**Chapter 29**

***The Emerging Genetic Landscape in Renal Cell Carcinoma***

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**CASE 1**

***Introduction*** – Much of what is known about the genetic nature of clear cell Renal Carcinoma (ccRCC) is derived from the study of hereditary kidney cancer syndromes. Von-Hipple Lindau (VHL) disease was the first of these conditions to be identified. The VHL gene, located at 3p25-26, is a classic tumor suppressor requiring mutations of both copies for tumor development. The gene encodes the substrate recognition component of an E3 ligase, the major targets of which include hypoxia-inducible factor-1α (HIF1α) and hypoxia-inducible factor-2α (HIF2α).

***Primary Question*** – Hypoxia-inducible factor-1α (HIF1α) and hypoxia-inducible factor-2α (HIF2α) are transcription factors that regulate hypoxia responsive genes. ***Which of the following hypoxia responsive genes is an active therapeutic target in the treatment of renal cell carcinoma?***

1. Vascular endothelial growth factor (VEGF)
2. Platelet derived growth factor (PDGF)
3. TGFα (a ligand of the epidermal growth factor receptor)
4. The glucose transporter, GLUT1
5. Carbonic anhydrase IX

***First Reply Question*** – VHL alterations represent the classic paradigm of a hereditary cancer gene that is often somatically mutated in sporadic forms of kidney cancer. ***Alterations of the gene product through genetic or epigenetic mechanisms occur in approximately what percentage of clear cell renal carcinomas?***

1. 1%
2. 15%
3. 50%
4. 90%

***Second Reply Question*** – Chromosome 3p is the locus for the VHL gene. ***Which other important identified gene also maps to chromosome 3p?***

1. MTOR
2. TP53
3. PBRM1
4. REDD1

**CASE 2**

***Introduction*** – NextGen sequencing and single-nucleotide polymorphism SNP) array technology have allowed for more accurate identification of gene targets. Genes involved in chromatin remodeling and histone modification have proven significant in the development of kidney cancer. Specifically, mutations in SETD2, BAP1, and PBRM1 have been identified. SETD2 encodes a histone H3 lysine 36 methyltransferase. Methylation of histone H3 lysine residues regulates chromatin structure and impacts transcriptional control. PBRM1 encodes BAF180, which is the chromatin targeting subunit of the Polybromo BRG1-associated factor complex (PBAF, SWI/SNF-B) involved in nucleosome remodeling. BAP1 encodes a nuclear protein containing an ubiquitin carboxy-terminal hydrolase (UCH) domain reported to target histone H2A and regulate transcription, cell cycle, and growth.

***Primary Question*** - ***Mutations in which of the above noted genes involved in kidney cancer portends a worse prognosis?***

1. SETD2
2. BAP1
3. PBRM1
4. VHL

***First Reply Question*** –Mutations of PBRM1 and BAP1 appears to be mutually exclusive driver mutations and are both located on the short arm of chromosome 3. ***It is proposed that the loss of chromosome 3p subsequent to mutation of this important gene is the initial event in tumorigenesis?***

1. SETD2
2. TP-53
3. VHL
4. MTOR

***Second Reply Question*** – Mutations involved in the chromatin remodeling pathway genes are important in the development and prognosis of renal cell carcinoma. ***All of the following genes are implicated in this pathway EXCEPT:***

1. SETD2
2. TP53
3. PBRM1
4. BAP1

**CASE 3**

***Introduction*** – The data presented from the cancer genome atlas project (TCGA) analysis identified 8 significantly mutated genes (SMGs) in renal cell carcinoma: VHL, PBRM1, SETD2, KDM5D, PTEN, BAP1, mTOR and TP53. Sato et al. also reported on whole-genome sequencing from an additional cohort of patients. They identified 28 significantly mutated genes. Other targets identified in this series included: TCEB1, which is the gene encoding Elongin C, known to be an essential part of the VHL complex; TET2, which encodes an α-ketoglutarate-dependent oxygenase catalyzing a critical step in DNA demethylation; KEAP1, which is a key component of a cullin-RING ubiquitin ligase complex that targets NRF2, which is required in oxidative stress responses, and finally mTOR/PTEN/PIK3CA/MTORC1/PIK3CG/RPS6KA2/TSC1/TSC2 and others, which together comprised 26% of reported cases.

***Primary Question*** – Although the MTOR gene is mutated in only 5-6 % of RCCs, cumulative mutations within the pathway are significantly more prevalent. ***MTOR inhibitors are an approved therapy for the treatment of renal cell carcinoma, which of the following medications is an MTOR inhibitor?***

1. everolimus
2. sunitinib
3. interferon
4. axitinib

***First Reply Question*** –The MTOR pathway is complex with multiple proteins responsible for control. ***Which protein is directly induced by HIF1α and HIF2α in ccRCC, and it’s induction is sufficient to inhibit mTORC1?***

1. PI3K
2. AKT
3. REDD1
4. TSC1

***Second Reply Question*** – Both activating mutations of PI3K and inactivating mutations of PTEN lead to increased levels of PIP3, which, in turn, result in increased binding and translocation of AKT to the membrane. AKT is activated through a dual phosphorylation mechanism and subsequently phosphorylates multiple substrates**. *Which of the following is a substrate of AKT phosphorylation?***

1. TSC2
2. BAP1
3. VHL
4. VEGF

**CASE 4**

***Introduction*** – SDH-associated kidney cancer and Hereditary Leiomyomatosis Renal Cell Carcinoma (HLRCC) are both genetic cancer syndromes that involve germ-line mutations in genes encoding enzymes of the Krebs cycle. Succinate Dehydrogenase (SDH) is a complex of four polypeptides (SDH A-D) that catalyzes the conversion of succinate to fumarate. The genes encoding the subunits of SDH are mutated in the SDH-associated kidney cancer syndrome. Fumarate Hydratase (FH) catalyzes the conversion of fumarate to malate and germ-line mutations in the gene encoding this enzyme cause HLRCC, which predisposes those affected to the development of pRCC type 2. The FH and SDH genes function as tumor suppressors and loss of function mutations lead to accumulation of fumarate and succinate, respectively.

***Primary Question*** – ***Increased levels of succinate and fumarate inhibit these enzymes, which are responsible for HIF hydroxylation and binding by VHL?***

1. Prolyl Hydroxylases (PHD)
2. Kelch-like ECH-associated protein 1 (KEAP1)
3. nuclear factor erythroid 2 related factor (NRF2)
4. alpha ketoglutarate

***First Reply Question*** –Fumarate and succinate at excess levels also interact with other proteins, including Kelch-like ECH-associated protein 1 (Keap1). Keap1 is a component of an E3 ubiquitin ligase that targets nuclear factor erythroid 2 related factor (NRF2). ***What is the main function of NRF2?***

1. disposal of damaged organelles and clearance of aggregated proteins
2. DNA demethylation
3. Regulator of antioxidant response
4. Chromatin Remodeling

***Second Reply Question*** – ***Which of the following Kreb’s cycle enzymes has NOT been known to be mutated in a hereditary kidney cancer syndrome?***

1. Succinate Dehydrogenase
2. Fumarate hydratase
3. Citrate synthase

**CASE 5**

***Introduction*** – Autophagy is a mechanism of protein degradation responsible for the disposal of damaged organelles and clearance of aggregated proteins. During this process, nuclear membranes engulf cytoplasmic substrates forming autophagosomes, which then fuse with lysosomes and lead to protein degradation.

***Primary Question*** – Sequestosome 1 (p62) is a scaffold protein and both an essential component and target of autophagy. ***When autophagy is impaired, p62 accumulates and it’s accumulation leads to interaction with a variety of proteins involved in all of the following pathways EXCEPT?***

1. mTOR
2. BAP1
3. nuclear factor erythroid 2 related factor (NRF2)
4. NF-κB

***First Reply Question*** – Gene expression analyses have provided important insight into the heterogeneity among kidney cancer and within the clear cell subtype. Some groups have proposed a new classification of clear cell kidney cancer using gene expression analysis. Brannon et al. demonstrated that two or more molecular sub-classifications of ccRCC exist and identified two distinct subsets, ccA and ccB, as well as a third divergent group characterized by wild-type VHL and a clear-cell papillary histology. ***True or False:******Molecular sub-classifications are important because they have prognostic implications.***

1. True
2. False

***Second Reply Question*** Universal guidelines for molecular testing in renal cell carcinoma have not yet been established. Current techniques broadly used include histologic review and immunohistochemical staining for hypoxia responsive proteins such as carbonic anhydrase IX, GLUT, and HIF-1α.This analysis alone is limited and quickly becoming arcane given the recent discoveries of a number of molecular targets. The authors propose the use of an “RCC chip” that includes most recurrently mutated genes in this disease. ***Which of the following genes is unlikely to be included in this chip?***

1. VHL
2. PBRM1
3. BAP1
4. BRCA1