21 miR-142-3p	<i>SLC12A1, TCF21</i> <i>LRRC2</i>	Liu et al. (2010a)	miR-141 miR-149	<i>SEMA6A</i> <i>KCNAB1, KCNMA1</i>	Liu et al. (2010a)

*BBC3/PUMA*, *BCL2 binding component 3*; *ERBB4*, *v-erb-a erythroblastic leukemia viral oncogene homolog 4*; *HK1*, *hexokinase 1*; *ISCU1/2*, *iron-sulfur cluster scaffold homolog 1/2*; *KCNAB1*, *potassium voltage-gated channel, shaker-related subfamily, beta member 1*; *KCNMA1*, *potassium large conductance calcium-activated channel, subfamily M, alpha member 1*; *LDHA*, *lactate dehydrogenase A*; *LOX*, *lysyl oxidase*; *LRRC2*, *leucine rich repeat containing 2*; *POX*, *proline dehydrogenase (oxidase) 1*; *PTPN13*, *protein tyrosine phosphatase, non-receptor type 13*; *VHL*, *Von Hippel-Lindau gene*; *SEMA6A*, *semaphorin 6A*; *SFRP1*, *secreted frizzled-related protein 1*; *SLC12A1*, *solute carrier family 12*; *TCF21*, *transcription factor 21*; *TIS11B*, *zinc finger protein 36*; *C3H type-like 1*; *VEGF*, *vascular endothelial growth factor*; *ZEB2/ZFHX1B*, *zinc finger E-box binding homeobox 2*.

of IGF-1), and *KCNJ16* (a cell growth-related membrane protein). In addition, upregulation of miR-34a was correlated with downregulation of *SFRP1* (a Wnt signaling pathway regulator) whereas miR-224 upregulation was associated with downregulation of *ERBB4* (an EGFR family member and putative TSG; Liu et al., 2010a).

Hypoxic regulation of miRNAs is also emerging as an important mechanism implicated in RCC tumorigenesis (Figure 1). The deregulation of miRNAs in cell lines lacking VHL was been shown to be either mediated largely via HIF induction (miR-210 and miR-155) or by HIF independent VHL actions (miR-31, miR-21, miR-18a, miR-17, let-7f, miR-20a; Neal et al., 2010). Furthermore, the expression levels of HIF2 $\alpha$  and of its downstream targets (VEGF $\alpha$ , TGF $\beta$ ) seems to be regulated by several members of the miR-200 family (miR-141, miR-200a\*, miR-200b, miR-200c), and downregulation of miR-200 (or miR-8) family and VHL loss activate the HIF pathway (Zhou et al., 2010). On the other hand, HIF1 $\alpha$  can induce miR-210 in many solid tumors, and miR-210 has been consistently found to be over-expressed in ccRCC (Jung et al., 2009; Liu et al., 2010a; Neal et al., 2010; Zhou et al., 2010). Upregulation of miR-210 was associated with a reduced expression of its target gene *ISCU1/2*, which encodes assembly proteins involved in the biogenesis of [4Fe-4S] and [2Fe-2S] iron-sulfur clusters (Neal et al., 2010). These are implicated in electron transport and mitochondrial oxidation-reduction reactions, and, thus, downregulation of *ISCU1/2* might contribute to the repression of mitochondrial proteins and to the anaerobic metabolism in ccRCC (Neal et al., 2010). Interestingly, hypoxia induced microRNAs have been also correlated with *SLC12A1* and *TCF21* downregulation (encoding

cell adhesion proteins) in ccRCC, whose mRNAs were identified as direct targets of hypoxia induced miR-21 (Liu et al., 2010a).

Only a few studies addressed the potential use of miRNAs as RCC biomarkers for detection. In this regard, differential miRNA expression patterns between neoplastic and non-neoplastic renal tissues, as well as among different renal tumor subtypes have been described. The discrimination between ccRCC and normal kidney tissue might be accomplished by a panel of nine miRs (miR-21, miR-34a, miR-142-3p, miR-155, miR-185, miR-200c, miR-210, miR-224, and miR-592; Juan et al., 2010), a combination of miR-141 and miR-155 (Jung et al., 2009) or through the differential expression of miR-92a, miR-210, and miR-200c (Valera et al., 2011).

Concerning distinctive miRNA signatures for each of the main RCT subtypes, unsupervised hierarchical cluster analysis of miRNA microarray data showed that tumors derived from the proximal nephron (ccRCC and pRCC type I) and tumors derived from the distal nephron (oncocytomas and chRCC) can be distinguished by their miRNA profile (Valera et al., 2011), extending previous observations for ccRCC and chRCC (Nakada et al., 2008). These differential expression patterns of microRNAs might be also used to subclassify RCT (Petillo et al., 2009; Fridman et al., 2010; Youssef et al., 2011). In ccRCC 23 miRNA are differentially expressed (let-7e, let-7f, let-7g, miR10b, miR-124, miR-126, miR-138, miR-140-5p, miR-142-5p, miR-144, miR-184, miR-200c, miR-203, miR-206, miR-210, miR-218, miR-27a, miR-27b, miR-335, miR-373, miR-378, miR-92a, miR-98; Valera et al., 2011). However, some miRNAs are characteristic of sporadic ccRCC (let-7c, let-7d, miR-1, miR-100, miR-10a, miR-148b,



**Table 4 | Diagnostic and prognostic information in renal cell tumors provided by epigenetic biomarkers.**

	Detection biomarkers	Reference	Prognostic/predictive biomarkers	Reference
DNA methylation	<i>APC/RARB2/RASSF1A</i> <i>RASSF1A</i>	Hoque et al. (2004) Peters et al. (2007)	<i>JUP</i> <i>DAL1</i> <i>APAF1/DAPK1</i>	Breault et al. (2005) Yamada et al. (2006) Christoph et al. (2006b)
Histone modifications & modifiers	<i>RASSF1A/PTGS2/CDH1</i> NA	Costa et al. (2007)	<i>PTEN</i> <i>H3K4me2/H3K18Ac</i> <i>H3K4me1/H3K4me2/H3K4me3</i> <i>H3K18Ac</i>	Kim et al. (2005) Seligson et al. (2009) Ellinger et al. (2010) Mosashvilli et al. (2010)
miRNA	miR-141/miR-155 miR-1233	Jung et al. (2009) Wulfken et al. (2011)	miR-32	Petillo et al. (2009)

NA, not available.

miR-191, miR-199a-3p, miR-19a, miR-215, miR-29b, miR-30c, miR-363, miR-9) and others of hereditary (Von Hippel–Lindau syndrome-related) RCC (let-7a, miR-125a-5p, miR-125b, miR-143, miR-146b-5p, miR-15b, miR-17, miR-193a-5p, miR-193b, miR-196a, miR-20b, miR-214, miR-23b, miR-32, miR-372; Valera et al., 2011). Furthermore, ccRCC and pRCC differentially express 27 miRNAs, with miR-203 and miR-424 being more expressed in ccRCC than in pRCC, and miR-31 and miR-504 being more expressed in pRCC than in ccRCC (Petillo et al., 2009). Remarkably, a similar miRNA expression pattern was reported for chRCC and oncocytoma. These tumors share 60 miRNAs, with miR-143, miR-19a, miR-21, miR-29a, miR-181a, and miR-378 unique to chRCC and miR-146a being preferentially expressed in oncocytoma (Valera et al., 2011). Moreover, chRCC displayed higher levels of miR-203, miR-200b, miR-197, and miR-320 than oncocytoma, whereas miR-186 is more highly expressed in oncocytoma than in chRCC (Petillo et al., 2009). Finally, a recent study which investigated miRNAs expression levels in sera of RCC patients and healthy controls, identified miR-1233 as promising biomarker for RCC detection and monitoring (Wulfken et al., 2011).

Altered levels of miRNA might also provide prognostic information. Whereas miR-155 and miR-21 expression in ccRCC tumors has been found to correlate with tumor size (Neal et al., 2010), higher miR-210 levels were found in tumors displaying higher Fuhrman grade (Valera et al., 2011). In addition, in ccRCC, overexpression of miR-32 as well as of miR-210, miR-21, let-7i, and miR-18a correlated with poor survival (Petillo et al., 2009; Neal et al., 2010) and lower miR-106b levels were associated with

metastatic disease and poorer relapse-free survival (Slaby et al., 2010). High miR-210 expression was also found in tumors with lymph node metastasis (Valera et al., 2011), suggesting unique miRNA signatures in RCC metastasis, distinct from those of primary tumors (White et al., 2011).

## CONCLUSION

The ubiquity of epigenetic alterations in RCT supports their fundamental role in renal carcinogenesis. Those alterations not only provide further insight into the complex mechanisms underlying the genesis and progression of RCT, but they also grant the opportunity for the development of innovative biomarkers which might aid in disease detection, diagnosis, assessment of prognosis, and prediction of response to therapy (Table 4). Finally, owing to the reversible and plastic nature of epigenetic alterations, these constitute an attractive target for novel therapeutic approaches that might tackle one of the most chemoresistant types of human cancer.

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