Renal Cell Carcinoma: Molecular Aspects

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Abstract Renal cell carcinoma is the most common form of the kidney cancer accounting for more than 85% of the cases of which clear cell renal cell carcinoma (ccRCC) is the major histological subtype. The central molecular signature for ccRCC pathogenesis is the biallelic inactivation of VHL gene due to the presence of mutations/hypermethylation/complete gene loss, which results in the downstream HIF activation. These events lead to increased tyrosine kinase receptor signalling pathways (RAS/MEK/ERK pathway, PI3K/AKT/mTOR pathway and NF- κ B pathway), which through their downstream effector proteins causes the cell to proliferate and migrate. Recent studies have shown that VHL inactivation alone is not sufficient to induce the tumor. Mutations in numerous other genes that codes for chromatin modifiers (PBRM1, SETD2 and BAP1) and signalling proteins (PTEN and mTOR) have been identified along with activation of alternate signalling pathways like STAT and Sonic Hedgehog (SHH) pathway. It has also been shown that STAT pathway also works cooperatively with HIF to enhance the tumor progression. However, SHH pathway reactivation resulted in tumor regardless of the VHL status, indicating the complex nature of the tumor at the molecular level. Therefore, understanding the complete aetiology of ccRCC is important for future therapeutics.

Keywords Renal cell carcinoma · Genomics · Signalling · Von Hippel–Lindau (VHL) · Hypoxia inducible factor (HIF) · Chromatin modifiers

Introduction

Renal cell carcinoma (RCC) represents 2–3% of all the adult cancers and is amongst the 10th most common cancer worldwide [1, 2]. Being a heterogeneous tumor, it is classified into various subtypes based on the histological findings in combination with its genetic abnormalities. Major subtypes include clear cell RCC (ccRCC), papillary RCC (pRCC), chromophobe RCC (chRCC), collecting duct RCC and unclassified RCC [3]. ccRCC is the most common subtype that contributes 75–80% of all the RCC cases and it originates from the proximal epithelium of the nephron [4] and is also the focus of this review.

As the molecular biology of ccRCC is being explored both at genetic and epigenetic level, a complex interplay was observed which leads to the generation of an altered proteomic profile. The central genetic event in the pathogenesis of ccRCC is the biallelic inactivation of Von Hippel–Lindau (VHL) gene either due to somatic mutations or promoter hypermethylation [5]. Up to 90% of the sporadic ccRCC patients harbour VHL gene inactivation but mutations are associated with only 50% of the cases, while 10–20% of the patients have promoter hypermethylation. Some cases of ccRCC displays mRNA profile similar to VHL inactivation despite the lack of any VHL mutations or hypermethylation, suggesting these tumors harbour other genetic or epigenetic changes that directly or indirectly compromise the function of VHL gene product (pVHL) [6, 7]. Apart from VHL gene inactivation, other epigenetic genes also play a contributing role in ccRCC

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development which mainly includes polybromol (PBRM1), a chromatin remodelling complex; SET domain containing 2 (SETD2), a writer of H3K36me3 and BRCA-1 associated protein 1 (BAP1), a chromatin remodelling complex scaffold [8].

Altered expression of these genes leads to activation of numerous downstream signalling pathways, which results in increased angiogenesis, cellular proliferation and migration. Hypoxia inducing factors (HIF1 and HIF2) activation, caused due to VHL gene inactivation, is the major event which affects various aspects of cellular metabolism like shift towards glycolysis, release of angiogenic and growth factors, inhibition of apoptosis and enhanced cell proliferation and migration [9, 10]. Also, enhanced tyrosine kinase activity which activates MAPK and mTOR signalling pathways, along with the NF- κ B pathway, leads to increased tumor growth [11]. Recently, role of STAT3 pathway and sonic hedgehog (SHH) signalling pathway has also been observed which are also implicated in enhancing the ccRCC pathogenesis. It has been shown that STAT pathway also works cooperatively with HIF to enhance the tumor progression. On the other hand, SHH pathway reactivation results in tumor regardless of the VHL status, indicating the complex nature of the tumor at the molecular level [12, 13]. Currently, there is scarcity of the molecular drug targets for RCC, owing to the poor understanding of the disease at the molecular level. Thus, understanding the molecular biology of the disease is the utmost importance in context of development of new molecular markers, which can be helpful for therapeutics, as well as prognosis of disease.

VHL: The Culprit for ccRCC

Although most of the cases of ccRCC are sporadic, inherited syndrome also accounts for 1–4% of all the cases. However, the main culprit behind both the forms is same i.e. VHL gene. Apart from ccRCC, no mutations were detected in VHL gene for other subtypes of RCC [14]. The first report that showed the association between VHL gene and kidney cancer dates 38 years back, from the study carried out on a single family by Cohen et al. [15]. Now it is a well-known fact that the major aetiology in 80% of the ccRCC cases is the inactivation of the VHL gene [16]. The familial ccRCC occurs early in life (mean age 37 years) as compared to sporadic RCC (mean age 61 years) [17]. The VHL gene was identified and cloned in 1993 which was reported to be located on the chromosome 3p25–26 and consists of three exons [18, 19]. The mRNA is 4.5 kb long with only 639 bp nucleotide coding sequence encoding 213 amino acids containing protein, pVHL [17]. The VHL protein is strongly expressed in kidneys, testis, central nervous system and lungs in adults although, during

embryogenesis, it is expressed in all the three germ layers [20]. pVHL is a component of multiprotein complex known as E3-ubiquitin ligase which targets the proteins for degradation. The pVHL has α and β domain, which are connected by two short polypeptide linkers and by a polar interface. Alpha domain of the pVHL forms a ternary complex known as VCB, after binding to elongin C and elongin B proteins of the E3 ubiquitin ligase complex [5, 21]. The beta domain mediates the interaction of pVHL with the substrates of E3 ubiquitin ligase [22]. VHL gene becomes inactivated due to mutations or promoter hypermethylation or by complete gene deletion [16]. The tumor causing mutations in VHL gene are missense mutations that map evenly to the α/β domain of the gene. Of the 279 entries of missense mutations, the most frequently mutated residues on α domain are: Arg167 which plays a role in stabilizing α - β domain interface, Cys162 which interacts with elongin C, and Leu178 which helps in stabilizing the α -domain and interacting with elongin C. The other α domain mutations map to the residues involved in packaging of the helices and stabilizing the α - β interdomain interface. In the β -domain, Tyr98 is the most mutated residue, however no structural role of this mutation has been observed [19]. In a study involving 106 ccRCC cases, 42 mutations were reported in VHL gene, of which 19 were missense mutations [8]. More recently a study involving 360 ccRCC cases, total 254 VHL mutations were identified, of which 35% accounted for missense mutations involving Ser65, Asn78, Ser80, Trp117 and Leu184 as the major hotspot codons [23].

Elongin C, initially thought to be a component of the transcription elongation factor elongin, is now known to be a vital component of the VHL complex. Elongin C interacts with VHL on one side and elongin B on other, forming a ternary complex [16]. Recently, mutations have been observed in elongin C encoding gene, TECB1 [Transcription elongation factor B (SIII), polypeptide 1]. Sato et al. [8] showed a total of 8 mutations in 3.3% of ccRCC cases ($n = 106$), involving two hotspots (Tyr79 and Ala100), located in the pVHL interacting domain. Besides VHL, elongin C is the only gene, which was reported to be mutated in ccRCC in the whole VHL complex.

Two more proteins are also associated with this complex i.e. Hs-Cul-2 (*Homo sapiens*-Cullin 2) and Rbx1 (RING box protein 1). While Hs-Cul-2 interaction with VCB complex depends on the integrity of the complex, Rbx1 potentially activates ubiquitination by E1/E2 ubiquitin activating and conjugating enzymes [24, 25].

Although VHL performs several cellular functions, it is well known for its role in controlling the cellular response to oxygen levels through the regulation of transcription factors like HIF-1 α and HIF-2 α [26].

Hypoxia Inducible Factor and ccRCC

Hypoxia inducible factor (HIF) is a heterodimeric protein made up of alpha subunit (HIF-1 α /HIF-2 α /HIF-3 α) and beta subunit (HIF- β). Under normoxia, the HIF- α is hydroxylated at one of the two proline residues by prolyl-4-hydroxylases (PHD1-3) in oxygen dependent manner, which is recognized by VHL complex and thus targeted for ubiquitination and proteasomal degradation. But under hypoxia or VHL inactivation, HIF- α escapes this degradation and translocates to the nucleus where it interacts with the beta subunit to form active HIF. This HIF, along with its co-activators like p300 and CBP (CREB binding protein), binds to hypoxia response element (HRE) as a transcription factor that results in the activation of more than 60 hypoxia inducible genes involved in various pathways [14]. Among these are glucose metabolic enzymes and transporters (at least one isoform of each glycolytic enzyme, LDH, lactate dehydrogenase, GLUT1), inhibitor of acetyl Co-A formation (PDK1, pyruvate dehydrogenase kinase 1), pH regulator (carbonic anhydrase 9; CA9), growth factors and angiogenic proteins (VEGF, PDGF, IGF, TGF- α), anti-apoptotic proteins (BCL-x1, ARC; apoptosis repressor with a CARD domain), cell cycle proteins (cyclin D1) and erythropoietin (EPO) [6, 27]. In ccRCC, HIF plays a contrasting role as compared to other cancers as HIF-2 α enhances tumorigenic activity while HIF-1 α has been shown to play a tumor suppressor role [28, 29].

As proposed by Warburg, the increased expression of various glucose metabolising proteins, along with LDH and lactate transporter MCT4, and decreased acetyl CoA production shifts the tumor energy metabolism from oxidative phosphorylation to aerobic glycolysis [30]. The transport of lactate outside the cell makes the tumor environment acidic, which is maintained by increased expression of carbonic anhydrase 9, a transmembrane protein [10]. Also, downregulation of certain proteins of electron transport chain viz. cytochrome c oxidase and NADH-ubiquinone oxidoreductase has been observed in ccRCC, which further diminishes the efficiency of oxidative phosphorylation [30].

HIF has also been shown to directly inhibit tumor apoptosis by increasing the expression of anti-apoptotic proteins. The study by Razorenova et al. [31] has shown an increased expression of apoptotic inhibitor ARC in 65% of the RCC by HIF which can explain how the cells survive early in tumor formation. Para-neoplastic erythrocytosis observed occasionally in kidney cancer is attributed to increased EPO [17]. CXCR4 (Cys-X-Cys containing chemokine receptor 4) is another target of HIF gene, which is a chemokine receptor present on the tumor cells and helps in metastasis [32]. Some of the HIF inhibitors like CRLX-

101, EZN-2968 and EZN-2088 showed efficacy in pre-clinical and phase I clinical studies but failed at later stages of trial [33–35]. But as the oncogenic role of HIF-2 α becomes more prominent, targeting the HIF-2 α alone would be an effective therapeutic strategy. Recently, some of its inhibitors (PT2385, PT2399) are in pre-clinical studies and showing promising results for future therapies [36, 37] (Fig. 1).

Downstream Pathways Involved in Disease Manifestation

Tyrosine Kinase Pathway

Constitutive overexpression of VEGF explains the extraordinary capillary network of ccRCC. Due to this, VEGF has been used as a therapeutic target (bevacizumab, sunitinib and sorafenib) in ccRCC [38]. VEGF binds to its tyrosine kinase receptors (VEGF-R1/R2) present on the endothelial cells. On binding to VEGF-R2, VEGF enhances cell proliferation and migration by activating downstream kinase pathways i.e. MAPK pathway and PI3K/AKT pathway. Also, it subsequently activates VEGF-R1, which further assists in neovascularisation [11].

However, VEGF-R2 is also present on ccRCC cells along with other growth factor receptors like EGFR and IGF receptor. The overexpression of the ligands (VEGF, TGF- α , IGF) results in dimerization and subsequent activation of these tyrosine kinase receptors. These receptors, on activation, activate either the RAS/MEK/ERK pathway or PI3K/AKT/mTOR pathway to ultimately enhance the production of HIF- α thus accentuating the tumor progression [9]. Also, mTOR on activation forms a multimolecular complex (mTORC1) which through its downstream effector proteins like P70S6K1 results in inhibition of apoptotic pathway and allows the cell cycle to enter into G1 phase [11]. PTEN, an inhibitor of PI3K (protein of mTOR pathway) has also been shown to be suppressed epigenetically in ccRCC [39]. Further, a study by Dey et al. showed that increased miR-21 attenuated PTEN expression and thus lead to cell proliferation and migration [40]. In a study by White et al., it has been shown that Galectin 1, a downstream HIF-1 α target, is overexpressed in ccRCC. It binds to integrin and extracellular matrix proteins and to numerous intracellular proteins involved in angiogenesis and metastasis. In their study, authors showed that Galectin 1 via AKT/mTOR/p70 kinase axis also causes increased cell migration and invasion [41]. Sato et al. [8] also reported mutations in this mTOR pathway in ccRCC; they observed various oncogenic mutations in PI3K. Mutations were also observed in various mTOR inhibitors (PTEN, TSC1/2, tuberous sclerosis) in ccRCC [42]. This indicates the essential role of mTOR signalling in ccRCC, which is

Fig. 1 Role of VHL complex and HIF- α in ccRCC. In normoxia, HIF- α is hydroxylated at one of two proline residues by prolyl hydroxylase in oxygen dependent manner, which is recognized by VHL complex (VHL SCF-like E3 ubiquitin ligase) and is targeted for ubiquitination and thus proteasomal degradation. But under hypoxia, prolyl hydroxylase is inactivated by ROS, which cannot hydroxylate HIF and is thus not recognised by VHL complex. Also, VHL inactivation (hotspot for mutations: Cys167, Leu 178, Tyr 98) or mutations in Elongin C (hotspots: Ala 100, Tyr 78), inhibits VHL complex formation, and thus HIF- α degradation. This HIF- α in turn interacts with its beta subunit in the nucleus to form active HIF. HIF, along with its co-activators like

p300 and CBP, binds to HRE as a transcription factor, which results in the activation various pathways. VHL (von Hippel–Lindau), HIF (hypoxia inducible factor), B (elongin B), C (elongin C), Cul-2 (cullin-2), Rbx1 (RING box protein 1), ROS (reactive oxygen species), CBP (CREB binding protein), HRE (hypoxia responsive element), LDH (lactate dehydrogenase), GLUT1 (glucose transporter 1), CA9 (carbonic anhydrase 9), PDK1 (pyruvate dehydrogenase kinase 1), VEGF (vascular endothelial growth factor), PDGF (platelet derived growth factor), TGF- α (transforming growth factor), IGF (insulin like growth factor), EPO (erythropoietin), ARC (apoptosis repressor with a CARD domain), CXCR4 (chemokine receptor 4)

thus intervened by a number of mTOR inhibitors (temsirolimus and everolimus) as therapeutic drugs [38]. Many of the tyrosine receptor inhibitors cause tumor regression but response rates are variable due to the up-regulation of the resistance pathways. In response to VEGFR inhibitors, MET signalling is one of the pathways involved in resistance in ccRCC [43]. MET is a tyrosine kinase receptor that is activated by a hepatocyte growth factor, which in turn results in the activation of multiple pathways involved in cell proliferation, differentiation, survival, and cytoskeletal rearrangement [44]. Cabozantinib, which is a dual MET/VEGFR inhibitor has been, studied in phase I trial with a response rate of 28% [45]. Therefore, the dual inhibitors have a direct anti-tumor effects and better efficacy than individual therapies. So, in future the elucidation of the pathways involved in the resistance will help in providing the better survival rate for ccRCC patients.

NF- κ B Pathway

Increased HIF also stimulates NF- κ B pathway. TGF- α released by ccRCC binds to its receptor EGFR and causes subsequent activation of PI3K/AKT/I κ B-kinase alpha/NF- κ B signalling cascade [46]. NF- κ B transcription factor activation ultimately leads to increased cell proliferation and apoptosis inhibition, tissue invasion and angiogenesis. Increased cellular proliferation by NF- κ B has been shown to be mediated by inducing cyclin D1, a protein required for transition of cell cycle from the G1 to the S phase [47]. NF- κ B has also shown to be involved in cell migration and invasion through Insulin-like growth factor 2 mRNA binding protein 3 (IMP3) [48]. In addition, it was reported that loss of VHL results in the activation of NF- κ B in both familial and sporadic cases of ccRCC [49]. Later on, it was studied that VHL loss decreased vascular cell adhesion molecule 1 (VCAM-1) levels through NF- κ B signalling pathway and this is independent of the HIF which in turn results in worse prognosis of ccRCC patients [50].

Fig. 2 Signaling pathways involved in ccRCC. Increased growth factors and their receptors lead to activation of downstream MAPK, mTOR and NF- κ B signalling pathways. Activation of RAS/MEK/ERK pathway (shown in green) through its downstream effector proteins (c-myc, CREB) leads to increased cell proliferation and migration. PI3K/AKT/mTORC pathway (shown in blue) activation enhances the cellular translation by targeting S6K1 and ribosomal synthesis. PTEN, a mTOR pathway inhibitor is present in decreased amount in ccRCC. However, Galectin1, mTOR pathway enhancer, has an increased expression. Apart from this, both the pathways also increase HIF- α , thus promoting tumor progression. NF- κ B pathway is also activated by TGF- α which further enhances tumor growth and inflammation. Also, reactivation of sonic hedgehog signalling (SHH) pathway, (through its effectors Smo and Gli proteins) results in increased cell proliferation and migration. Besides the above pathways, increased MMPs, found in ccRCC matrix, cleaves HAVCR/KIM-1 protein, expressed on tumor cell surface. Globular KIM-1 binds to its receptor and activated IL6 production which on binding to

its receptor (gp80) activated the STAT3 signalling pathway. STAT3 apart from directly activating tumor promoter genes also binds cooperatively to HIF- α to enhance the production of HIF- α target genes. GRIM19, a STAT3 inhibitor (which binds STAT3 and restricts it to the perinuclear space), is also downregulated in ccRCC. MAPK (mitogen activated protein kinase), RAS (rat sarcoma), MEK (MAP/ERK kinase), ERK (extracellular receptor kinase), MNK (MAPK-interacting protein kinase), CREB (cAMP response element binding protein), PI3K (phosphatidylinositol 3-OH kinase), AKT also known as protein kinase B (PKB), mTORC (mammalian target of rapamycin complex), PTEN (phosphatase and tensin homologue), HIF (hypoxia inducible factor), NF- κ B (nuclear factor kappa light chain enhancer of activated B cells), Smo (smoothened), Gli (glioma associated oncogene), MMP (matrix metalloproteinase), STAT3 (signal transducer and activator of transcription 3), HAVCR/KIM-1 (Hepatitis A virus receptor/kidney injury molecule1), GRIM19 (gene associated with retinoid interferon induced mortality 19)

Therefore, targeting NF- κ B pathway in ccRCC has a great promising for the future therapeutics.

STAT3 Pathway

In a study by Cuadros et al., Hepatitis A virus receptor/kidney injury molecule 1(HAVCR/KIM-1) protein was found to be significantly overexpressed in ccRCC. KIM-1 is a type 1 glycoprotein with extracellular globulin domain, which is cleaved by metalloproteinase present in the extracellular matrix. In the above study, the authors have shown that this ecto-domain of KIM-1 protein activates IL-6 transcription, which in turn binds to its receptor (gp80) present on the cell membrane. gp80 then recruits its signal transducer gp130, which on phosphorylation acts as a

docking site of STAT3. On binding to gp130, STAT3 gets phosphorylated which is now an active transcription factor. It leads to increased transcription of HIF-1 α along with various other proteins involved in cell cycle and apoptosis like BCL-xL, survivin and cyclin-D1, thus causing tumor growth [12]. It was also reported that STAT3 cooperatively activates HIF1 target genes by binding to HIF-1 α protein, its co-activators and RNA polymerase, thus initiating the gene transcription of HIF1 target genes [51]. Also, in a study by Alchanati et al., GRIM19 (gene associated with retinoid interferon-induced mortality 19) was found to be severely downregulated in ccRCC. It was shown to be a STAT3 inhibitor; therefore, its down-regulation helped in tumor cell proliferation [52]. The STAT3 was also implicated in prognosis of ccRCC and found to be associated

Table 1 Mutational summary of different genes involved in ccRCC

S. no.	Gene	Function	Nucleotide change	Amino acid change	References
1.	VHL (von Hippel–Lindau)	Helps in ubiquitination of HIF- α	c388G > C c485G > A c533T > C c256C > A c224_226del TCT c355 > T c351G > A	Arg167Gln Cys162Tyr Leu178Pro Pro86Thr Phe76del Phe119fs Trp117X	[8, 19]
2.	TECB1 (Transcription elongation factor B (SIII), polypeptide 1)	Binds with VHL and forms VCB complex	c298G > C c236A > G	Ala100Pro Tyr79Cys	[8]
3.	PBRM1 (Poly bromo 1)	Chromatin remodeler	– – c4510_4512delCTG c4430delC c2627G > A c2088C > G	His24fs Ile57del Leu1504del Pro1477fs Arg876His Tyr696X	[8, 55, 56]
4.	BAP1 (BRCA1 associated protein 1)	Chromatin modifier and de-ubiquitin	c1252delT c860C > G c689T > A c443_444insCG c271T > G c155_158delGGAT c152A > T c92A > T c38delG	Tyr418fs Ser287X Leu230Gln Glu148fs Cys91Gly Trp52fs Lys51Ile Glu31Val Gly13fs	[8, 59]
5.	SETD2 (SET domain containing 2)	Histone methyl transfrase (H3K36)	c7496T > G c6866C > G c4973C > T c3650G > A c2885C > G c4376C > T c1637_1638insT	Ile2499Ser Ala2289Gly Ser1658Leu Trp1217X Ala962Gly Pro1459Leu Ser546fs	[8, 59]
6.	MTOR (Mammalian target of rapamycin)	Serine-threonine protein kinase	c7571A > T c7235A > T c6016G > T c3939G > A	Gln2524Leu Asp2412Val Val2006Phe Met1313Ile	[8]
7.	PTEN (Phosphatase and tensin homologue)	Inhibitor of AKT/mTORsignaling pathway	c105G > T c633C > A –	Met35Ile Cys211X Leu320Ser	[8, 42]
8.	TP53 (Tumour protein p53)	Regulator of cell cycle	c31G > A c100_101insC c67G > T c467G > A c2303G > A c2767C > A c1596C > T	Val11Met Val34fs Glu23X Arg156His Gly768Glu Leu923Ile Ser532Ser	[8, 60]

Table 1 continued

S. no.	Gene	Function	Nucleotide change	Amino acid change	References
9.	JARID1C (Jumonji/ARID Domain-Containing Protein 1C)	Histone demethylase (H3K4)	c807delC c1420delA	Thr270fs Asn477fs	[61]
10.	MLL2 (Mixed lineage leukemia 2)	Histone methyltransferase (H3K4)	c1311G > T c14778G > A	Glu437Asp Met492Ile	[61]
11.	GLI1 (Glioma associated oncogene 1)	Hedgehog signalling protein	c3840C > T	Pro1280Pro	[55]

Besides the top four genes (VHL, PBRM1, BAP1 and SETD2) that are frequently mutated in ccRCC, other genes such as TCEB1, mTOR, PTEN, GLI1, TP53, MLL2, JARD1C and JMJD1C are also recently shown to be mutated in ccRCC

with advanced tumor stage and poorer patient survival rate [53]. These data suggest an effective role of STAT3 inhibitors in therapeutic drug targeting of ccRCC owing to their role in inhibiting pro-tumorigenic signalling pathways.

Sonic Hedgehog (SHH) Signalling Pathway

Dormoy et al. reported reactivation of SHH signalling pathway in ccRCC regardless of VHL status. The authors found an increased expression of Smo and Gli transcription factors, promoting cell proliferation in ccRCC. These factors were also shown to interact with PI3k/AKT pathway and activate NF- κ B signalling pathway, thus orchestrating various pathways to promote the tumor growth. They also reported a long lasting effect of SHH signalling inhibitor, cyclopamine, on tumor growth in nude mice, even after treatment arrest, suggesting a complementary role of these inhibitors in ccRCC therapeutics [13]. The SHH-GLI1 signalling was also found to be overexpressed in ccRCC under hypoxia, which was mediated by HIF-2 α and involved in radio-resistant nature of the tumor [54] (Fig. 2).

Other Genes Involved in ccRCC Pathogenesis

However, VHL alone is not sufficient to induce the tumor, other molecular changes also play an important role [5]. PBRM1 gene encodes the protein BAF180, which is a subunit of the chromatin remodelling complex PBAF SWI/SNF. The gene consists of six bromodomains, which helps in the binding to acetylated lysine residues of histone tails and thus regulates various cellular processes like cell proliferation, replication, repair and transcription of DNA. Truncating mutations in PBRM1 were found in 34% of the cases of ccRCC (n = 257). Two in-frame deletions were also identified. A six amino-acid deletion (E1214delM-FYKKE) was found in the second BAH (bromo-adjacent homology) domain, which is involved in protein–protein

interactions within the SWI/SNF complex. Also, deletion of an isoleucine codon (Ile 57) in the first bromodomain was observed [8, 55, 56]. PBRM1 maps to the chromosome 3p21 and is the second most frequently mutated gene in ccRCC after VHL. Knockdown of PBRM1 in ccRCC resulted in increased cell proliferation and migration via regulating p53/p21 pathway, suggesting its role as a tumor suppressor gene [57, 58].

Another gene, BRCA1, which is, associated protein-1 (BAP1) located on chromosome 3p between VHL and PBRM1 genes also found to be mutated in 10–15% of the ccRCC cases. It is a de-ubiquitinating enzyme and is associated with chromatin regulating factors like Host cell factor-C1 [16]. In a large scale study of ccRCC (n = 609), BAP1 mutations were shown to be correlated with both high tumor stage and grade and were associated with worse cancer specific survival which decreased to 31.2 months from 78.2 months. The majority of the mutations were truncating and missense mutations [59]. Similar observations were also reported by Sato et al. [8] In addition, BAP1 mutations have been shown to activate the mTORC1. The worse survival observed in patients with BAP1 mutations may be accounted for the activation of mTOR pathway, further aggravating the ccRCC pathology [16].

Also, SETD2 mutations were found in 10–15% of the ccRCC cases among all subtypes of RCC. SETD2 is located at the chromosome 3p near the VHL, PBRM1, BAP1 genes and is a histone-3-lysine-36-methyltransferase, which causes transcriptional activation of its target genes [8, 16]. In another study by Hakami et al., SETD2 was mutated in 7.4% of the cases (n = 609). These mutations were associated with short cancer specific survival from 78.2 to 62.7 months [59]. As all the above genes modulate the chromatin architecture, it suggests that their mutations play an important role in pathogenesis of ccRCC by modulating the epigenetics of the kidney cells (Table 1).

Conclusion

Although VHL is central molecular signature that is involved in majority of the ccRCC cases, this alone is not sufficient to induce the tumor. Other genes also play an important role in the formation and pathogenesis of ccRCC. They act in VHL independent manner by targeting chromatin architecture of the kidney cells. Also, various VEGF and mTOR inhibitors have been implicated in ccRCC, still resistance to these chemotherapeutics is common obstacle in the patients. This may be due to activation of various alternative pathways like STAT3 and SHH signalling pathways. Thus indicating an important role of multiple pathways at both genomic and proteomic level. Detailed identification of these pathways will help in further exploring the additional drug interventions, which may help in treating the tumor or increasing the efficacy of traditional therapeutics.

Authors contribution All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest None.

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