3

The VHL-HIF Signaling in Renal Cell Carcinoma: Promises and Pitfalls

Christudas Morais[1*], David W. Johnson[1,2] and Glenda C. Gobe[1]
[1]*Centre for Kidney Disease Research, School of Medicine, The University of Queensland,*
[2]*Department of Renal Medicine, The University of Queensland at Princess Alexandra Hospital, Brisbane, Australia*

1. Introduction

Renal cell carcinoma (RCC) is the third most common genitourinary cancer behind prostate and bladder cancer, accounting for 3% of all adult malignancies (Curti, 2004). It is a highly metastatic and heterogeneous disease with at least 16 histologic subtypes (Eble et al., 2001; Lopez-Beltran et al., 2006), among which clear cell (70-80%), papillary (10-15%) and chromophobe (5%) are the most common (Curti, 2004). Up to 25% of patients with RCC have distant metastases at presentation. Another 50% develop metastases or local recurrence during follow-up, despite treatment of the primary tumor (Thyavihally et al., 2005). The average survival, following metastatic RCC, is about 4 months, and only 10% of patients survive for one year. The global incidence of RCC per year is close to 300000, with a male to female ratio of 3:2 and an estimated mortality of approximately 100000 (Arai&Kanai, 2010; Ferlay et al., 2010). The incidence of RCC has been rising steadily, probably because of incidental findings from imaging techniques performed for other reasons. It can occur at any age, but is most frequently diagnosed in the 40-70 year old group (Eble et al., 2001; Pascual & Borque, 2008; Arai&Kanai, 2010).

Well before the advent of the modern era of genetics and molecular biology, surgeons and pathologists were aware of the hyper-vascular nature of RCC (Corn, 2007). The subsequent isolation of the von Hippel-Lindau (VHL) gene in 1993 led to the important discoveries that aberrant VHL is the most important risk for RCC and that VHL negatively regulates the hypoxia inducible factor (HIF) and thus the downstream angiogenesis pathway thereby engendering increased vascularity. In this chapter, we will focus on the role of the VHL-HIF pathway in RCC, advancements in novel therapeutics targeting this pathway and future directions.

2. Von Hippel-Lindau syndrome

VHL disease, commonly known as the VHL syndrome, is named after the German ophthalmologist Eugene von Hippel, and the Swedish neuropathologist Arvid Lindau, who

* Corresponding Author

in the early 1900s, described highly vascularised tumors of the retina and the central nervous system (Kim&Kaelin, 2004; Ohh, 2006). VHL syndrome is the result of germ line mutations in VHL, a tumor-suppressor gene located on chromosome 3p25 (Seizinger et al., 1988; Kim&Kaelin, 2004; Clark, 2009; Gossage&Eisen, 2010). It affects 1 in 35,000 individuals and is associated with the development of tumors in multiple organs, including the brain, spinal cord, pancreas, adrenal gland, epididymis (in males), broad ligament (in women) and kidneys (Lonser et al., 2003; Kim&Kaelin, 2004; Grubb et al., 2005; Clark, 2009). Individuals with VHL syndrome carry one wild type VHL allele and one faulty allele. Tumors develop only after the spontaneous somatic loss (loss of heterzygosity [LoH]) or inactivation, or both, of the remaining wild type. Thus, at cellular level, VHL syndrome can be considered as an autosomal recessive disease, whilst clinically, it manifests more like an autosomal dominant disease because the inactivation of the wild type allele is almost guaranteed (Ohh&Kaelin, 2003; Ohh, 2006). Apart from LoH and mutations, hypermethylation of the VHL promoter regions can prevent the wild-type VHL gene from expressing its functional tumor suppressor protein pVHL (Kim&Kaelin, 2004). The VHL gene is mutated in 50-80% (Weiss&Lin, 2006; Arai&Kanai, 2010) and hypermethylated in 19% (Herman et al., 1994; Arai&Kanai, 2010) of sporadic clear cell RCC. Clear cell RCC is the leading cause of death in patients with VHL mutation (Maher et al., 1990; Clark, 2009; Gossage&Eisen, 2010).

The VHL gene, which was isolated in 1993 (Latif et al., 1993), has three exons and encodes for two mRNA transcripts that are translated into three types of pVHL (Ohh&Kaelin, 2003; Ohh, 2006). The first VHL mRNA transcript that contains all three exons, is translated into a larger 213 amino acid protein of approximately 24-30 kDa ($pVHL_{30}$), and a smaller 160 amino acid protein of approximately 18-19 kDa ($pVHL_{19}$) due to alternative translation initiation (Iliopoulos et al., 1998; Schoenfeld et al., 1998; Blankenship et al., 1999; Safran&Kaelin, 2003). In the early days, both $pVHL_{30}$ and $pVHL_{19}$ were considered to be tumor suppressors (Iliopoulos et al., 1995; Ohh, 2006). However, further studies have cast doubt on the tumor suppressive role of $pVHL_{19}$ (Ohh, 2006). Generally, the term 'pVHL' is used to refer to both isoforms (Kim&Kaelin, 2004; Kaelin, 2007). The second VHL mRNA transcript lacks exon 2 due to alternative splicing. Literature on this isoform is scant, although RCCs that exclusively produce this shorter VHL mRNA transcript have been identified (Gnarra et al., 1996; Safran&Kaelin, 2003; Ohh, 2006).

pVHL consists of α and β domains (Fig.1), which are essential for its tumor suppressor activities (Stebbins et al., 1999; Kaelin, 2002). pVHL forms complexes with elonginB, elonginC, Rbx1, and Cullin 2 to form an E3 ubiquitin ligase complex (Stebbins et al., 1999; Ohh et al., 2000; Tanimoto et al., 2000; Kaelin, 2002; Kaelin, 2005a; Kaelin, 2005b; Gossage&Eisen, 2010). The α domain binds to elongin C and the β domain interacts with hydroxylated prolines of HIF (Fig.1). This complex has multiple functions, which can be broadly classified into HIF-independent and HIF-dependent. The HIF-independent functions include maintenance of primary cilia (Hergovich et al., 2003; Baldewijns et al., 2010), assembly of extracellular fibronectin matrix (Guo et al., 2009), regulation of apoptosis (Guo et al., 2009), epithelial mesenchymal transition (EMT) (Koochekpour et al., 1999; Hara et al., 2006), and transcriptional regulation (Yuen et al., 2007; Mikhaylova et al., 2008; Baldewijns et al., 2010). The best studied role is its HIF-dependent function in RCC, which is the degradation of HIF (Ohh, 2006; Baldewijns et al., 2010; Gossage&Eisen, 2010).

3. Hypoxia-inducible factor

A hypoxic tumor microenvironment is one of the characteristics of solid tumors. Cells undergo a variety of adaptive changes that will facilitate their survival under hypoxic conditions. One such change at the molecular level is the activation of the hypoxia-sensitive transcription factor, HIF. HIF is a heterodimer consisting of two subunits: α and β (Wang et al., 1995; Kaluz et al., 2008). The β subunit, also known as aryl hydrocarbon receptor nuclear translocator (ARNT), is constitutively expressed and is independent of intracellular oxygen tension. The oxygen-sensitive α subunit, which has three further subunits, HIF-1α, HIF-2α, and HIF-3α, is also constitutively expressed, but is rapidly degraded under normoxic conditions (Semenza, 1999; Huang&Bunn, 2003; Maynard et al., 2003; Maynard&Ohh, 2004; Kaluz et al., 2008; Baldewijns et al., 2010). At least two mechanisms that negatively regulate the stability of HIF-α under normoxia have been recognized: oxygen-dependent prolyl hydroxylation and asparaginyl hydroxylation (Lando et al., 2002a; Lando et al., 2002b; Ohh, 2006).

HIF-1α and HIF-2α contain an N-terminal transactivation domain (NTAD), a C-terminal transactivation domain (CTAD) and an oxygen-dependent degradation domain (ODDD) [Fig.1] (Pugh et al., 1997; Sang et al., 2002; Baldewijns et al., 2010). The ODDD has a stretch of proline residues. HIF-3α lacks the transactivation domain and has many splice variants. Under normoxic conditions, the proline residues within the ODDD of HIF-α are hydroxylated by prolyl hydroxylases (PH) at positions 402 and 564 (Ivan et al., 2001; Jaakkola et al., 2001; Schofield&Ratcliffe, 2005; Koivunen et al., 2007; Kaluz et al., 2008; Baldewijns et al., 2010). The pVHL-E3 ubiquitin ligase complex binds to the hydroxylated HIF-α through the β domain of pVHL and enables polyubiquitination. The polyubiquitinated HIF-α is degraded by the 26S proteasome (Maxwell et al., 1999; Ohh et al., 2000; Tanimoto et al., 2000; Jaakkola et al., 2001; Baldewijns et al., 2010). While the prolyl hydroxylation enables the binding of pVHL to HIF-α and its eventual degradation, the asparaginyl hydroxylation prevents the transcriptional activation of HIF target genes (Dames et al., 2002; Freedman et al., 2002). For transcriptional activity, HIF-α requires the recruitment of p300/CBP transcriptional coactivators (Arany et al., 1996). The CTAD has a conserved C-terminal asparagine. In normoxia, the factor inhibiting HIF-1 (FIH-1), an oxygenase, hydroxylates asparagine at position 803, which diminishes the recruitment of p300/CBP to HIF-α (Freedman et al., 2002) leading to the transcriptional downregulation of HIF responsive elements (Mahon et al., 2001; Dames et al., 2002; Lando et al., 2002a; Lando et al., 2002b).

In hypoxia, the activity of prolyl hydroxylases and FIH-1 are reduced, leading to the inhibition of proline and asparginyl hydroxylation, respectively. In the absence of a functional pVHL, the binding of the pVHL-E3 ubiquitin ligase complex to HIF-α and the subsequent polyubiquitination and degradation of HIF-α are inhibited even under normoxic conditions. Both events lead to the stabilization and accumulation of HIF-α in cells. As a result, HIF-α is translocated to the nucleus, where it dimerizes with HIF-β. The HIF-α/HIF-β heterodimer binds to hypoxia-responsive elements (HRE) of the DNA, recruits p300/CBP to the CTAD and transactivates over 60 hypoxia-inducible genes of which, vascular endothelial growth factor (VEGF) (angiogenesis), transforming growth factor alpha (TGF-α) and epidermal growth factor receptor (EGFR) (proliferation), carbonic anhydrase IX (CAIX)

(pH regulation), erythropoietin (EPO) (erythropoiesis), e-cadherin (EMT) and glucose transporter-1 (GLUT-1) (glucose metabolism) have attracted much attention (Ohh, 2006; Clark, 2009; Baldewijns et al., 2010). Because of the defective VHL-HIF-VEGF pathway, RCC is considered to be one of the most hypervascularized tumors. Hence, it is not surprising that considerable research in the past decade has focused on the angiogenic pathway, which led to the development of some promising novel therapeutics.

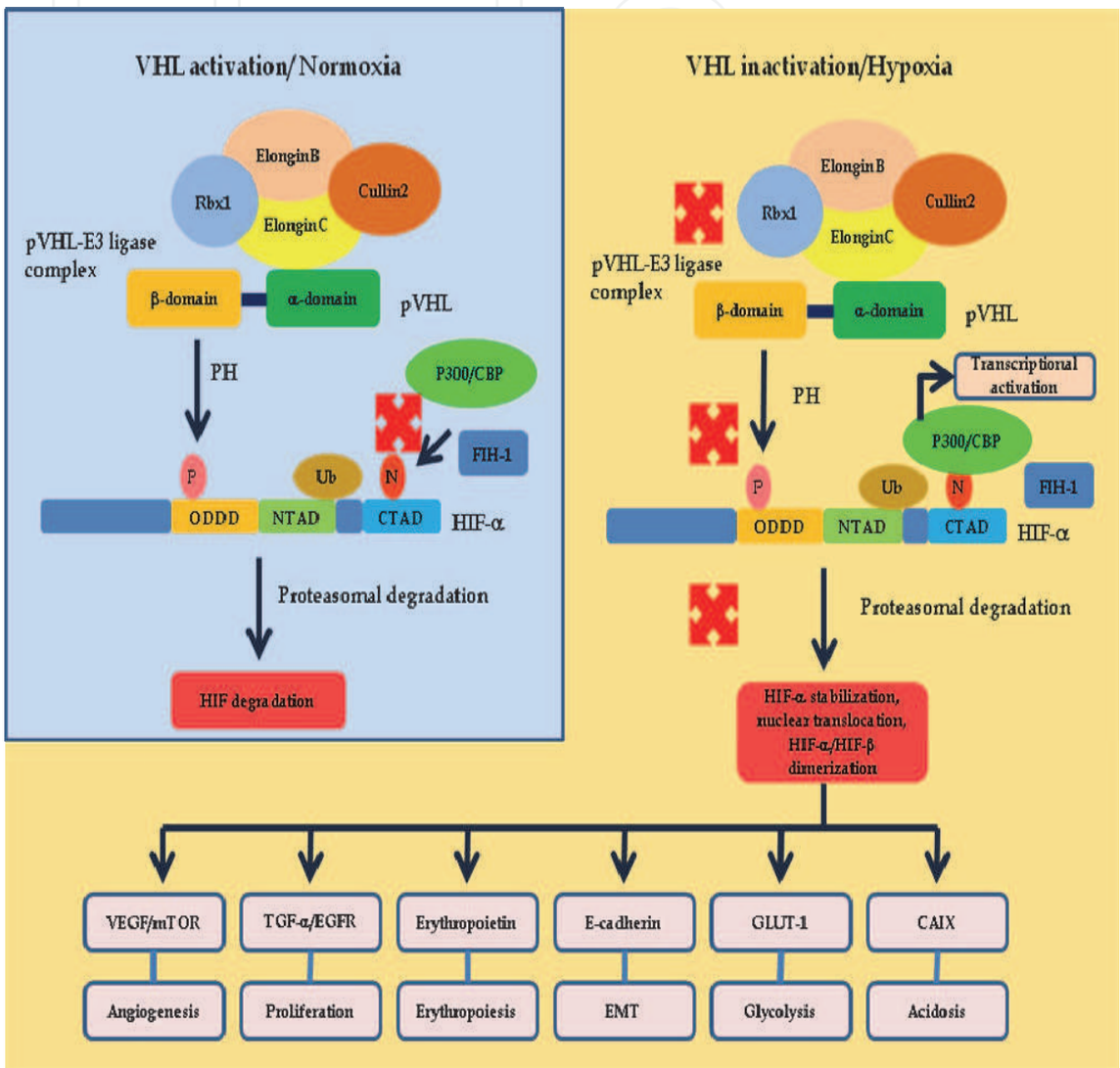


Fig. 1. The von Hippel-Lindau-Hypoxia Inducible Factor (VHL-HIF) pathway. The details are given in text. In brief, under active VHL and normoxic conditions, HIF is degraded. When the VHL gene is inactive due to mutations or hypermethylation, HIF is stabilized, translocated to the nucleus and activates the transcription of over 60 hypoxia-responsive molecules that are involved in oncogenesis and tumor progression. Only selected molecules and their alleged roles in tumor progression that are discussed in this chapter are shown. X, represents inhibition or inactivation. N, asparagine; P, proline; PH, prolyl hydroxylases; Ub, ubiquitination.

4. VHL inactivation and HIF-induced hypoxia responsive genes

4.1 Vascular endothelial growth factor – mammalian target of rapamycin

Of the many HIF-regulated angiogenic molecules, VEGF is perhaps the most studied - not only in RCC but also cancers in general. In humans, the VEGF system consists of five secreted ligands, namely VEGF-A, VEGF-B, VEGF-C, VEGF-D and placenta growth factor-1 (PlGF) and three receptor tyrosine kinases, VEGF-R1 (flt-1), VEGF-R2 (flk1 in the mouse, KDR in the human), and VEGF-R3 (flt4) (Carmeliet, 2005; Hicklin&Ellis, 2005; Tammela et al., 2005; Cebe-Suarez et al., 2006; Donovan&Kummar, 2006; Roy et al., 2006; Thurston&Kitajewski, 2008; Clark, 2009; Stuttfeld&Ballmer-Hofer, 2009; Bates, 2010). VEGF-R1 and VEGF-R2 are predominantly expressed in vascular endothelial cells and VEGF-R3 in lymphatic endothelial cells (Tammela et al., 2005; Thurston&Kitajewski, 2008). Hence, VEGF-R1 and VEGF-R2 are thought to be more important for angiogenesis and VEGF-R3 for lymphangiogenesis (Donovan&Kummar, 2006; Clark, 2009). The binding of the ligands to the receptors is an essential step in the initiation of the VEGF signaling. Generally, VEGF-R1 binds to VEGF-A, VEGF-B and PlGF; VEGF-R2 binds to VEGF-A, VEGF-C and VEGF-D and VEGF-R3 to VEGF-C and VEGF-D (Cebe-Suarez et al., 2006; Stuttfeld&Ballmer-Hofer, 2009). The receptor-ligand binding promotes conformational changes, followed by phosphorylation of specific tyrosine residues of the receptor (Cebe-Suarez et al., 2006; Stuttfeld&Ballmer-Hofer, 2009). Subsequently, a variety of downstream signaling pathways is activated, of which the most studied are the RAF-MEK-ERK series of kinases and the phosphatidylinositol-3 kinase-protein kinase B-mammalian target of rapamycin (PI3K-AKT-mTOR) pathway (Clark, 2009; Banumathy&Cairns, 2010). Increased mTOR, which itself is the result of HIF activation secondary to VHL loss, can in turn lead to an increase in HIF thereby maintaining a positive feedback loop that exacerbates the deleterious effects of VHL inactivation (Cho et al., 2007; Clark, 2009; Banumathy&Cairns, 2010). Both pathways predominantly up-regulate a variety of pro-angiogenic factors, thereby imparting a hyper-angiogenic phenotype to RCC.

There is considerable evidence demonstrating that VEGF is significantly elevated in RCC, with some studies reporting its up-regulation in up to 96% of RCC (Takahashi et al., 1994). There is a direct correlation between VEGF expression, tumor microvascular density, disease progression and metastasis of RCC (Takahashi et al., 1994; Zhang et al., 2002; Fukata et al., 2005; Baldewijns et al., 2007; Rioux-Leclercq et al., 2007; Patard et al., 2009). Apart from VEGF, RCC is reported to over-express several potent pro-angiogenic factors, including basic fibroblast growth factor (bFGF) (Cenni et al., 2007), PlGF (Takahashi et al., 1994), platelet-derived growth factor (PDGF) (Xu et al., 2005a), epidermal growth factor (EGF) (Kedar et al., 2002), the interleukins IL-6 (Angelo et al., 2002) and IL-8 (Konig et al., 1999), leptin (Horiguchi et al., 2006b; Horiguchi et al., 2006a) and various chemokines (Slaton et al., 2001; Fukata et al., 2005).

Hence, it is not surprising that the past decade has placed much emphasis on HIF-regulated downstream pathways, especially angiogenesis. This has resulted in the development of many clinically available novel chemotherapeutic agents. They can be broadly categorized as VEGF inhibitors, multiple tyrosine kinase inhibitors and mTOR inhibitors. The most successful VEGF inhibitor is the humanized VEGF-neutralizing antibody, bevacizumab. Bevacizumab is thought to exert its anti-angiogenic activity by acting against the angiogenic endothelial cells surrounding the tumor rather than the tumor *per se* thus blocking the

supply of oxygen and nutrients to the tumors (Ellis&Hicklin, 2008; Ainsworth et al., 2009; Banumathy&Cairns, 2010). The clinically available multiple tyrosine kinase inhibitors are sunitinib, sorafenib, pazopanib and axitinib. As summarized in Table 1, they target multiple tyrosine kinase receptors neutralizing the downstream signaling pathways activated by ligand-receptor binding, as discussed above. Two of the most successful mTOR inhibitors are temsirolimus and everolimus. Both are rapamycin analogues and bind to FK506-binding protein 12 (FKBP12), which in turn binds to mTOR leading to the inhibition of the PI3K/Akt/mTOR pathway (Thomas et al., 2006; Abraham&Gibbons, 2007; Ainsworth et al., 2009; Banumathy&Cairns, 2010). In addition, temsirolimus has been shown to have a direct inhibitory effect on HIF-α and VEGF (Del Bufalo et al., 2006; Motzer et al., 2008; Banumathy&Cairns, 2010; Houghton, 2010).

Drug	Target receptors	Reference
Sunitinib	VEGFR1, VEGFR2, VEGFR3, PDGFR-α, PDGFR-β, FLT3, c-KIT, RET	(Mendel et al., 2003; Faivre et al., 2006; Ainsworth et al., 2009; Banumathy&Cairns, 2010)
Sorafenib	VEGFR-2, PDGFR-β, c-KIT, Raf-1	(Wilhelm&Chien, 2002; Wilhelm et al., 2004; Ainsworth et al., 2009)
Pazopanib	VEGFR1, VEGFR2, VEGFR3, PDGFR-α, PDGFR-β, c-KIT	(Kumar et al., 2007; Ainsworth et al., 2009; Banumathy&Cairns, 2010; Sternberg et al., 2010)
Axitinib	VEGFR1, VEGFR2, VEGFR3, PDGFR-β, c-KIT,	(Rugo et al., 2005; Rixe et al., 2007)

Table 1. Clinically available multiple tyrosine kinase inhibitors for metastatic RCC (Bullock et al., 2010)

4.2 Transforming growth factor-α and epidermal growth factor receptor

TGF-α is an autocrine mitogen for fibroblasts and epithelial cells. It has structural and functional homology to EGF (Derynck, 1986; Everitt et al., 1997). TGF-α is thought to mediate its biological effects through the binding of EGFR, which is a family of four closely related cell membrane receptors, EGFR (HER1; ErbB1), HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4) (Higashiyama et al., 2008; Pu et al., 2009). These receptors are transmembrane glycoproteins with an extracellular ligand-binding domain and an intracellular tyrosine kinase domain (Higashiyama et al., 2008; Pu et al., 2009). Ligand-binding to the extracellular domain of EGFR activates tyrosine kinase, resulting in autophosphorylation of EGFR and subsequent signal transduction leading to cell cycle progression, inhibition of apoptosis, induction of angiogenesis, promotion of invasion and metastasis, and other oncogenic activities (Derynck, 1986; Everitt et al., 1997; Gunaratnam et al., 2003; Black&Dinney, 2008; Lee et al., 2008; Uberall et al., 2008; Pu et al., 2009). TGF-α/EGFR activation in RCC is either independent of, or dependent on the VHL status. While the mechanisms behind VHL-independent activation have not been fully elucidated, the VHL-dependent TGF-α/EGFR follows the classical pattern of HIF-1 up-regulation secondary to loss of VHL function (de

Paulsen et al., 2001; Gunaratnam et al., 2003; Lee et al., 2008). It has been suggested that TGF-α overproduction secondary to VHL deficiency is the consequence of HIF activation and that such activation establishes an autocrine TGF-α/EGFR stimulatory system leading to the oncogenic transformation of normal renal epithelial cells and the formation of RCC (Ishikawa et al., 1990; Atlas et al., 1992; Yoshida et al., 1994; Everitt et al., 1997; de Paulsen et al., 2001; Gunaratnam et al., 2003; Pelletier et al., 2009; Pu et al., 2009). For example, transgenic expression of TGF-α in mice leads to the formation of multiple renal cysts reminiscent of pre-neoplastic lesions of the human kidney, whilst the re-introduction of wild type VHL inhibits TGF-α expression and cyst formation (Lowden et al., 1994; Everitt et al., 1997; Knebelmann et al., 1998; de Paulsen et al., 2001; Kaelin, 2002; Gunaratnam et al., 2003).

Many studies have reported the over expression of TGF-α and EGFR in RCC (Freeman et al., 1989; Gomella et al., 1989; Mydlo et al., 1989; Sargent et al., 1989; Petrides et al., 1990; Lager et al., 1994; Yoshida et al., 1994; Uhlman et al., 1995; Everitt et al., 1997; Yoshida et al., 1997; Ramp et al., 2000; Merseburger et al., 2005; Lee et al., 2008; Pelletier et al., 2009; Pu et al., 2009). The expression pattern of TGF-α in RCC is such that it has been identified as an ideal candidate for immunotherapy (Pelletier et al., 2009). The prognostic association between over-expression of EGFR in RCC, and development of the cancer is controversial. Two distinct patterns of EGFR expression have been observed: a) cytoplasmic in normal renal cells, and b) membranous in RCC. These observations suggest that it is not just the over expression of EGFR, but the location of its over-expression that determines prognosis. Accordingly, RCC patients with positive membranous EGFR expression may have a poorer survival outcome when compared with those with negative expression of membranous EGFR (Pu et al., 2009). However, a previous study casts doubt on this hypothesis (Kallio et al., 2003).

Some studies have explored the therapeutic efficiency of EGFR inhibitors, either as single agents or in combination, with a success rate of around 10% (Motzer et al., 2003; Dawson et al., 2004; Rowinsky et al., 2004; Jermann et al., 2006; Ravaud et al., 2008; Gordon et al., 2009; Pu et al., 2009). Interestingly however, results of anti-EGFR therapy in other cancers show a lack of correlation between EGFR expression and response to therapy, suggesting that differential intracellular signaling events rather than the mere expression of EGFR may also play significant roles in predicting response to anti-EGFR therapy (Marks et al., 2008; Gordon et al., 2009). Furthermore, RCC cells with wild type VHL have been shown to be more sensitive to anti-EGFR treatment than those with mutated VHL (Perera et al., 2000; Gordon et al., 2009).

4.3 Erythropoietin

Paraneoplastic erythrocytosis is a salient feature of many cancers, including RCC in which about 5% of patients are polycythemic (Wiesener et al., 2002; Wiesener et al., 2007; Papworth et al., 2009). In adults, the kidney is the major source of EPO. EPO regulates erythropoiesis and is the only hematopoietic cytokine whose production is regulated by hypoxia (Lacombe&Mayeux, 1999; Fandrey, 2004; Michael et al., 2007; Wiesener et al., 2007). Although RCC is thought to arise from proximal tubular epithelial cells (PTEC), normal PTEC do not express detectable levels of EPO even under hypoxic conditions (Wiesener et al., 2002; Wiesener et al., 2007). This pointed towards the role of molecular mechanisms that transform a non-EPO expressing PTEC to an EPO-expressing RCC , leading to the

identification of the link between VHL mutation and the subsequent HIF-mediated EPO upregulation in RCC (Da Silva et al., 1990; Noguchi et al., 1999; Wiesener et al., 2002; Pastore et al., 2003; Lee et al., 2005; Wiesener et al., 2007; Rad et al., 2008; Papworth et al., 2009). Enhanced expression of EPO and its receptor EPOR has been reported in cultured RCC cells and in patient samples, the reported prevalence ranges from 33 - 84% (Murphy et al., 1970; Hagiwara et al., 1984; Da Silva et al., 1990; Ljungberg et al., 1992; Noguchi et al., 1999; Wiesener et al., 2002; Sakamoto et al., 2003; Lee et al., 2005; Michael et al., 2007; Wiesener et al., 2007; Papworth et al., 2009).

Although 33-84% of the RCC patients have elevated plasma level of EPO, only 5% of them are polycythemic (Kazal&Erslev, 1975; Ljungberg et al., 1992; Michael et al., 2007; Wiesener et al., 2007; Papworth et al., 2009). The lack of correlation between paraneoplastic EPO production and paraneoplastic erythrocytosis has led to the suggestion that other cancer-related factors can counterbalance the effect of increased EPO. One of the well studied factors is the reduced iron availability and the resultant anemia in cancer patients. Since recombinant human EPO (rhEPO) is frequently used to treat anemia in patients with cancer, the presence of EPOR in RCC has raised concern (Lai&Grandis, 2006; Papworth et al., 2009). As EPOR is over-expressed in RCC, and EPO exerts its effects through its binding of EPOR, exogenous rhEPO could enhance tumor aggressiveness as reported in breast and head and neck cancers (Henke et al., 2003; Leyland-Jones et al., 2005; Wiesener et al., 2007). Furthermore, EPO has been shown to reduce cisplatin-induced apoptosis in cultured RCC cells (Li et al., 2007). In contrast, EPO sensitized RCC cell lines to vinblastin and daunorubicin-induced apoptosis (Carvalho et al., 2005).

4.4 E-cadherin

Cell-cell adherence, mediated by intercellular junctional complexes, plays an important role in tissue formation and maintenance of cell phenotypes. Intercellular junctional complexes are composed of tight junctions, adherence junctions and desmosomes (Cavallaro & Christofori, 2004; Krishnamachary et al., 2006; Migita et al., 2008). E-cadherin, a transmembrane glycoprotein, is the principal component of adherence junctions and desmosomes in epithelial cells (Cavallaro&Christofori, 2004; Krishnamachary et al., 2006). The extracellular domain of E-cadherin mediates cell-cell adherence through homophilic interaction with the E-cadherin of adjacent cells, whereas the intracellular domain binds with the cytoskeleton *via* a protein complex containing α-, ß- and γ -catenins (Behrens et al., 1989; Cavallaro&Christofori, 2004; Krishnamachary et al., 2006; Russell&Ohh, 2007). EMT is the hallmark of many cancers, in which E-cadherin-mediated cell-cell adherence is lost and the cells attain hyper-proliferative, invasive and metastatic properties (Behrens et al., 1989; Thiery, 2002; Krishnamachary et al., 2006). While the reasons for loss of E-cadherin in cancers are undoubtedly multifactoral, many studies have shown a direct role of VHL in regulating E-cadherin expression in RCC (Esteban et al., 2006; Krishnamachary et al., 2006; Evans et al., 2007; Russell&Ohh, 2007).

The loss of VHL in turn leads to a loss or significant downregulation of E-cadherin and its re-introduction restores E-cadherin expression in RCC (Esteban et al., 2006; Krishnamachary et al., 2006; Russell&Ohh, 2007). Further reports of E-cadherin loss in pre-malignant foci of VHL patients has led to the suggestion that E-cadherin loss is an early step in the pathogenesis of RCC, attributed to VHL inactivation (Esteban et al., 2006; Russell&Ohh,

2007). In line with these observations, tissue microarray of human RCC samples has shown the lack of E-cadherin immunoreactivity in VHL-deficient samples (Gervais et al., 2007). This study also found that low Fuhrman grade samples were positive for E-cadherin and VHL immunostaining and had a better prognosis. Conversely, high-grade tumors were negative for E-cadherin and VHL immunoreactivity and had a worse prognosis. However, these observations have been contradicted by another report, which found no association between E-cadherin expression, tumor grade and prognosis in RCC (Ronkainen et al., 2010).

4.5 Glucose transporter-1

Glucose is the major substrate for energy production in mammalian cells. The intracellular transport of glucose and its oxidative metabolism are vital for normal functioning of cells. Intracellular glucose transport is facilitated by a family of 14 glucose transporters (GLUT), of which GLUT-1 is the most studied (Macheda et al., 2005; Ozcan et al., 2007; Suganuma et al., 2007; Lidgren et al., 2008). Due to rapid proliferative rate relative to vascular support of cancer cells, the tumor microenvironment is in a constant state of hypoxia (Ozcan et al., 2007). In order to counteract the deleterious effects of hypoxia, such as apoptosis and necrosis, malignant cells undergo adaptive and genetic changes. One such change is increased uptake of glucose when compared with normal cells through accelerated glycolysis – often referred to as 'glycolytic switch' or 'Warburg effect' (Warburg, 1956; Airley&Mobasheri, 2007; Singer et al., 2011). This is largely mediated by the up-regulation of GLUT especially GLUT-1 (Binder et al., 1997; Smith, 1999; Medina&Owen, 2002; Airley&Mobasheri, 2007; Ozcan et al., 2007; Suganuma et al., 2007). It is now well established that accelerated glycolysis and increased glucose uptake mediated by GLUT-1 are the hallmarks of many malignant tumors and that these adaptive changes in glucose metabolism favor survival, proliferation and metastasis of tumor cells, even under hypoxic conditions (Airley&Mobasheri, 2007; Ozcan et al., 2007; Singer et al., 2011).

The link between VHL-HIF and GLUT-1 has been well established. GLUT-1 is over-expressed in VHL-mutated mice and the GLUT-1 promoter region has an HRE (Ebert et al., 1995; Park et al., 2007; Lidgren et al., 2008). While the basal level of expression of GLUT-1 in normal PTEC is a subject of debate (Nagase et al., 1995; Ozcan et al., 2007), the over-expression of GLUT-1 in RCC has been demonstrated by many studies (Nagase et al., 1995; Miyakita et al., 2002; Ozcan et al., 2007; Suganuma et al., 2007; Lidgren et al., 2008; Singer et al., 2011). All of these studies concluded that GLUT-1 is over-expressed markedly in clear cell RCC compared with other RCC subtypes, confirming the link between VHL aberration and the subsequent HIF up-regulation. Lactate is one of the byproducts of glycolysis. Due to the enhanced glycolysis, RCC patients are reported to have increased glycolytic enzymes and higher levels of lactate in their serum (Gao et al., 2008). Increased dependence of cancer cells on glycolysis suggests that glycolysis inhibition may be a therapeutic option. Although not well-documented in RCC, experimental glycolysis inhibition has produced anti-cancer effects in certain cancer cell lines (Xu et al., 2005b; Pelicano et al., 2006).

4.6 Carbonic anhydrase IX

CAIX is a membrane-bound glycoprotein belonging to the carbonic anhydrase (CA) family of enzymes and is thought to promote cell proliferation, oncogenesis and tumor progression

in response to hypoxia (Grabmaier et al., 2000; Ivanov et al., 2001; Bui et al., 2003; Lawrentschuk et al., 2011). There are at least 15 isoforms of CA. These enzymes play an important role in the regulation of pH of cells by catalyzing one of the vital reactions of biological systems - the reversible hydration of CO_2 into bicarbonate and hydrogen ions, $CO_2+H_2O \Leftrightarrow HCO_3^- + H^+$ (Opavsky et al., 1996; Pastorekova et al., 2008; Stillebroer et al., 2010). As discussed above, enhanced glycolysis is a hallmark of cancer cells, which results in accumulation of lactate and a hypoxic acidic microenvironment. CO_2 is a significant source of hypoxia and acidity in tumors (Pastorekova et al., 2008). Enhanced CAIX expression and the resultant rapid reversible conversion of CO_2 to H^+ helps to maintain an acidic, hypoxic microenvironment thereby sustaining tumor progression (Opavsky et al., 1996; Dorai et al., 2006; Pastorekova et al., 2008; Patard et al., 2008; Stillebroer et al., 2010). CAIX is regulated by HIF and the correlation between VHL-HIF-CAIX is well-established (Wykoff et al., 2000; Grabmaier et al., 2004; Patard et al., 2008; Kaluz et al., 2009; Lawrentschuk et al., 2011). Enhanced expression of CAIX has been well documented in RCC (Bui et al., 2003; Zavada et al., 2003; Atkins et al., 2005; Al-Ahmadie et al., 2008; Jensen et al., 2008; Li et al., 2008; Patard et al., 2008; Patard et al., 2009; Stillebroer et al., 2010; Zhou et al., 2010). However, in contrast to other cancers, enhanced CAIX in RCC is associated with a better prognosis. Furthermore, metastatic patients with higher tumoral CAIX expression showed a better response to interleukin-2 treatment (Atkins et al., 2005). Based on the VHL-CAIX status of RCC, Patard and colleagues (Patard et al., 2008) stratified RCC patients into three distinct groups: patients with both VHL mutation and high CAIX expression had the most favourable prognosis; patients with either VHL mutation or high CAIX expression had an intermediate prognosis; and patients with neither VHL mutation nor high CAIX expression had the worst prognosis.

5. Promises, pitfalls and future directions

The past decade has witnessed an unprecedented increase in the understanding of molecular mechanisms of RCC, of which, the VHL-HIF pathway arguably is the most explored. Not surprisingly, this has resulted in promising, clinically available novel therapeutic agents. Patients were reported to have progression-free survival with these novel therapeutic agents, and many new drugs are under clinical trial, offering hope for better treatment strategies in the future. However, progression-free survival, which occurs in approximately 10% of selected patients, is generally measured in months, rather than years. Moreover, there is currently no compound, either singly or in combination, which is capable of producing a complete response in metastatic RCC. Systemic toxicity and resistance of RCC in response to currently available VEGF/mTOR inhibitors are starting to emerge. Also, the rising costs of therapy associated with these novel drugs are another barrier to effective treatment. These highlight the need for more effective therapeutic agents for metastatic RCC. One approach would be simultaneous targeting of multiple molecular pathways. Pertinent to the molecular mechanism of VHL-HIF, there are at least 60 HIF-regulated molecules that are engaged in tumor development and progression. This means that targeting the VEGF/mTOR pathway could be potentially abrogated by the opposing actions of numerous molecules favoring tumor progression. Therefore, given the heterogeneity of RCC, individualized, targeted treatment based on a preceding 'molecular map' of each tumor might represent an innovative therapeutic approach to achieving improved clinical outcomes. With the rapid advancements in technology, especially

microarrays and bioinformatics, the availability of clinically feasible platforms to generate molecular maps of individual tumors and customized treatment strategies may become a reality in the near future.

6. References

Abraham, R.T. & Gibbons, J.J. (2007). The mammalian target of rapamycin signaling pathway: twists and turns in the road to cancer therapy. *Clin Cancer Res* Vol.13, No.11, (Jun 1), pp.3109-14

Ainsworth, N.L., Lee, J.S. & Eisen, T. (2009). Impact of anti-angiogenic treatments on metastatic renal cell carcinoma. *Expert Rev Anticancer Ther* Vol.9, No.12, (Dec), pp.1793-805

Airley, R.E. & Mobasheri, A. (2007). Hypoxic regulation of glucose transport, anaerobic metabolism and angiogenesis in cancer: novel pathways and targets for anticancer therapeutics. *Chemotherapy* Vol.53, No.4, pp.233-56

Al-Ahmadie, H.A., Alden, D., Qin, L.X., Olgac, S., Fine, S.W., Gopalan, A., Russo, P., Motzer, R.J., Reuter, V.E. & Tickoo, S.K. (2008). Carbonic anhydrase IX expression in clear cell renal cell carcinoma: an immunohistochemical study comparing 2 antibodies. *Am J Surg Pathol* Vol.32, No.3, (Mar), pp.377-82

Angelo, L.S., Talpaz, M. & Kurzrock, R. (2002). Autocrine interleukin-6 production in renal cell carcinoma: evidence for the involvement of p53. *Cancer Res* Vol.62, No.3, (Feb 1), pp.932-40

Arai, E. & Kanai, Y. (2010). Genetic and epigenetic alterations during renal carcinogenesis. *Int J Clin Exp Pathol* Vol.4, No.1, pp.58-73

Arany, Z., Huang, L.E., Eckner, R., Bhattacharya, S., Jiang, C., Goldberg, M.A., Bunn, H.F. & Livingston, D.M. (1996). An essential role for p300/CBP in the cellular response to hypoxia. *Proc Natl Acad Sci U S A* Vol.93, No.23, (Nov 12), pp.12969-73

Atkins, M., Regan, M., McDermott, D., Mier, J., Stanbridge, E., Youmans, A., Febbo, P., Upton, M., Lechpammer, M. & Signoretti, S. (2005). Carbonic anhydrase IX expression predicts outcome of interleukin 2 therapy for renal cancer. *Clin Cancer Res* Vol.11, No.10, (May 15), pp.3714-21

Atlas, I., Mendelsohn, J., Baselga, J., Fair, W.R., Masui, H. & Kumar, R. (1992). Growth regulation of human renal carcinoma cells: role of transforming growth factor alpha. *Cancer Res* Vol.52, No.12, (Jun 15), pp.3335-9

Baldewijns, M.M., Thijssen, V.L., Van den Eynden, G.G., Van Laere, S.J., Bluekens, A.M., Roskams, T., van Poppel, H., De Bruine, A.P., Griffioen, A.W. & Vermeulen, P.B. (2007). High-grade clear cell renal cell carcinoma has a higher angiogenic activity than low-grade renal cell carcinoma based on histomorphological quantification and qRT-PCR mRNA expression profile. *Br J Cancer* Vol.96, No.12, (Jun 18), pp.1888-95

Baldewijns, M.M., van Vlodrop, I.J., Vermeulen, P.B., Soetekouw, P.M., van Engeland, M. & de Bruine, A.P. (2010). VHL and HIF signalling in renal cell carcinogenesis. *J Pathol* Vol.221, No.2, (Jun), pp.125-38

Banumathy, G. & Cairns, P. (2010). Signaling pathways in renal cell carcinoma. *Cancer Biol Ther* Vol.10, No.7, (Oct), pp.658-64

Bates, D.O. (2010). Vascular endothelial growth factors and vascular permeability. *Cardiovasc Res* Vol.87, No.2, (Jul 15), pp.262-71

Behrens, J., Mareel, M.M., Van Roy, F.M. & Birchmeier, W. (1989). Dissecting tumor cell invasion: epithelial cells acquire invasive properties after the loss of uvomorulin-mediated cell-cell adhesion. *J Cell Biol* Vol.108, No.6, (Jun), pp.2435-47

Binder, C., Binder, L., Marx, D., Schauer, A. & Hiddemann, W. (1997). Deregulated simultaneous expression of multiple glucose transporter isoforms in malignant cells and tissues. *Anticancer Res* Vol.17, No.6D, (Nov-Dec), pp.4299-304

Black, P.C. & Dinney, C.P. (2008). Growth factors and receptors as prognostic markers in urothelial carcinoma. *Curr Urol Rep* Vol.9, No.1, (Jan), pp.55-61

Blankenship, C., Naglich, J.G., Whaley, J.M., Seizinger, B. & Kley, N. (1999). Alternate choice of initiation codon produces a biologically active product of the von Hippel Lindau gene with tumor suppressor activity. *Oncogene* Vol.18, No.8, (Feb 25), pp.1529-35

Bui, M.H., Seligson, D., Han, K.R., Pantuck, A.J., Dorey, F.J., Huang, Y., Horvath, S., Leibovich, B.C., Chopra, S., Liao, S.Y., Stanbridge, E., Lerman, M.I., Palotie, A., Figlin, R.A. & Belldegrun, A.S. (2003). Carbonic anhydrase IX is an independent predictor of survival in advanced renal clear cell carcinoma: implications for prognosis and therapy. *Clin Cancer Res* Vol.9, No.2, (Feb), pp.802-11

Bullock, A., McDermott, D.F. & Atkins, M.B. (2010). Management of metastatic renal cell carcinoma in patients with poor prognosis. *Cancer Manag Res* Vol.2, pp.123-32

Carmeliet, P. (2005). VEGF as a key mediator of angiogenesis in cancer. *Oncology* Vol.69 Suppl 3, pp.4-10

Carvalho, G., Lefaucheur, C., Cherbonnier, C., Metivier, D., Chapel, A., Pallardy, M., Bourgeade, M.F., Charpentier, B., Hirsch, F. & Kroemer, G. (2005). Chemosensitization by erythropoietin through inhibition of the NF-kappaB rescue pathway. *Oncogene* Vol.24, No.5, (Jan 27), pp.737-45

Cavallaro, U. & Christofori, G. (2004). Cell adhesion and signalling by cadherins and Ig-CAMs in cancer. *Nat Rev Cancer* Vol.4, No.2, (Feb), pp.118-32

Cebe-Suarez, S., Zehnder-Fjallman, A. & Ballmer-Hofer, K. (2006). The role of VEGF receptors in angiogenesis; complex partnerships. *Cell Mol Life Sci* Vol.63, No.5, (Mar), pp.601-15

Cenni, E., Perut, F., Granchi, D., Avnet, S., Amato, I., Brandi, M.L., Giunti, A. & Baldini, N. (2007). Inhibition of angiogenesis via FGF-2 blockage in primitive and bone metastatic renal cell carcinoma. *Anticancer Res* Vol.27, No.1A, (Jan-Feb), pp.315-9

Cho, D., Signoretti, S., Regan, M., Mier, J.W. & Atkins, M.B. (2007). The role of mammalian target of rapamycin inhibitors in the treatment of advanced renal cancer. *Clin Cancer Res* Vol.13, No.2 Pt 2, (Jan 15), pp.758s-63s

Clark, P.E. (2009). The role of VHL in clear-cell renal cell carcinoma and its relation to targeted therapy. *Kidney Int* Vol.76, No.9, (Nov), pp.939-45

Corn, P.G. (2007). Role of the ubiquitin proteasome system in renal cell carcinoma. *BMC Biochem* Vol.8 Suppl 1, pp.S4

Curti, B.D. (2004). Renal cell carcinoma. *Jama* Vol.292, No.1, (Jul 7), pp.97-100

Da Silva, J.L., Lacombe, C., Bruneval, P., Casadevall, N., Leporrier, M., Camilleri, J.P., Bariety, J., Tambourin, P. & Varet, B. (1990). Tumor cells are the site of

erythropoietin synthesis in human renal cancers associated with polycythemia. *Blood* Vol.75, No.3, (Feb 1), pp.577-82

Dames, S.A., Martinez-Yamout, M., De Guzman, R.N., Dyson, H.J. & Wright, P.E. (2002). Structural basis for Hif-1 alpha /CBP recognition in the cellular hypoxic response. *Proc Natl Acad Sci U S A* Vol.99, No.8, (Apr 16), pp.5271-6

Dawson, N.A., Guo, C., Zak, R., Dorsey, B., Smoot, J., Wong, J. & Hussain, A. (2004). A phase II trial of gefitinib (Iressa, ZD1839) in stage IV and recurrent renal cell carcinoma. *Clin Cancer Res* Vol.10, No.23, (Dec 1), pp.7812-9

de Paulsen, N., Brychzy, A., Fournier, M.C., Klausner, R.D., Gnarra, J.R., Pause, A. & Lee, S. (2001). Role of transforming growth factor-alpha in von Hippel--Lindau (VHL)(-/-) clear cell renal carcinoma cell proliferation: a possible mechanism coupling VHL tumor suppressor inactivation and tumorigenesis. *Proc Natl Acad Sci U S A* Vol.98, No.4, (Feb 13), pp.1387-92

Del Bufalo, D., Ciuffreda, L., Trisciuoglio, D., Desideri, M., Cognetti, F., Zupi, G. & Milella, M. (2006). Antiangiogenic potential of the Mammalian target of rapamycin inhibitor temsirolimus. *Cancer Res* Vol.66, No.11, (Jun 1), pp.5549-54

Derynck, R. (1986). Transforming growth factor-alpha: structure and biological activities. *J Cell Biochem* Vol.32, No.4, pp.293-304

Donovan, E.A. & Kummar, S. (2006). Targeting VEGF in cancer therapy. *Curr Probl Cancer* Vol.30, No.1, (Jan-Feb), pp.7-32

Dorai, T., Sawczuk, I., Pastorek, J., Wiernik, P.H. & Dutcher, J.P. (2006). Role of carbonic anhydrases in the progression of renal cell carcinoma subtypes: proposal of a unified hypothesis. *Cancer Invest* Vol.24, No.8, (Dec), pp.754-79

Ebert, B.L., Firth, J.D. & Ratcliffe, P.J. (1995). Hypoxia and mitochondrial inhibitors regulate expression of glucose transporter-1 via distinct Cis-acting sequences. *J Biol Chem* Vol.270, No.49, (Dec 8), pp.29083-9

Eble, J., Sauter, G., Epstein, J. & Sesterhenn, I. (2001). *Pathology and genetics. Tumours of the urinary system and male genital organs,* (Editor ed.). Lyon: IARC Press.

Ellis, L.M. & Hicklin, D.J. (2008). VEGF-targeted therapy: mechanisms of anti-tumour activity. *Nat Rev Cancer* Vol.8, No.8, (Aug), pp.579-91

Esteban, M.A., Tran, M.G., Harten, S.K., Hill, P., Castellanos, M.C., Chandra, A., Raval, R., O'Brien T, S. & Maxwell, P.H. (2006). Regulation of E-cadherin expression by VHL and hypoxia-inducible factor. *Cancer Res* Vol.66, No.7, (Apr 1), pp.3567-75

Evans, A.J., Russell, R.C., Roche, O., Burry, T.N., Fish, J.E., Chow, V.W., Kim, W.Y., Saravanan, A., Maynard, M.A., Gervais, M.L., Sufan, R.I., Roberts, A.M., Wilson, L.A., Betten, M., Vandewalle, C., Berx, G., Marsden, P.A., Irwin, M.S., Teh, B.T., Jewett, M.A. & Ohh, M. (2007). VHL promotes E2 box-dependent E-cadherin transcription by HIF-mediated regulation of SIP1 and snail. *Mol Cell Biol* Vol.27, No.1, (Jan), pp.157-69

Everitt, J.I., Walker, C.L., Goldsworthy, T.W. & Wolf, D.C. (1997). Altered expression of transforming growth factor-alpha: an early event in renal cell carcinoma development. *Mol Carcinog* Vol.19, No.3, (Jul), pp.213-9

Faivre, S., Delbaldo, C., Vera, K., Robert, C., Lozahic, S., Lassau, N., Bello, C., Deprimo, S., Brega, N., Massimini, G., Armand, J.P., Scigalla, P. & Raymond, E. (2006). Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget

tyrosine kinase inhibitor, in patients with cancer. *J Clin Oncol* Vol.24, No.1, (Jan 1), pp.25-35

Fandrey, J. (2004). Oxygen-dependent and tissue-specific regulation of erythropoietin gene expression. *Am J Physiol Regul Integr Comp Physiol* Vol.286, No.6, (Jun), pp.R977-88

Ferlay, J., Shin, H.R., Bray, F., Forman, D., Mathers, C. & Parkin, D.M. (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* Vol.127, No.12, (Dec 15), pp.2893-917

Freedman, S.J., Sun, Z.Y., Poy, F., Kung, A.L., Livingston, D.M., Wagner, G. & Eck, M.J. (2002). Structural basis for recruitment of CBP/p300 by hypoxia-inducible factor-1 alpha. *Proc Natl Acad Sci U S A* Vol.99, No.8, (Apr 16), pp.5367-72

Freeman, M.R., Washecka, R. & Chung, L.W. (1989). Aberrant expression of epidermal growth factor receptor and HER-2 (erbB-2) messenger RNAs in human renal cancers. *Cancer Res* Vol.49, No.22, (Nov 15), pp.6221-5

Fukata, S., Inoue, K., Kamada, M., Kawada, C., Furihata, M., Ohtsuki, Y. & Shuin, T. (2005). Levels of angiogenesis and expression of angiogenesis-related genes are prognostic for organ-specific metastasis of renal cell carcinoma. *Cancer* Vol.103, No.5, (Mar 1), pp.931-42

Gao, H., Dong, B., Liu, X., Xuan, H., Huang, Y. & Lin, D. (2008). Metabonomic profiling of renal cell carcinoma: high-resolution proton nuclear magnetic resonance spectroscopy of human serum with multivariate data analysis. *Anal Chim Acta* Vol.624, No.2, (Aug 29), pp.269-77

Gervais, M.L., Henry, P.C., Saravanan, A., Burry, T.N., Gallie, B.L., Jewett, M.A., Hill, R.P., Evans, A.J. & Ohh, M. (2007). Nuclear E-cadherin and VHL immunoreactivity are prognostic indicators of clear-cell renal cell carcinoma. *Lab Invest* Vol.87, No.12, (Dec), pp.1252-64

Gnarra, J.R., Zhou, S., Merrill, M.J., Wagner, J.R., Krumm, A., Papavassiliou, E., Oldfield, E.H., Klausner, R.D. & Linehan, W.M. (1996). Post-transcriptional regulation of vascular endothelial growth factor mRNA by the product of the VHL tumor suppressor gene. *Proc Natl Acad Sci U S A* Vol.93, No.20, (Oct 1), pp.10589-94

Gomella, L.G., Sargent, E.R., Wade, T.P., Anglard, P., Linehan, W.M. & Kasid, A. (1989). Expression of transforming growth factor alpha in normal human adult kidney and enhanced expression of transforming growth factors alpha and beta 1 in renal cell carcinoma. *Cancer Res* Vol.49, No.24 Pt 1, (Dec 15), pp.6972-5

Gordon, M.S., Hussey, M., Nagle, R.B., Lara, P.N., Jr., Mack, P.C., Dutcher, J., Samlowski, W., Clark, J.I., Quinn, D.I., Pan, C.X. & Crawford, D. (2009). Phase II study of erlotinib in patients with locally advanced or metastatic papillary histology renal cell cancer: SWOG S0317. *J Clin Oncol* Vol.27, No.34, (Dec 1), pp.5788-93

Gossage, L. & Eisen, T. (2010). Alterations in VHL as potential biomarkers in renal-cell carcinoma. *Nat Rev Clin Oncol* Vol.7, No.5, (May), pp.277-88

Grabmaier, K., MC, A.d.W., Verhaegh, G.W., Schalken, J.A. & Oosterwijk, E. (2004). Strict regulation of CAIX(G250/MN) by HIF-1alpha in clear cell renal cell carcinoma. *Oncogene* Vol.23, No.33, (Jul 22), pp.5624-31

Grabmaier, K., Vissers, J.L., De Weijert, M.C., Oosterwijk-Wakka, J.C., Van Bokhoven, A., Brakenhoff, R.H., Noessner, E., Mulders, P.A., Merkx, G., Figdor, C.G., Adema, G.J.

& Oosterwijk, E. (2000). Molecular cloning and immunogenicity of renal cell carcinoma-associated antigen G250. *Int J Cancer* Vol.85, No.6, (Mar 15), pp.865-70

Grubb, R.L., 3rd, Choyke, P.L., Pinto, P.A., Linehan, W.M. & Walther, M.M. (2005). Management of von Hippel-Lindau-associated kidney cancer. *Nat Clin Pract Urol* Vol.2, No.5, (May), pp.248-55

Gunaratnam, L., Morley, M., Franovic, A., de Paulsen, N., Mekhail, K., Parolin, D.A., Nakamura, E., Lorimer, I.A. & Lee, S. (2003). Hypoxia inducible factor activates the transforming growth factor-alpha/epidermal growth factor receptor growth stimulatory pathway in VHL(-/-) renal cell carcinoma cells. *J Biol Chem* Vol.278, No.45, (Nov 7), pp.44966-74

Guo, Y., Schoell, M.C. & Freeman, R.S. (2009). The von Hippel-Lindau protein sensitizes renal carcinoma cells to apoptotic stimuli through stabilization of BIM(EL). *Oncogene* Vol.28, No.16, (Apr 23), pp.1864-74

Hagiwara, M., Chen, I.L., McGonigle, R., Beckman, B., Kasten, F.H. & Fisher, J.W. (1984). Erythropoietin production in a primary culture of human renal carcinoma cells maintained in nude mice. *Blood* Vol.63, No.4, (Apr), pp.828-35

Hara, S., Nakashiro, K., Klosek, S.K., Ishikawa, T., Shintani, S. & Hamakawa, H. (2006). Hypoxia enhances c-Met/HGF receptor expression and signaling by activating HIF-1alpha in human salivary gland cancer cells. *Oral Oncol* Vol.42, No.6, (Jul), pp.593-8

Henke, M., Laszig, R., Rube, C., Schafer, U., Haase, K.D., Schilcher, B., Mose, S., Beer, K.T., Burger, U., Dougherty, C. & Frommhold, H. (2003). Erythropoietin to treat head and neck cancer patients with anaemia undergoing radiotherapy: randomised, double-blind, placebo-controlled trial. *Lancet* Vol.362, No.9392, (Oct 18), pp.1255-60

Hergovich, A., Lisztwan, J., Barry, R., Ballschmieter, P. & Krek, W. (2003). Regulation of microtubule stability by the von Hippel-Lindau tumour suppressor protein pVHL. *Nat Cell Biol* Vol.5, No.1, (Jan), pp.64-70

Herman, J.G., Latif, F., Weng, Y., Lerman, M.I., Zbar, B., Liu, S., Samid, D., Duan, D.S., Gnarra, J.R., Linehan, W.M. & et al. (1994). Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. *Proc Natl Acad Sci U S A* Vol.91, No.21, (Oct 11), pp.9700-4

Hicklin, D.J. & Ellis, L.M. (2005). Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *J Clin Oncol* Vol.23, No.5, (Feb 10), pp.1011-27

Higashiyama, S., Iwabuki, H., Morimoto, C., Hieda, M., Inoue, H. & Matsushita, N. (2008). Membrane-anchored growth factors, the epidermal growth factor family: beyond receptor ligands. *Cancer Sci* Vol.99, No.2, (Feb), pp.214-20

Horiguchi, A., Sumitomo, M., Asakuma, J., Asano, T., Zheng, R., Nanus, D.M. & Hayakawa, M. (2006a). Increased serum leptin levels and over expression of leptin receptors are associated with the invasion and progression of renal cell carcinoma. *J Urol* Vol.176, No.4 Pt 1, (Oct), pp.1631-5

Horiguchi, A., Sumitomo, M., Asakuma, J., Asano, T., Zheng, R., Nanus, D.M. & Hayakawa, M. (2006b). Leptin promotes invasiveness of murine renal cancer cells via extracellular signal-regulated kinases and rho dependent pathway. *J Urol* Vol.176, No.4 Pt 1, (Oct), pp.1636-41

Houghton, P.J. (2010). Everolimus. *Clin Cancer Res* Vol.16, No.5, (Mar 1), pp.1368-72

Huang, L.E. & Bunn, H.F. (2003). Hypoxia-inducible factor and its biomedical relevance. *J Biol Chem* Vol.278, No.22, (May 30), pp.19575-8

Iliopoulos, O., Kibel, A., Gray, S. & Kaelin, W.G., Jr. (1995). Tumour suppression by the human von Hippel-Lindau gene product. *Nat Med* Vol.1, No.8, (Aug), pp.822-6

Iliopoulos, O., Ohh, M. & Kaelin, W.G., Jr. (1998). pVHL19 is a biologically active product of the von Hippel-Lindau gene arising from internal translation initiation. *Proc Natl Acad Sci U S A* Vol.95, No.20, (Sep 29), pp.11661-6

Ishikawa, J., Maeda, S., Umezu, K., Sugiyama, T. & Kamidono, S. (1990). Amplification and overexpression of the epidermal growth factor receptor gene in human renal-cell carcinoma. *Int J Cancer* Vol.45, No.6, (Jun 15), pp.1018-21

Ivan, M., Kondo, K., Yang, H., Kim, W., Valiando, J., Ohh, M., Salic, A., Asara, J.M., Lane, W.S. & Kaelin, W.G., Jr. (2001). HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. *Science* Vol.292, No.5516, (Apr 20), pp.464-8

Ivanov, S., Liao, S.Y., Ivanova, A., Danilkovitch-Miagkova, A., Tarasova, N., Weirich, G., Merrill, M.J., Proescholdt, M.A., Oldfield, E.H., Lee, J., Zavada, J., Waheed, A., Sly, W., Lerman, M.I. & Stanbridge, E.J. (2001). Expression of hypoxia-inducible cell-surface transmembrane carbonic anhydrases in human cancer. *Am J Pathol* Vol.158, No.3, (Mar), pp.905-19

Jaakkola, P., Mole, D.R., Tian, Y.M., Wilson, M.I., Gielbert, J., Gaskell, S.J., Kriegsheim, A., Hebestreit, H.F., Mukherji, M., Schofield, C.J., Maxwell, P.H., Pugh, C.W. & Ratcliffe, P.J. (2001). Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. *Science* Vol.292, No.5516, (Apr 20), pp.468-72

Jensen, H.K., Nordsmark, M., Donskov, F., Marcussen, N. & von der Maase, H. (2008). Immunohistochemical expression of carbonic anhydrase IX assessed over time and during treatment in renal cell carcinoma. *BJU Int* Vol.101 Suppl 4, (Jun), pp.41-4

Jermann, M., Stahel, R.A., Salzberg, M., Cerny, T., Joerger, M., Gillessen, S., Morant, R., Egli, F., Rhyner, K., Bauer, J.A. & Pless, M. (2006). A phase II, open-label study of gefitinib (IRESSA) in patients with locally advanced, metastatic, or relapsed renal-cell carcinoma. *Cancer Chemother Pharmacol* Vol.57, No.4, (Apr), pp.533-9

Kaelin, W.G. (2005a). The von Hippel-Lindau tumor suppressor protein: roles in cancer and oxygen sensing. *Cold Spring Harb Symp Quant Biol* Vol.70, pp.159-66

Kaelin, W.G., Jr. (2002). Molecular basis of the VHL hereditary cancer syndrome. *Nat Rev Cancer* Vol.2, No.9, (Sep), pp.673-82

Kaelin, W.G., Jr. (2005b). The von Hippel-Lindau protein, HIF hydroxylation, and oxygen sensing. *Biochem Biophys Res Commun* Vol.338, No.1, (Dec 9), pp.627-38

Kaelin, W.G., Jr. (2007). The von Hippel-Lindau tumor suppressor protein and clear cell renal carcinoma. *Clin Cancer Res* Vol.13, No.2 Pt 2, (Jan 15), pp.680s-4s

Kallio, J.P., Hirvikoski, P., Helin, H., Kellokumpu-Lehtinen, P., Luukkaala, T., Tammela, T.L. & Martikainen, P.M. (2003). Membranous location of EGFR immunostaining is associated with good prognosis in renal cell carcinoma. *Br J Cancer* Vol.89, No.7, (Oct 6), pp.1266-9

Kaluz, S., Kaluzova, M., Liao, S.Y., Lerman, M. & Stanbridge, E.J. (2009). Transcriptional control of the tumor- and hypoxia-marker carbonic anhydrase 9: A one transcription factor (HIF-1) show? *Biochim Biophys Acta* Vol.1795, No.2, (Apr), pp.162-72

Kaluz, S., Kaluzova, M. & Stanbridge, E.J. (2008). Does inhibition of degradation of hypoxia-inducible factor (HIF) alpha always lead to activation of HIF? Lessons learnt from the effect of proteasomal inhibition on HIF activity. *J Cell Biochem* Vol.104, No.2, (May 15), pp.536-44

Kazal, L.A. & Erslev, A.J. (1975). Erythropoietin production in renal tumors. *Ann Clin Lab Sci* Vol.5, No.2, (Mar-Apr), pp.98-109

Kedar, D., Baker, C.H., Killion, J.J., Dinney, C.P. & Fidler, I.J. (2002). Blockade of the epidermal growth factor receptor signaling inhibits angiogenesis leading to regression of human renal cell carcinoma growing orthotopically in nude mice. *Clin Cancer Res* Vol.8, No.11, (Nov), pp.3592-600

Kim, W.Y. & Kaelin, W.G. (2004). Role of VHL gene mutation in human cancer. *J Clin Oncol* Vol.22, No.24, (Dec 15), pp.4991-5004

Knebelmann, B., Ananth, S., Cohen, H.T. & Sukhatme, V.P. (1998). Transforming growth factor alpha is a target for the von Hippel-Lindau tumor suppressor. *Cancer Res* Vol.58, No.2, (Jan 15), pp.226-31

Koivunen, P., Tiainen, P., Hyvarinen, J., Williams, K.E., Sormunen, R., Klaus, S.J., Kivirikko, K.I. & Myllyharju, J. (2007). An endoplasmic reticulum transmembrane prolyl 4-hydroxylase is induced by hypoxia and acts on hypoxia-inducible factor alpha. *J Biol Chem* Vol.282, No.42, (Oct 19), pp.30544-52

Konig, B., Steinbach, F., Janocha, B., Drynda, A., Stumm, M., Philipp, C., Allhoff, E.P. & Konig, W. (1999). The differential expression of proinflammatory cytokines IL-6, IL-8 and TNF-alpha in renal cell carcinoma. *Anticancer Res* Vol.19, No.2C, (Mar-Apr), pp.1519-24

Koochekpour, S., Jeffers, M., Wang, P.H., Gong, C., Taylor, G.A., Roessler, L.M., Stearman, R., Vasselli, J.R., Stetler-Stevenson, W.G., Kaelin, W.G., Jr., Linehan, W.M., Klausner, R.D., Gnarra, J.R. & Vande Woude, G.F. (1999). The von Hippel-Lindau tumor suppressor gene inhibits hepatocyte growth factor/scatter factor-induced invasion and branching morphogenesis in renal carcinoma cells. *Mol Cell Biol* Vol.19, No.9, (Sep), pp.5902-12

Krishnamachary, B., Zagzag, D., Nagasawa, H., Rainey, K., Okuyama, H., Baek, J.H. & Semenza, G.L. (2006). Hypoxia-inducible factor-1-dependent repression of E-cadherin in von Hippel-Lindau tumor suppressor-null renal cell carcinoma mediated by TCF3, ZFHX1A, and ZFHX1B. *Cancer Res* Vol.66, No.5, (Mar 1), pp.2725-31

Kumar, R., Knick, V.B., Rudolph, S.K., Johnson, J.H., Crosby, R.M., Crouthamel, M.C., Hopper, T.M., Miller, C.G., Harrington, L.E., Onori, J.A., Mullin, R.J., Gilmer, T.M., Truesdale, A.T., Epperly, A.H., Boloor, A., Stafford, J.A., Luttrell, D.K. & Cheung, M. (2007). Pharmacokinetic-pharmacodynamic correlation from mouse to human with pazopanib, a multikinase angiogenesis inhibitor with potent antitumor and antiangiogenic activity. *Mol Cancer Ther* Vol.6, No.7, (Jul), pp.2012-21

Lacombe, C. & Mayeux, P. (1999). The molecular biology of erythropoietin. *Nephrol Dial Transplant* Vol.14 Suppl 2, pp.22-8

Lager, D.J., Slagel, D.D. & Palechek, P.L. (1994). The expression of epidermal growth factor receptor and transforming growth factor alpha in renal cell carcinoma. *Mod Pathol* Vol.7, No.5, (Jun), pp.544-8

Lai, S.Y. & Grandis, J.R. (2006). Understanding the presence and function of erythropoietin receptors on cancer cells. *J Clin Oncol* Vol.24, No.29, (Oct 10), pp.4675-6

Lando, D., Peet, D.J., Gorman, J.J., Whelan, D.A., Whitelaw, M.L. & Bruick, R.K. (2002a). FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. *Genes Dev* Vol.16, No.12, (Jun 15), pp.1466-71

Lando, D., Peet, D.J., Whelan, D.A., Gorman, J.J. & Whitelaw, M.L. (2002b). Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. *Science* Vol.295, No.5556, (Feb 1), pp.858-61

Latif, F., Tory, K., Gnarra, J., Yao, M., Duh, F.M., Orcutt, M.L., Stackhouse, T., Kuzmin, I., Modi, W., Geil, L. & et al. (1993). Identification of the von Hippel-Lindau disease tumor suppressor gene. *Science* Vol.260, No.5112, (May 28), pp.1317-20

Lawrentschuk, N., Lee, F.T., Jones, G., Rigopoulos, A., Mountain, A., O'Keefe, G., Papenfuss, A.T., Bolton, D.M., Davis, I.D. & Scott, A.M. (2011). Investigation of hypoxia and carbonic anhydrase IX expression in a renal cell carcinoma xenograft model with oxygen tension measurements and (124)I-cG250 PET/CT. *Urol Oncol* Vol.29, No.4, (Jul-Aug), pp.411-20

Lee, S.J., Lattouf, J.B., Xanthopoulos, J., Linehan, W.M., Bottaro, D.P. & Vasselli, J.R. (2008). Von Hippel-Lindau tumor suppressor gene loss in renal cell carcinoma promotes oncogenic epidermal growth factor receptor signaling via Akt-1 and MEK-1. *Eur Urol* Vol.54, No.4, (Oct), pp.845-53

Lee, Y.S., Vortmeyer, A.O., Lubensky, I.A., Vogel, T.W., Ikejiri, B., Ferlicot, S., Benoit, G., Giraud, S., Oldfield, E.H., Linehan, W.M., Teh, B.T., Richard, S. & Zhuang, Z. (2005). Coexpression of erythropoietin and erythropoietin receptor in von Hippel-Lindau disease-associated renal cysts and renal cell carcinoma. *Clin Cancer Res* Vol.11, No.3, (Feb 1), pp.1059-64

Leyland-Jones, B., Semiglazov, V., Pawlicki, M., Pienkowski, T., Tjulandin, S., Manikhas, G., Makhson, A., Roth, A., Dodwell, D., Baselga, J., Biakhov, M., Valuckas, K., Voznyi, E., Liu, X. & Vercammen, E. (2005). Maintaining normal hemoglobin levels with epoetin alfa in mainly nonanemic patients with metastatic breast cancer receiving first-line chemotherapy: a survival study. *J Clin Oncol* Vol.23, No.25, (Sep 1), pp.5960-72

Li, G., Feng, G., Gentil-Perret, A., Genin, C. & Tostain, J. (2008). Serum carbonic anhydrase 9 level is associated with postoperative recurrence of conventional renal cell cancer. *J Urol* Vol.180, No.2, (Aug), pp.510-3; discussion 3-4

Li, J., Vesey, D.A., Johnson, D.W. & Gobe, G. (2007). Erythropoietin reduces cisplatin-induced apoptosis in renal carcinoma cells via a PKC dependent pathway. *Cancer Biol Ther* Vol.6, No.12, (Dec), pp.1944-50

Lidgren, A., Bergh, A., Grankvist, K., Rasmuson, T. & Ljungberg, B. (2008). Glucose transporter-1 expression in renal cell carcinoma and its correlation with hypoxia inducible factor-1 alpha. *BJU Int* Vol.101, No.4, (Feb), pp.480-4

Ljungberg, B., Rasmuson, T. & Grankvist, K. (1992). Erythropoietin in renal cell carcinoma: evaluation of its usefulness as a tumor marker. *Eur Urol* Vol.21, No.2, pp.160-3

Lonser, R.R., Glenn, G.M., Walther, M., Chew, E.Y., Libutti, S.K., Linehan, W.M. & Oldfield, E.H. (2003). von Hippel-Lindau disease. *Lancet* Vol.361, No.9374, (Jun 14), pp.2059-67

Lopez-Beltran, A., Scarpelli, M., Montironi, R. & Kirkali, Z. (2006). 2004 WHO classification of the renal tumors of the adults. *Eur Urol* Vol.49, No.5, (May), pp.798-805

Lowden, D.A., Lindemann, G.W., Merlino, G., Barash, B.D., Calvet, J.P. & Gattone, V.H., 2nd. (1994). Renal cysts in transgenic mice expressing transforming growth factor-alpha. *J Lab Clin Med* Vol.124, No.3, (Sep), pp.386-94

Macheda, M.L., Rogers, S. & Best, J.D. (2005). Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. *J Cell Physiol* Vol.202, No.3, (Mar), pp.654-62

Maher, E.R., Yates, J.R., Harries, R., Benjamin, C., Harris, R., Moore, A.T. & Ferguson-Smith, M.A. (1990). Clinical features and natural history of von Hippel-Lindau disease. *Q J Med* Vol.77, No.283, (Nov), pp.1151-63

Mahon, P.C., Hirota, K. & Semenza, G.L. (2001). FIH-1: a novel protein that interacts with HIF-1alpha and VHL to mediate repression of HIF-1 transcriptional activity. *Genes Dev* Vol.15, No.20, (Oct 15), pp.2675-86

Marks, J.L., Broderick, S., Zhou, Q., Chitale, D., Li, A.R., Zakowski, M.F., Kris, M.G., Rusch, V.W., Azzoli, C.G., Seshan, V.E., Ladanyi, M. & Pao, W. (2008). Prognostic and therapeutic implications of EGFR and KRAS mutations in resected lung adenocarcinoma. *J Thorac Oncol* Vol.3, No.2, (Feb), pp.111-6

Maxwell, P.H., Wiesener, M.S., Chang, G.W., Clifford, S.C., Vaux, E.C., Cockman, M.E., Wykoff, C.C., Pugh, C.W., Maher, E.R. & Ratcliffe, P.J. (1999). The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* Vol.399, No.6733, (May 20), pp.271-5

Maynard, M.A. & Ohh, M. (2004). Von Hippel-Lindau tumor suppressor protein and hypoxia-inducible factor in kidney cancer. *Am J Nephrol* Vol.24, No.1, (Jan-Feb), pp.1-13

Maynard, M.A., Qi, H., Chung, J., Lee, E.H., Kondo, Y., Hara, S., Conaway, R.C., Conaway, J.W. & Ohh, M. (2003). Multiple splice variants of the human HIF-3 alpha locus are targets of the von Hippel-Lindau E3 ubiquitin ligase complex. *J Biol Chem* Vol.278, No.13, (Mar 28), pp.11032-40

Medina, R.A. & Owen, G.I. (2002). Glucose transporters: expression, regulation and cancer. *Biol Res* Vol.35, No.1, pp.9-26

Mendel, D.B., Laird, A.D., Xin, X., Louie, S.G., Christensen, J.G., Li, G., Schreck, R.E., Abrams, T.J., Ngai, T.J., Lee, L.B., Murray, L.J., Carver, J., Chan, E., Moss, K.G., Haznedar, J.O., Sukbuntherng, J., Blake, R.A., Sun, L., Tang, C., Miller, T., Shirazian, S., McMahon, G. & Cherrington, J.M. (2003). In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. *Clin Cancer Res* Vol.9, No.1, (Jan), pp.327-37

Merseburger, A.S., Hennenlotter, J., Simon, P., Kruck, S., Koch, E., Horstmann, M., Kuehs, U., Kufer, R., Stenzl, A. & Kuczyk, M.A. (2005). Membranous expression and prognostic implications of epidermal growth factor receptor protein in human renal cell cancer. *Anticancer Res* Vol.25, No.3B, (May-Jun), pp.1901-7

Michael, A., Politi, E., Havranek, E., Corbishley, C., Karapanagiotou, L., Anderson, C., Relph, K., Syrigos, K.N. & Pandha, H. (2007). Prognostic significance of erythropoietin expression in human renal cell carcinoma. *BJU Int* Vol.100, No.2, (Aug), pp.291-4

Migita, T., Oda, Y., Masuda, K., Hirata, A., Kuwano, M., Naito, S. & Tsuneyoshi, M. (2008). Inverse relationship between E-cadherin and p27Kip1 expression in renal cell carcinoma. *Int J Oncol* Vol.33, No.1, (Jul), pp.41-7

Mikhaylova, O., Ignacak, M.L., Barankiewicz, T.J., Harbaugh, S.V., Yi, Y., Maxwell, P.H., Schneider, M., Van Geyte, K., Carmeliet, P., Revelo, M.P., Wyder, M., Greis, K.D., Meller, J. & Czyzyk-Krzeska, M.F. (2008). The von Hippel-Lindau tumor suppressor protein and Egl-9-Type proline hydroxylases regulate the large subunit of RNA polymerase II in response to oxidative stress. *Mol Cell Biol* Vol.28, No.8, (Apr), pp.2701-17

Miyakita, H., Tokunaga, M., Onda, H., Usui, Y., Kinoshita, H., Kawamura, N. & Yasuda, S. (2002). Significance of 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) for detection of renal cell carcinoma and immunohistochemical glucose transporter 1 (GLUT-1) expression in the cancer. *Int J Urol* Vol.9, No.1, (Jan), pp.15-8

Motzer, R.J., Amato, R., Todd, M., Hwu, W.J., Cohen, R., Baselga, J., Muss, H., Cooper, M., Yu, R., Ginsberg, M.S. & Needle, M. (2003). Phase II trial of antiepidermal growth factor receptor antibody C225 in patients with advanced renal cell carcinoma. *Invest New Drugs* Vol.21, No.1, (Feb), pp.99-101

Motzer, R.J., Escudier, B., Oudard, S., Hutson, T.E., Porta, C., Bracarda, S., Grunwald, V., Thompson, J.A., Figlin, R.A., Hollaender, N., Urbanowitz, G., Berg, W.J., Kay, A., Lebwohl, D. & Ravaud, A. (2008). Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. *Lancet* Vol.372, No.9637, (Aug 9), pp.449-56

Murphy, G.P., Kenny, G.M. & Mirand, E.A. (1970). Erythropoietin levels in patients with renal tumors or cysts. *Cancer* Vol.26, No.1, (Jul), pp.191-4

Mydlo, J.H., Michaeli, J., Cordon-Cardo, C., Goldenberg, A.S., Heston, W.D. & Fair, W.R. (1989). Expression of transforming growth factor alpha and epidermal growth factor receptor messenger RNA in neoplastic and nonneoplastic human kidney tissue. *Cancer Res* Vol.49, No.12, (Jun 15), pp.3407-11

Nagase, Y., Takata, K., Moriyama, N., Aso, Y., Murakami, T. & Hirano, H. (1995). Immunohistochemical localization of glucose transporters in human renal cell carcinoma. *J Urol* Vol.153, No.3 Pt 1, (Mar), pp.798-801

Noguchi, Y., Goto, T., Yufu, Y., Uike, N., Hasegawa, Y., Fukuda, T., Jimi, A. & Funakoshi, A. (1999). Gene expression of erythropoietin in renal cell carcinoma. *Intern Med* Vol.38, No.12, (Dec), pp.991-4

Ohh, M. (2006). Ubiquitin pathway in VHL cancer syndrome. *Neoplasia* Vol.8, No.8, (Aug), pp.623-9

Ohh, M. & Kaelin, W.G., Jr. (2003). VHL and kidney cancer. *Methods Mol Biol* Vol.222, pp.167-83

Ohh, M., Park, C.W., Ivan, M., Hoffman, M.A., Kim, T.Y., Huang, L.E., Pavletich, N., Chau, V. & Kaelin, W.G. (2000). Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel-Lindau protein. *Nat Cell Biol* Vol.2, No.7, (Jul), pp.423-7

Opavsky, R., Pastorekova, S., Zelnik, V., Gibadulinova, A., Stanbridge, E.J., Zavada, J., Kettmann, R. & Pastorek, J. (1996). Human MN/CA9 gene, a novel member of the carbonic anhydrase family: structure and exon to protein domain relationships. *Genomics* Vol.33, No.3, (May 1), pp.480-7

Ozcan, A., Shen, S.S., Zhai, Q.J. & Truong, L.D. (2007). Expression of GLUT1 in primary renal tumors: morphologic and biologic implications. *Am J Clin Pathol* Vol.128, No.2, (Aug), pp.245-54

Papworth, K., Bergh, A., Grankvist, K., Ljungberg, B. & Rasmuson, T. (2009). Expression of erythropoietin and its receptor in human renal cell carcinoma. *Tumour Biol* Vol.30, No.2, pp.86-92

Park, S.K., Haase, V.H. & Johnson, R.S. (2007). von Hippel Lindau tumor suppressor regulates hepatic glucose metabolism by controlling expression of glucose transporter 2 and glucose 6-phosphatase. *Int J Oncol* Vol.30, No.2, (Feb), pp.341-8

Pascual, D. & Borque, A. (2008). Epidemiology of kidney cancer. *Adv Urol* pp.782381

Pastore, Y., Jedlickova, K., Guan, Y., Liu, E., Fahner, J., Hasle, H., Prchal, J.F. & Prchal, J.T. (2003). Mutations of von Hippel-Lindau tumor-suppressor gene and congenital polycythemia. *Am J Hum Genet* Vol.73, No.2, (Aug), pp.412-9

Pastorekova, S., Ratcliffe, P.J. & Pastorek, J. (2008). Molecular mechanisms of carbonic anhydrase IX-mediated pH regulation under hypoxia. *BJU Int* Vol.101 Suppl 4, (Jun), pp.8-15

Patard, J.J., Fergelot, P., Karakiewicz, P.I., Klatte, T., Trinh, Q.D., Rioux-Leclercq, N., Said, J.W., Belldegrun, A.S. & Pantuck, A.J. (2008). Low CAIX expression and absence of VHL gene mutation are associated with tumor aggressiveness and poor survival of clear cell renal cell carcinoma. *Int J Cancer* Vol.123, No.2, (Jul 15), pp.395-400

Patard, J.J., Rioux-Leclercq, N., Masson, D., Zerrouki, S., Jouan, F., Collet, N., Dubourg, C., Lobel, B., Denis, M. & Fergelot, P. (2009). Absence of VHL gene alteration and high VEGF expression are associated with tumour aggressiveness and poor survival of renal-cell carcinoma. *Br J Cancer* Vol.101, No.8, (Oct 20), pp.1417-24

Pelicano, H., Martin, D.S., Xu, R.H. & Huang, P. (2006). Glycolysis inhibition for anticancer treatment. *Oncogene* Vol.25, No.34, (Aug 7), pp.4633-46

Pelletier, S., Tanguay, S., Lee, S., Gunaratnam, L., Arbour, N. & Lapointe, R. (2009). TGF-alpha as a candidate tumor antigen for renal cell carcinomas. *Cancer Immunol Immunother* Vol.58, No.8, (Aug), pp.1207-18

Perera, A.D., Kleymenova, E.V. & Walker, C.L. (2000). Requirement for the von Hippel-Lindau tumor suppressor gene for functional epidermal growth factor receptor blockade by monoclonal antibody C225 in renal cell carcinoma. *Clin Cancer Res* Vol.6, No.4, (Apr), pp.1518-23

Petrides, P.E., Bock, S., Bovens, J., Hofmann, R. & Jakse, G. (1990). Modulation of pro-epidermal growth factor, pro-transforming growth factor alpha and epidermal

growth factor receptor gene expression in human renal carcinomas. *Cancer Res* Vol.50, No.13, (Jul 1), pp.3934-9

Pu, Y.S., Huang, C.Y., Kuo, Y.Z., Kang, W.Y., Liu, G.Y., Huang, A.M., Yu, H.J., Lai, M.K., Huang, S.P., Wu, W.J., Chiou, S.J. & Hour, T.C. (2009). Characterization of membranous and cytoplasmic EGFR expression in human normal renal cortex and renal cell carcinoma. *J Biomed Sci* Vol.16, pp.82

Pugh, C.W., O'Rourke, J.F., Nagao, M., Gleadle, J.M. & Ratcliffe, P.J. (1997). Activation of hypoxia-inducible factor-1; definition of regulatory domains within the alpha subunit. *J Biol Chem* Vol.272, No.17, (Apr 25), pp.11205-14

Rad, F.H., Ulusakarya, A., Gad, S., Sibony, M., Juin, F., Richard, S., Machover, D. & Uzan, G. (2008). Novel somatic mutations of the VHL gene in an erythropoietin-producing renal carcinoma associated with secondary polycythemia and elevated circulating endothelial progenitor cells. *Am J Hematol* Vol.83, No.2, (Feb), pp.155-8

Ramp, U., Reinecke, P., Gabbert, H.E. & Gerharz, C.D. (2000). Differential response to transforming growth factor (TGF)-alpha and fibroblast growth factor (FGF) in human renal cell carcinomas of the clear cell and papillary types. *Eur J Cancer* Vol.36, No.7, (May), pp.932-41

Ravaud, A., Hawkins, R., Gardner, J.P., von der Maase, H., Zantl, N., Harper, P., Rolland, F., Audhuy, B., Machiels, J.P., Petavy, F., Gore, M., Schoffski, P. & El-Hariry, I. (2008). Lapatinib versus hormone therapy in patients with advanced renal cell carcinoma: a randomized phase III clinical trial. *J Clin Oncol* Vol.26, No.14, (May 10), pp.2285-91

Rioux-Leclercq, N., Fergelot, P., Zerrouki, S., Leray, E., Jouan, F., Bellaud, P., Epstein, J.I. & Patard, J.J. (2007). Plasma level and tissue expression of vascular endothelial growth factor in renal cell carcinoma: a prospective study of 50 cases. *Hum Pathol* Vol.38, No.10, (Oct), pp.1489-95

Rixe, O., Bukowski, R.M., Michaelson, M.D., Wilding, G., Hudes, G.R., Bolte, O., Motzer, R.J., Bycott, P., Liau, K.F., Freddo, J., Trask, P.C., Kim, S. & Rini, B.I. (2007). Axitinib treatment in patients with cytokine-refractory metastatic renal-cell cancer: a phase II study. *Lancet Oncol* Vol.8, No.11, (Nov), pp.975-84

Ronkainen, H., Kauppila, S., Hirvikoski, P. & Vaarala, M.H. (2010). Evaluation of myosin VI, E-cadherin and beta-catenin immunostaining in renal cell carcinoma. *J Exp Clin Cancer Res* Vol.29, pp.2

Rowinsky, E.K., Schwartz, G.H., Gollob, J.A., Thompson, J.A., Vogelzang, N.J., Figlin, R., Bukowski, R., Haas, N., Lockbaum, P., Li, Y.P., Arends, R., Foon, K.A., Schwab, G. & Dutcher, J. (2004). Safety, pharmacokinetics, and activity of ABX-EGF, a fully human anti-epidermal growth factor receptor monoclonal antibody in patients with metastatic renal cell cancer. *J Clin Oncol* Vol.22, No.15, (Aug 1), pp.3003-15

Roy, H., Bhardwaj, S. & Yla-Herttuala, S. (2006). Biology of vascular endothelial growth factors. *Febs Letters* Vol.580, No.12, (May 22), pp.2879-87

Rugo, H.S., Herbst, R.S., Liu, G., Park, J.W., Kies, M.S., Steinfeldt, H.M., Pithavala, Y.K., Reich, S.D., Freddo, J.L. & Wilding, G. (2005). Phase I trial of the oral antiangiogenesis agent AG-013736 in patients with advanced solid tumors: pharmacokinetic and clinical results. *J Clin Oncol* Vol.23, No.24, (Aug 20), pp.5474-83

Russell, R.C. & Ohh, M. (2007). The role of VHL in the regulation of E-cadherin: a new connection in an old pathway. *Cell Cycle* Vol.6, No.1, (Jan 1), pp.56-9

Safran, M. & Kaelin, W.G., Jr. (2003). HIF hydroxylation and the mammalian oxygen-sensing pathway. *J Clin Invest* Vol.111, No.6, (Mar), pp.779-83

Sakamoto, S., Igarashi, T., Osumi, N., Imamoto, T., Tobe, T., Kamiya, M. & Ito, H. (2003). Erythropoietin-producing renal cell carcinoma in chronic hemodialysis patients: a report of two cases. *Int J Urol* Vol.10, No.1, (Jan), pp.49-51

Sang, N., Fang, J., Srinivas, V., Leshchinsky, I. & Caro, J. (2002). Carboxyl-terminal transactivation activity of hypoxia-inducible factor 1 alpha is governed by a von Hippel-Lindau protein-independent, hydroxylation-regulated association with p300/CBP. *Mol Cell Biol* Vol.22, No.9, (May), pp.2984-92

Sargent, E.R., Gomella, L.G., Belldegrun, A., Linehan, W.M. & Kasid, A. (1989). Epidermal growth factor receptor gene expression in normal human kidney and renal cell carcinoma. *J Urol* Vol.142, No.5, (Nov), pp.1364-8

Schoenfeld, A., Davidowitz, E.J. & Burk, R.D. (1998). A second major native von Hippel-Lindau gene product, initiated from an internal translation start site, functions as a tumor suppressor. *Proc Natl Acad Sci U S A* Vol.95, No.15, (Jul 21), pp.8817-22

Schofield, C.J. & Ratcliffe, P.J. (2005). Signalling hypoxia by HIF hydroxylases. *Biochem Biophys Res Commun* Vol.338, No.1, (Dec 9), pp.617-26

Seizinger, B.R., Rouleau, G.A., Ozelius, L.J., Lane, A.H., Farmer, G.E., Lamiell, J.M., Haines, J., Yuen, J.W., Collins, D., Majoor-Krakauer, D. & et al. (1988). Von Hippel-Lindau disease maps to the region of chromosome 3 associated with renal cell carcinoma. *Nature* Vol.332, No.6161, (Mar 17), pp.268-9

Semenza, G.L. (1999). Regulation of mammalian O2 homeostasis by hypoxia-inducible factor 1. *Annu Rev Cell Dev Biol* Vol.15, pp.551-78

Singer, K., Kastenberger, M., Gottfried, E., Hammerschmied, C.G., Buttner, M., Aigner, M., Seliger, B., Walter, B., Schlosser, H., Hartmann, A., Andreesen, R., Mackensen, A. & Kreutz, M. (2011). Warburg phenotype in renal cell carcinoma: high expression of glucose-transporter 1 (GLUT-1) correlates with low CD8(+) T-cell infiltration in the tumor. *Int J Cancer* Vol.128, No.9, (May 1), pp.2085-95

Slaton, J.W., Inoue, K., Perrotte, P., El-Naggar, A.K., Swanson, D.A., Fidler, I.J. & Dinney, C.P. (2001). Expression levels of genes that regulate metastasis and angiogenesis correlate with advanced pathological stage of renal cell carcinoma. *Am J Pathol* Vol.158, No.2, (Feb), pp.735-43

Smith, T.A. (1999). Facilitative glucose transporter expression in human cancer tissue. *Br J Biomed Sci* Vol.56, No.4, pp.285-92

Stebbins, C.E., Kaelin, W.G., Jr. & Pavletich, N.P. (1999). Structure of the VHL-ElonginC-ElonginB complex: implications for VHL tumor suppressor function. *Science* Vol.284, No.5413, (Apr 16), pp.455-61

Sternberg, C.N., Davis, I.D., Mardiak, J., Szczylik, C., Lee, E., Wagstaff, J., Barrios, C.H., Salman, P., Gladkov, O.A., Kavina, A., Zarba, J.J., Chen, M., McCann, L., Pandite, L., Roychowdhury, D.F. & Hawkins, R.E. (2010). Pazopanib in locally advanced or metastatic renal cell carcinoma: results of a randomized phase III trial. *J Clin Oncol* Vol.28, No.6, (Feb 20), pp.1061-8

Stillebroer, A.B., Mulders, P.F., Boerman, O.C., Oyen, W.J. & Oosterwijk, E. (2010). Carbonic anhydrase IX in renal cell carcinoma: implications for prognosis, diagnosis, and therapy. *Eur Urol* Vol.58, No.1, (Jul), pp.75-83

Stuttfeld, E. & Ballmer-Hofer, K. (2009). Structure and function of VEGF receptors. *IUBMB Life* Vol.61, No.9, (Sep), pp.915-22

Suganuma, N., Segade, F., Matsuzu, K. & Bowden, D.W. (2007). Differential expression of facilitative glucose transporters in normal and tumour kidney tissues. *BJU Int* Vol.99, No.5, (May), pp.1143-9

Takahashi, A., Sasaki, H., Kim, S.J., Tobisu, K., Kakizoe, T., Tsukamoto, T., Kumamoto, Y., Sugimura, T. & Terada, M. (1994). Markedly increased amounts of messenger RNAs for vascular endothelial growth factor and placenta growth factor in renal cell carcinoma associated with angiogenesis. *Cancer Res* Vol.54, No.15, (Aug 1), pp.4233-7

Tammela, T., Enholm, B., Alitalo, K. & Paavonen, K. (2005). The biology of vascular endothelial growth factors. *Cardiovasc Res* Vol.65, No.3, (Feb 15), pp.550-63

Tanimoto, K., Makino, Y., Pereira, T. & Poellinger, L. (2000). Mechanism of regulation of the hypoxia-inducible factor-1 alpha by the von Hippel-Lindau tumor suppressor protein. *Embo J* Vol.19, No.16, (Aug 15), pp.4298-309

Thiery, J.P. (2002). Epithelial-mesenchymal transitions in tumour progression. *Nat Rev Cancer* Vol.2, No.6, (Jun), pp.442-54

Thomas, G.V., Tran, C., Mellinghoff, I.K., Welsbie, D.S., Chan, E., Fueger, B., Czernin, J. & Sawyers, C.L. (2006). Hypoxia-inducible factor determines sensitivity to inhibitors of mTOR in kidney cancer. *Nat Med* Vol.12, No.1, (Jan), pp.122-7

Thurston, G. & Kitajewski, J. (2008). VEGF and Delta-Notch: interacting signalling pathways in tumour angiogenesis. *Br J Cancer* Vol.99, No.8, (Oct 21), pp.1204-9

Thyavihally, Y.B., Mahantshetty, U., Chamarajanagar, R.S., Raibhattanavar, S.G. & Tongaonkar, H.B. (2005). Management of renal cell carcinoma with solitary metastasis. *World J Surg Oncol* Vol.3, (Jul 20), pp.48

Uberall, I., Kolar, Z., Trojanec, R., Berkovcova, J. & Hajduch, M. (2008). The status and role of ErbB receptors in human cancer. *Exp Mol Pathol* Vol.84, No.2, (Apr), pp.79-89

Uhlman, D.L., Nguyen, P., Manivel, J.C., Zhang, G., Hagen, K., Fraley, E., Aeppli, D. & Niehans, G.A. (1995). Epidermal growth factor receptor and transforming growth factor alpha expression in papillary and nonpapillary renal cell carcinoma: correlation with metastatic behavior and prognosis. *Clin Cancer Res* Vol.1, No.8, (Aug), pp.913-20

Wang, G.L., Jiang, B.H., Rue, E.A. & Semenza, G.L. (1995). Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. *Proc Natl Acad Sci U S A* Vol.92, No.12, (Jun 6), pp.5510-4

Warburg, O. (1956). On the origin of cancer cells. *Science* Vol.123, No.3191, (Feb 24), pp.309-14

Weiss, R.H. & Lin, P.Y. (2006). Kidney cancer: identification of novel targets for therapy. *Kidney Int* Vol.69, No.2, (Jan), pp.224-32

Wiesener, M.S., Munchenhagen, P., Glaser, M., Sobottka, B.A., Knaup, K.X., Jozefowski, K., Jurgensen, J.S., Roigas, J., Warnecke, C., Grone, H.J., Maxwell, P.H., Willam, C. & Eckardt, K.U. (2007). Erythropoietin gene expression in renal carcinoma is

considerably more frequent than paraneoplastic polycythemia. *Int J Cancer* Vol.121, No.11, (Dec 1), pp.2434-42

Wiesener, M.S., Seyfarth, M., Warnecke, C., Jurgensen, J.S., Rosenberger, C., Morgan, N.V., Maher, E.R., Frei, U. & Eckardt, K.U. (2002). Paraneoplastic erythrocytosis associated with an inactivating point mutation of the von Hippel-Lindau gene in a renal cell carcinoma. *Blood* Vol.99, No.10, (May 15), pp.3562-5

Wilhelm, S. & Chien, D.S. (2002). BAY 43-9006: preclinical data. *Curr Pharm Des* Vol.8, No.25, pp.2255-7

Wilhelm, S.M., Carter, C., Tang, L., Wilkie, D., McNabola, A., Rong, H., Chen, C., Zhang, X., Vincent, P., McHugh, M., Cao, Y., Shujath, J., Gawlak, S., Eveleigh, D., Rowley, B., Liu, L., Adnane, L., Lynch, M., Auclair, D., Taylor, I., Gedrich, R., Voznesensky, A., Riedl, B., Post, L.E., Bollag, G. & Trail, P.A. (2004). BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res* Vol.64, No.19, (Oct 1), pp.7099-109

Wykoff, C.C., Beasley, N.J., Watson, P.H., Turner, K.J., Pastorek, J., Sibtain, A., Wilson, G.D., Turley, H., Talks, K.L., Maxwell, P.H., Pugh, C.W., Ratcliffe, P.J. & Harris, A.L. (2000). Hypoxia-inducible expression of tumor-associated carbonic anhydrases. *Cancer Res* Vol.60, No.24, (Dec 15), pp.7075-83

Xu, L., Tong, R., Cochran, D.M. & Jain, R.K. (2005a). Blocking platelet-derived growth factor-D/platelet-derived growth factor receptor beta signaling inhibits human renal cell carcinoma progression in an orthotopic mouse model. *Cancer Res* Vol.65, No.13, (Jul 1), pp.5711-9

Xu, R.H., Pelicano, H., Zhou, Y., Carew, J.S., Feng, L., Bhalla, K.N., Keating, M.J. & Huang, P. (2005b). Inhibition of glycolysis in cancer cells: a novel strategy to overcome drug resistance associated with mitochondrial respiratory defect and hypoxia. *Cancer Res* Vol.65, No.2, (Jan 15), pp.613-21

Yoshida, K., Hosoya, Y., Sumi, S., Honda, M., Moriguchi, H., Yano, M. & Ueda, Y. (1997). Studies of the expression of epidermal growth factor receptor in human renal cell carcinoma: a comparison of immunohistochemical method versus ligand binding assay. *Oncology* Vol.54, No.3, (May-Jun), pp.220-5

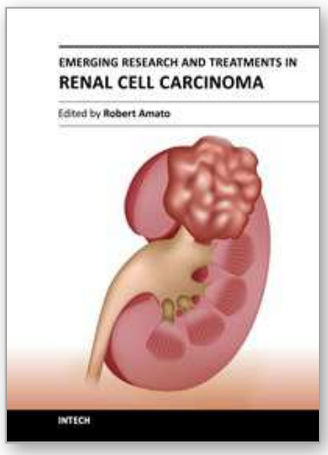
Yoshida, K., Tosaka, A., Takeuchi, S. & Kobayashi, N. (1994). Epidermal growth factor receptor content in human renal cell carcinomas. *Cancer* Vol.73, No.7, (Apr 1), pp.1913-8

Yuen, J.S., Cockman, M.E., Sullivan, M., Protheroe, A., Turner, G.D., Roberts, I.S., Pugh, C.W., Werner, H. & Macaulay, V.M. (2007). The VHL tumor suppressor inhibits expression of the IGF1R and its loss induces IGF1R upregulation in human clear cell renal carcinoma. *Oncogene* Vol.26, No.45, (Oct 4), pp.6499-508

Zavada, J., Zavadova, Z., Zat'ovicova, M., Hyrsl, L. & Kawaciuk, I. (2003). Soluble form of carbonic anhydrase IX (CA IX) in the serum and urine of renal carcinoma patients. *Br J Cancer* Vol.89, No.6, (Sep 15), pp.1067-71

Zhang, X., Yamashita, M., Uetsuki, H. & Kakehi, Y. (2002). Angiogenesis in renal cell carcinoma: Evaluation of microvessel density, vascular endothelial growth factor and matrix metalloproteinases. *Int J Urol* Vol.9, No.9, (Sep), pp.509-14

Zhou, G.X., Ireland, J., Rayman, P., Finke, J. & Zhou, M. (2010). Quantification of carbonic anhydrase IX expression in serum and tissue of renal cell carcinoma patients using enzyme-linked immunosorbent assay: prognostic and diagnostic potentials. *Urology* Vol.75, No.2, (Feb), pp.257-61



Emerging Research and Treatments in Renal Cell Carcinoma
Edited by Dr. Robert Amato

ISBN 978-953-51-0022-5
Hard cover, 442 pages
Publisher InTech
Published online 03, February, 2012
Published in print edition February, 2012

The field of renal cell cancer has undergone a significant resurgence. This book summarizes up-to-date research and innovative ideas for the future in this rapidly changing field, which encompasses medicine, surgery, radiation oncology, basic science, pathology, radiology, and supportive care. This book is aimed at the clinician or scientist who has an interest in renal cell cancer, whether they are academic or nonacademic. The book covers tumor biology, molecular biology, surgery techniques, radiation therapy, personal testimonies, and present and future treatments of the disease that are on the horizon. The goal was to produce a textbook that would act as an authoritative source for scientists and clinicians and interpret the field for trainees in surgery, medicine, radiation oncology, and pathology.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen