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Exploitation of Aberrant Signalling Pathways as Useful Targets for Renal Clear Cell Carcinoma Therapy

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1. Introduction

Renal cell carcinoma (RCC) is the third leading cause of death among urological tumours, annually afflicts about 150, 000 people globally and causes nearly 78, 000 deaths (Jemal et al., 2008; Zbar et al., 2003). RCC is an epithelial tumour consisting of several different histological subtypes of which clear cell RCC is the prototypical. Traditionally treatment has been via surgery and immunotherapy. Surgical resection is appropriate for some patient cohorts including those with isolated metastases. However, recurrence is common even when the primary and metastatic sites have been aggressively resected (Couillard & deVere White, 1993). RCC is highly unresponsive to standard chemotherapy and the use of cytokine therapy with interleukin (IL)-2 or interferon (IFN)-α is associated with low rates of response and high rates of toxicity (Oudard et al., 2007). Thus, development of new therapies continues to be crucial to improve outcomes in patients with RCC.

The increased understanding of the molecular structure and aberrant activity of signalling pathways in RCC has lead to a flurry of research activity in the arena of targeted therapies namely anti-angiogenic vascular endothelial growth factor (VEGF) and the mammalian target of rapamycin (mTOR), both of which are involved in the pathogenesis of RCC (Mulders, 2009). These advancements in an obvious therapeutic gap have significantly improved the progression free survival (PFS) of patients with RCC. Despite the explosion in drug development during the past five years, however, PFS for patients with metastatic RCC (mRCC) still remains poor as none of the current targeted therapies possess the capacity to induce remission. In addition these drugs provide dose-limiting toxic side effects and so we are still faced with a considerable task in developing newer safer therapeutics for use as either first line agents or in combination with existing ones.

2. Targeted therapy for RCC

As the understanding of the molecular biology underlying RCC has increased, various components of growth and angiogenic signal transduction pathways have been identified as rational targets for therapeutic intervention in the treatment of patients with RCC and mRCC. The VEGF/VEGF receptor (VEGFR) pathway is one such target. VEGF expression is induced under hypoxic conditions triggering several mechanisms that promote

angiogenesis (Ellis & Hicklin, 2008). Members of the VEGF family namely VEGF-A, -B, -C and –D regulate angiogenesis through binding to the related family of receptor tyrosine kinases (RTKs): VEGF receptors (VEGFR)-1, -2 and -3. The VEGFR consists of an extracellular ligand binding site, a transmembrane α-helical domain and an intracellular protein-tyrosine kinase region. Once activated, phosphorylated tyrosine residues on these receptor kinases provide high-affinity binding sites for components of the Raf/MEK/ERK (MAP Kinase) and PI3K/AKT signalling pathways which mediate cell growth and angiogenesis. Inhibition of the pathway involving VEGF-A activation of VEGFR-2 has undergone the most extensive investigation in recent years. This pathway mediates the formation and preservation of the blood vessel network which is vital for tumour cell survival and proliferation (Casanovas et al., 2005). In RCC, VEGF is also a powerful tumour growth factor. RCCs over-express the different VEGFRs and also produce as paracrine and autocrine growth factors, large amounts of VEGF (Qian et al., 2009). In tumours the VEGF isoforms –C and –D have been shown to activate the VEGFR3 receptor and to initiate the development and maintenance of a lymphatic system (He et al., 2005). Targeting this process in cancer treatment is now in the early stages of development. Presently, different therapeutic avenues exist for inhibiting the activation of receptor tyrosine kinases (RTKs). Monoclonal antibodies (mAbs) against growth factor ligands, or antibody fragments against RTK ligand-binding domain, can prevent binding of growth factors, thus attenuating RTK activity. Alternatively, the protein kinase can be targeted. Drugs that bind reversibly to the ATP-binding site within the kinase domain or to a small pocket that is immediately adjacent to the ATP-binding site are used to block the enzymatic activity of the kinase. Due to similarities within the amino acid structure of the kinase domain, ATP-competitive inhibitors can have cross reactivity with other structurally related kinases.

2.1 VEGF- antibody therapy/ligand competitors

2.1.1 Bevacizumab

Bevacizumab is an i.v. administered humanized monoclonal IgG1 antibody that targets and neutralises all major isoforms of circulating VEGF (Presta et al., 1997). By binding with high affinity to VEGF, bevacizumab inhibits its interaction with tyrosine kinase receptors thereby preventing the initiation of an angiogenic signal (Figure 1). This weakens existing microvasculature and production of new vasculature is inhibited. The loss of vascularisation eventually leads to tumour cell death (Jain, 2005).

Bevacizumab is used in the treatment of a wide range of cancer types including RCC, colon, brain and lung cancers. It was approved in 2009 for the first-line treatment of patients with advanced RCC or mRCC in combination with IFN-α. FDA approval came as a result of the phase III AVOREN trial. RCC patients following previous nephrectomy were randomized to receive either bevacizumab plus IFN-α or placebo plus IFN-α (Escudier et al., 2007a). The addition of bevacizumab to IFN-α significantly improved both the overall response rate (ORR) (30.6 vs. 12.4%, $P<0.0001$) and PFS (10.2 vs. 5.4 months). Subgroup analysis, however, indicated that the advantage in PFS related only to favourable and intermediate risk patients and not to the poor risk group. The final overall survival (OS) results reported no significant difference between the bevacizumab and control groups (23.3 vs. 21.3 months). However, these findings may have been influenced by the fact that 63% of patients in the placebo plus IFN-α group and 55% of patients in the bevacizumab plus IFN-α group received second-line therapy with other agents (Escudier et al., 2010).

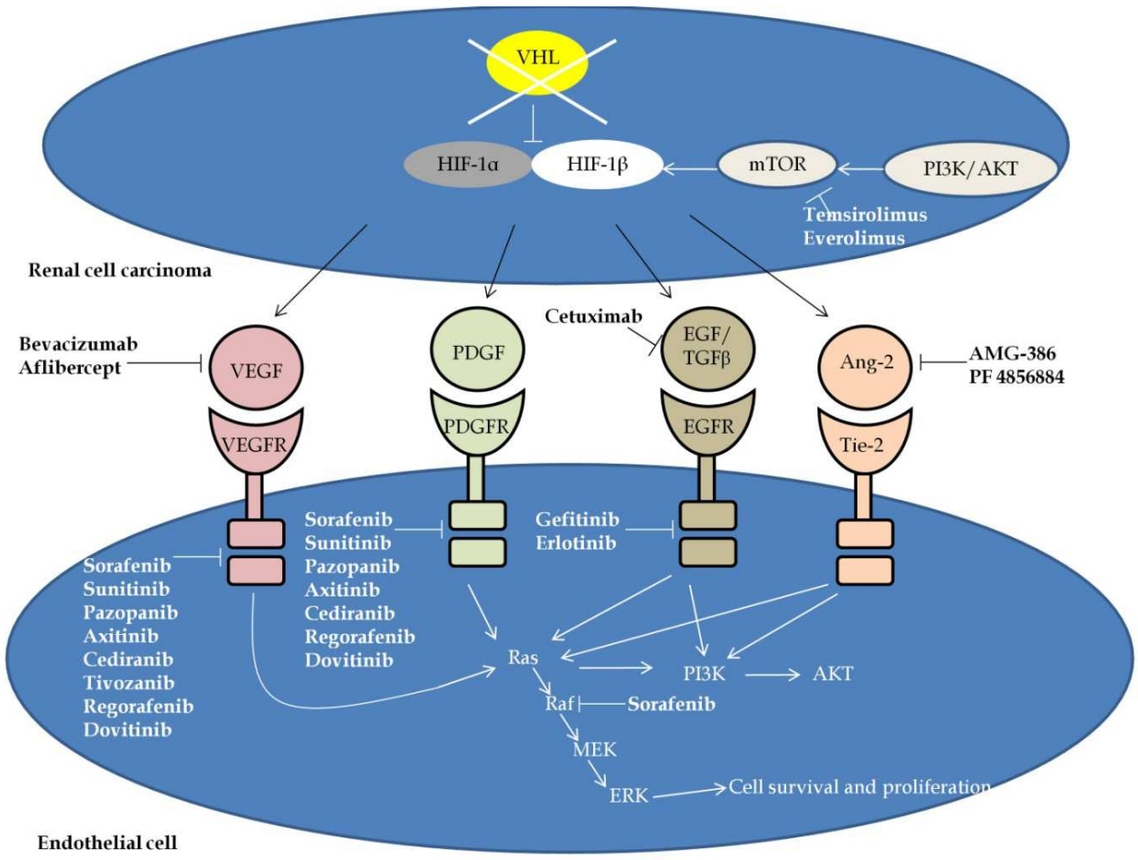

Fig. 1. Schematic representation of the signalling pathways contributing to angiogenesis and cell proliferation in RCC and the targeted agents which inhibit them. HIF activation upregulates the expression of VEGF, PDGF, EGF and Ang-2. Binding of these ligands to their receptors induces downstream activation of MAPK signalling, resulting in angiogenesis. Abbreviations; VHL: Von Hippel Lindau; HIF: hypoxia-inducible factor; mTOR: mammalian target of rapamycin; PI3K: phosphoinositol-3 kinase; VEGF(R): vascular endothelial growth factor (receptor); PDGF(R): platelet-derived growth factor (receptor); EGF(R): epidermal growth factor (receptor); TGF-β: transforming growth factor beta; Ang-2: angiopoietin 2. Inhibitory arrows (⊣) show clinically available or in development therapeutic agents for the treatment of RCC and mRCC.

2.1.2 Aflibercept

Aflibercept is an engineered fusion protein designed to interact with all isoforms of VEGF and to placental growth factor (PLGF), thereby preventing them from binding to VEGFRs. It is composed of the extracellular domain 2 of VEGFR1 and extracellular domain 3 of VEGFR2 fused to an Fc segment of human IgG1 (Wulff et al., 2002). Aflibercept appears to have a greater affinity for VEGF than bevacizumab, resulting in a more complete obstruction of VEGF signalling. This together with the fact that aflibercept binds to PLGF while bevacizumab does not, may explain why preclinical studies have shown aflibercept to be more effective than bevacizumab (Kim et al., 2002).

2.2 Receptor tyrosine kinase inhibitors (RTKIs)

The intracellular kinase activity of growth factor receptors also provides an attractive target for therapeutic intervention. Three receptor tyrosine kinase inhibitors are currently available for the treatment of clear cell RCC and many more are in development (Figure 1). By targeting the intracellular tyrosine kinase domain of multiple growth factor receptors these drugs inhibit not only the VEGF pathway but also the platelet derived growth factor (PDGF) pathway as well as other kinases critical for proliferation and angiogenesis.

2.2.1 Sorafenib

Sorafenib was the first multi-targeted kinase inhibitor approved for the treatment of patients with cytokine-refractory advanced RCC or mRCC. It is a potent small molecule dual-action inhibitor, first identified in *in vitro* assays as an inhibitor of Ras signalling. Sorafenib also inhibits VEGFRs and PDGFRs (Wilhelm et al., 2004). As multiple kinases are inhibited by sorafenib it is difficult to determine the relative contribution of each target to the anti-tumour activity of this drug. Preclinical studies in a variety of cancer models suggest sorafenib acts on both tumour cells and tumour vasculature by inhibiting cellular proliferation and angiogenesis pathways (Wilhelm et al., 2008).

Sorafenib's approval by the FDA in 2005 was as a result of a phase III trial namely; Treatment Approaches in Renal Cell Cancer Global Evaluation Trial (TARGET). All participants had advanced RCC and had experienced disease progression in spite of the then standard cytokine therapy. Patients were randomly assigned to receive sorafenib or placebo (Escudier et al., 2007b). At the first interim analysis the ORR was 10% for sorafenib compared with 2% for placebo. Also, sorafenib treated patients had a significant PFS advantage of 5.5 months versus 2.8 months for the placebo group. At this point those patients initially assigned to receive placebo were allowed to switch to sorafenib, potentially obscuring differences in end point results. In fact the final analysis of all patients registered in the trial did not show a statistically significant difference in the OS of the initial intent-to-treat population (17.8 vs. 15.2 months in sorafenib and placebo treated patients, respectively). However, a secondary analysis was performed in which patients who crossed over to sorafenib after initial treatment with placebo were censored. This demonstrated a significant benefit for sorafenib treatment with a median OS of 17.8 months compared to 14.3 months for placebo (Escudier et al., 2009). In terms of side effect profile sorafenib treated patients reported fewer adverse effects and a better overall quality of life than those receiving IFN-α. Both hypertension and skin toxicity, in general, are common manifestations of toxicity with multiple tyrosine kinase inhibitors, with incidences of 17% and 40% respectively, outlined in the TARGET trial. Other adverse effects associated with sorafenib treatment are diarrhea (Escudier et al., 2007b), and an additive loss of muscle mass above that usually observed in patients with advanced cancer (Antoun et al., 2010).

2.2.2 Sunitinib

Sunitinib is a RTKI designed to prevent cells from responding to the elevated level of pro-angiogenic signals associated with RCC. It is an orally available, small molecule, multi-targeted kinase inhibitor with activity against VEGFRs, PDGFRs, fms-like tyrosine kinase receptor-3 (FLT-3) and stem cell factor receptor (c-KIT). Sunitinib is classified as an ATP competitive inhibitor. It received accelerated approval by the FDA in 2006 based on responses in patients with mRCC who had failed cytokine therapy. Regular approval was obtained in 2007 as a result of a phase III study evaluating sunitinib as a first-line therapy

compared with IFN-α. Results of the trial demonstrated a considerable advantage for sunitinib over IFN-α and both OS rates and PFS were significantly higher for the sunitinib treated group (Motzer et al., 2007a; Motzer et al., 2009). Sunitinib is now the standard of care for initial therapy of good to moderate prognosis mRCC patients. OS of over two years is a marked improvement on the one year OS rates observed before the advent of targeted kinase inhibitor therapy (Motzer et al., 2009).

As sunitinib inhibits multiple kinases and therefore blocks several signalling pathways, numerous side effects are associated with treatment. These, however, are favourable when compared to the significant toxicity profile associated with the previous therapeutic option for RCC i.e. immunotherapy. In the phase III trial which led to the approval of sunitinib, slightly different toxicity profiles were observed between the two treatment groups. Sunitinib treated patients more commonly experienced diarrhea, hypertension, hand-foot syndrome, neutropenia and thrombocytopenia while fatigue occurred more commonly in the IFN-α group. Overall, however, patients who received sunitinib reported a better quality of life compared to patients treated with IFN-α.

2.2.3 Pazopanib

Pazopanib is the latest multiple kinase inhibitor approved for the first-line treatment of patients with advanced RCC. It inhibits signalling by VEGFRs, PDGFRs, and c-KIT by competitively binding to the ATP enzymatic pocket of the RTK. Pazopanib differs from sunitinib and sorafenib as the range of targets it potently inhibits is narrower. FDA approval followed a phase III trial involving clear cell (or predominately clear cell) RCC patients with no previous treatment history (54%), or who had progressed following a single prior cytokine treatment (46%) (Sternberg et al., 2010). Patients were randomized to receive either pazopanib or placebo daily. In the overall population the primary end point of PFS was significantly higher in the pazopanib group compared to placebo (9.2 vs. 4.2 months). The ORR for the pazopanib group was 30%. Although a non-significant improvement in median OS of 22.9 months for the pazopanib group versus 20.5 months for the placebo group was reported, this analysis had been confounded by the early and high level of patient cross-over from placebo to pazopanib upon progression (Sternberg, 2010).

The toxicity profile associated with pazopanib treatment is similar to both sunitinib and sorafenib. The most common effects observed include hypertension, diarrhea, nausea, hair depigmentation and asthenia. Clinical trials cannot easily be compared, however, as evident from a phase III trial of pazopanib which demonstrated lower incidence of hand-foot syndrome, diarrhea and asthenia compared with sunitinib and sorafenib. Conversely, the incidence of hypertension associated with pazopanib treatment in the phase III trial is high (40%) when compared to sunitinib and sorafenib trials (Lang & Harrison, 2010).

2.2.4 In development

The new generation of RTKIs in development for the treatment of RCC display greater potency and selectivity for VEGFRs compared to the established kinase inhibitors discussed above. It is hoped that this increased potency and high specificity will give rise to enhanced anti-tumour activity. Furthermore, the absence of off-target (non-VEGFR) inhibition may result in less toxicity than is normally associated with kinase inhibitors in general.

2.2.5 Axitinib (AG-013736)

Axitinib is an orally available RTKI. Picomolar concentrations are sufficient for axitinib to inhibit VEGFRs, while it inhibits PDGFR-β and c-KIT at low nanomolar concentrations (Hu-Lowe et al., 2008). In this study and others (Inai et al., 2004) axitinib in mouse models has demonstrated anti-tumour, anti-angiogenic and anti-metastatic properties as well as having an ability to induce central necrosis. Axitinib is now being looked at for the second-line treatment of advanced RCC. In a phase II trial of patients with cytokine-refractory mRCC (Rixe et al., 2007), axitinib displayed an ORR of 44.2% and median PFS of 15.7 months, greater than any agent investigated for mRCC treatment to-date. However, this efficacy has not been examined in comparative trials with other targeted agents.

2.2.6 Cediranib (AZD2171)

Cediranib is an ATP-competitive inhibitor of RTKs and like axitinib is a potent inhibitor of VEGFRs and PDGFR-β at subnanomolar concentrations (Gomez-Rivera et al., 2007; Takeda et al., 2007). In a recent placebo controlled phase II trial, a median PFS of 12.1 months was observed in patients treated with cediranib compared to 2.7 months for those who received placebo. Furthermore, the mean change in tumour size in patients receiving cediranib was a 20% decrease versus a mean increase of 19% for patients randomized to placebo (Bhargava & Robinson, 2011).

2.2.7 Tivozanib (AV-951)

Tivozanib is an orally active RTKI and is selective for VEGFRs at picomolar concentrations (Nakamura et al., 2006). In a phase II trial, clear cell RCC patients who had undergone nephrectomy displayed an ORR of 32% to 1.5 mg tivozanib daily. The median PFS for patients was 14.8 months (Bhargava et al., 2010). This potency combined with the selectivity of tivozanib for VEGFRs reduces the inhibition of off-target kinases, resulting in less toxicity. The most common side effects reported in this trial were hypertension and dysphonia while incidences of other toxicities usually associated with RTKIs (fatigue, diarrhea and hand-foot syndrome) were low. The occurrence of fewer toxicities together with the specificity of tivozanib allows it to be safely combined at full dose and scheduled with another targeted agent. For example, preliminary results of a phase I trial combining tivozanib and the mTOR inhibitor temsirolimus in mRCC patients reported no dose limiting toxicities (Fishman et al., 2009).

2.2.8 Regorafenib

Regorafenib is a potentially significant multi-kinase inhibitor in that it inhibits the traditional targets of VEGFRs, PDGF-β and fibroblast growth factor (FGF)-1, as well as the endothelium specific receptor tyrosine kinase Tie-2. The inhibition of targets both within and external to the VEGF axis may offer valuable therapeutic advantages when it comes to avoiding resistance and enhancing the efficacy of targeted therapy. A phase II trial of regorafenib in mRCC patients showed that those receiving regorafenib had a 27% partial response and a 42% stable disease rate (Eisen et al., 2009).

2.2.9 Dovitinib (TKI258)

Dovitinib is also a promising agent targeting VEGFRs, PDGFRs, FLT3 as well as FGF receptors namely FGFR3. This is significant as not only does the FGF angiogenic signalling

pathway provide a potential mechanism of resistance to VEGF therapy, activating mutations or upregulation of FGF/FGFRs have been identified in RCC (Emoto et al., 1994). Members of the FGF family are involved in proliferation, differentiation and migration of a range of cell types. A phase II study of dovitinib in previously treated advanced RCC or mRCC patients has reported results which include a median PFS and OS of 6.1 and 16 months, respectively (Angevin et al., 2011).

3. Targeting EGFR

Disruption of EGF signalling is a popular therapeutic mechanism in a number of cancer types. As the expression of ligands of the EGFR (including EGF and the angiogenesis promoting transforming growth factor (TGF)-β) is upregulated by *VHL* inactivation, the validity of this approach in clear cell RCC was explored in a number of trials. In single-agent EGFR inhibitor trials, gefitinib (a selective EGFR TKI) and cetuximab (a recombinant mouse-human mAb) were administered as monotherapy. Neither agent demonstrated a complete or partial response (Staehler et al., 2005). In a randomized phase II trial, clear cell RCC patients received either bevacizumab or bevacizumab plus the EGFR inhibitor erlotinib. The results showed identical median PFS and ORR between the two groups (Bukowski et al., 2007). EGFR inhibition therefore does not appear to be a viable approach for the treatment of clear cell RCC. A possible reason for this may be the rarity of EGFR mutations in RCC, when compared to other cancers (Dancey, 2004). Furthermore, the activators of EGFR signalling which are upregulated in RCC can also initiate VEGFR signalling, making the inhibition of EGFR alone insufficient to disrupt tumour proliferation and angiogenesis.

4. Targeting PDGF

Members of the PDGF family include PDGF-A, -B, -C and –D and mediate their effects through binding to PDGFR-α and –β leading to the activation of various signalling pathways giving rise to tumour growth (Guo et al., 2003; Pietras et al., 2003). High levels of PDGF-D has been shown to be associated with RCC and its progression has been linked to PDGF-D/PDGFR-β signalling and PDGFR-α expression (Sulzbacher et al., 2003). Although there is not a wealth of data published on PDGF and PDGFR in RCC there are currently many drugs either clinically available or in development that target this RTK as outlined above and in Figure 1.

5. Limitations of currently available targeted agents

Comparison of the relative benefit of each targeted treatment in advanced RCC and mRCC is exceptionally difficult. Trials conducted targeting RTKs involved varied patient populations with differences in prior treatment status, prognosis and histology of RCC. According to Flaherty & Puzanov the easiest comparison is ORR. This comparison identifies two groups: bevacizumab and sorafenib generate a response rate of ≤10%, while sunitinib and pazopanib induce a response in ≥30% of patients. However, this does not reflect any clinical benefit as higher response rates do not appear to be associated with improved PFS or OS (Flaherty & Puzanov, 2010).

Resistance is a major problem with both older and newer therapies as well as mono and poly therapies. Few complete responses are associated with any of the targeted therapies

discussed. All patients will eventually develop resistance and progress, usually within 8 to 16 months (Sosman & Puzanov, 2009). A number of mechanisms have been described outlining how resistance can occur. Sorafenib, sunitinib and pazopanib therapy is associated with a significant increase in VEGF production (Deprimo et al., 2007; Kumar et al., 2007; Veronese et al., 2006). Resistance may occur if the increase in VEGF reaches a threshold that can overcome the inhibition. Another hypothesis has been examined in animal models of tumour angiogenesis. These models outline that the inhibition of VEGF or VEGFR leads to upregulation of PDGF and basic FGF (bFGF) production by tumours activating alternative pathways for angiogenic signalling (Fernando et al., 2008). Despite the overlap of targets and inevitable resistance, however, RCC tumours do not appear to be totally cross-resistant to sequential therapy with different agents. In a phase II study, patients who had progressed on sunitinib underwent treatment with sorafenib. Objective responses resulted in 10% of patients with a median PFS of 16 weeks (Di Lorenzo et al., 2009). A possible explanation is that when the initial inhibitor is removed (once resistance has occurred and the tumour has progressed), cells revert back to VEGF signalling.

Presently, it is hoped that combination therapies for the treatment of patients with RCC will see improvements in PFS, ORR and OS greater than those seen with any single agent. There are two options for the combination of targeted therapies i.e. vertical blockade and horizontal blockade. Vertical blockade involves targeting several steps along a single signalling pathway. This is an attractive option in treatment of RCC combining drugs which inhibit hypoxia-inducible factor (HIF) translation, VEGF or VEGFR. This approach could provide an opportunity to overcome the resistance associated with increased levels of VEGF. A phase I trial evaluated the potential benefit of combining sorafenib with bevacizumab in mRCC patients (Sosman, 2008). Due to toxicity, the dosage of both agents had to be reduced from their usual levels to half the recommended dose of bevacizumab and one-quarter the usual dose of sorafenib. Despite this a 50% response rate was observed, a vast improvement on the 10-15% achieved with full doses of each agent alone. The combination strategy of horizontal blockade entails simultaneously targeting more than one signalling pathway crucial for tumour cell survival and proliferation. Horizontal blockade is an appealing option as combining VEGFR inhibitors with other tyrosine kinase inhibitors may prove useful in preventing resistance by the mechanism of alternative angiogenic pathways.

6. PI3K/AKT/mTOR signalling pathway

The phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin-pathway (PI3K/AKT/mTOR-pathway) is one of the most common aberrant pathways activated in cancer, regulating many known oncogenic pathways including apoptosis, proliferation and cell migration (Carracedo & Pandolfi, 2008; Klein & Levitzki, 2009; Inoki et al., 2005). Activation of PI3K occurs at the cell membrane in response to several RTKs including the EGFR, the insulin-like growth factor receptor (IGFR) and the PDGFR leading to the production of the second messenger phosphatidylinositol-3,4,5-triphosphate (PIP3). Levels of PIP3 are tightly regulated by the tumour suppressor phosphatase and tensin homolog (PTEN), serving as the negative regulator of the PI3K/AKT/mTOR pathway. AKT is recruited to the cell membrane by PIP3 and phosphorylated to its fully activated form by phosphoinositide dependent protein kinase 1 (PDK1). Activated AKT can directly activate mTOR by phosphorylation or indirectly through inactivation of the tuberous sclerosis complex 2 (TSC2) which normally inhibits mTOR (Figure 2).

mTOR is a 290kDa serine/threonine protein kinase and is highly conserved from fungi to mammals. It forms multimolecular complexes and plays a key role in diverse signalling events such as growth, proliferation, survival, angiogenesis and protein synthesis (Dowling et al., 2010). mTOR responds to a range of diverse stimuli including growth factors, cytokines, and hormones but also acts as an important sensor of cellular stresses imposed by hypoxia, pH or osmotic alterations, heat shock, oxidative stress and DNA damage. Defects in signalling components upstream of mTOR including excessive growth factor receptor activation or mutation correlates with a more aggressive tumour and a worse prognosis (Faivre et al., 2006). mTOR exists as two distinct complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (Figure 2). Regulation of mTOR activation is controlled by two components of the tuberous sclerosis complex (TSC) comprising TSC1 (hamartin) and TSC2 (tuberin). When they heterodimerise mTOR is inhibited and can no longer phosphorylate downstream substrates (Dancey, 2005). Phosphorylation of TSC2 by AKT, however, promotes dissociation of the TSC1/TSC2 complex which activates the guanosine triphosphate Rheb. Rheb activity subsequently activates mTORC1. mTORC1 is bound to raptor (regulatory-associated protein of mTOR), mLST8 (mammalian lethal with sec 13 protein 8), also known as GβL and PRAS40 (proline-rich AKT substrate 40kDa) which is phosphorylated by AKT causing its dissociation from raptor and its subsequent activation. mTORC2 complexes with rictor (rapamycin-insensitive companion of mTOR), mSIN1, mLST8 and Protor-1 or Protor-2. Both complexes phosphorylate the hydrophobic motifs of AGC kinase family members. mTORC1 phosphorylates S6K and the inhibitory binding protein 4E-BP1 at Thr37 and Thr 46 which acts as a priming event essential for the phosphorylation of Ser65 and Thr70 leading to the release of eIF4E and subsequent assembly of the eIF4F complex (Gingras et al., 2001). Activation of S6K in turn leads to phosphorylation of inhibitory sites (Ser636 and 639) on the insulin receptor substrate-1 (IRS-1), thereby suppressing IRS-1 mediated activation of the PI3K/AKT pathway. mTORC2 activation leads to phosphorylation of AKT, SGK1 and PKC which control cell survival and cytoskeletal organization (Figure 2).

mTORC1 is frequently dysregulated in cancer (Guertin & Sabatini, 2007). Loss or inactivation of tumour suppressors such as p53, liver kinase B1 (LKB1), PTEN and TSC1/2 which antagonise PI3K-dependent activation of mTORC1 can promote tumourigenesis via increased signalling through mTORC1 (Sabatini, 2006; Shaw et al., 2004). Moreover, increased levels and/or phosphorylation of downstream targets of mTORC1 have been reported in various human malignancies in which they correlate with tumour aggressiveness and poor prognosis (Guertin & Sabatini, 2007; Mamane et al., 2006). Collectively these studies suggest that aberrant mTORC1 signalling is linked to dysregulated control in cancer and for this reason the spotlight has been shone on mTORC1 as a possible therapeutic target for anti-cancer therapy.

6.1 mTOR and RCC

As outlined above the signalling network controlled by the PI3K/AKT/mTOR axis is very often found to be dysregulated in human malignancy. There is a wealth of data to support the notion that signalling through mTOR is dysregulated in RCC. This makes this cancer type in particular an attractive target for mTOR inhibitor therapy. RCC demonstrates increased phosphorylation of AKT, S6K1 and mTOR as well as increased expression of PTEN and disrupted TSC complexes (Pantuck et al., 2007). mTOR activation ultimately leads to increased production of angiogenic factors leading to a highly vascularised tumour which is evident in RCC.

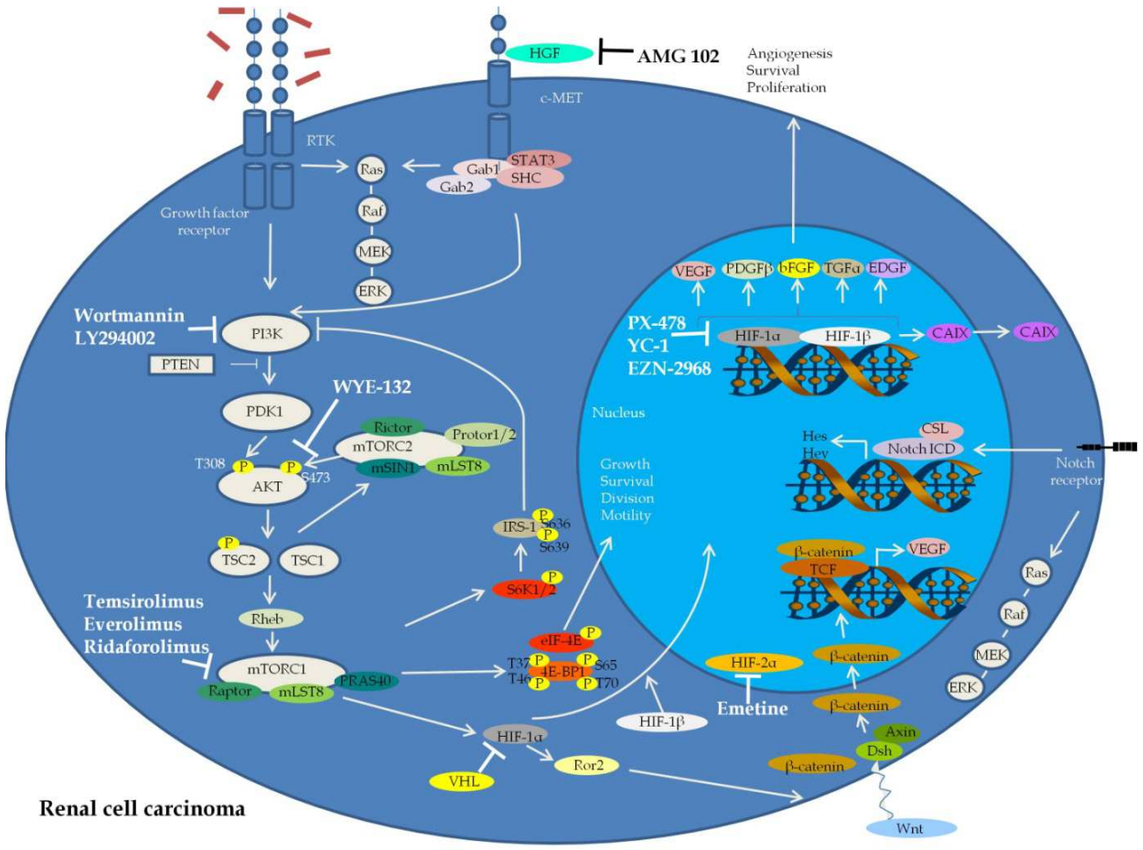

Fig. 2. Schematic representation of mTOR signalling, phosphatidylinositide 3'OH-kinase/AKT/mTOR signalling, Wnt/β-catenin signalling, HGF signalling and core NOTCH signalling pathways. In relation to growth factor signalling, PI3K activates downstream mTORC1 giving rise to HIF-1α activation, which in turn switches on gene expression required for angiogenesis and cell proliferation in endothelial cells. HGF binding to MET leads to its phosphorylation and subsequent recruitment of adapter proteins such as Gab1, Gab2, SHC, STAT3 and PI3K with downstream activation of Ras/MAPK and PI3K/AKT pathways. Wnt pathway activation leads to hypophosphorylated β-catenin where it translocates into the nucleus and forms a complex with TCF. Ligand binding to the Notch receptor leads to release of the intracellular domain (ICD) of Notch. Notch ICD subsequently translocates into the nucleus, where it forms a complex with essential cofactors such as the transcription factor CSL. This complex mediates the transcription of target genes such as HES and HEY. Abbreviations; RTK: receptor tyrosine kinase; PI3K: phosphatidylinositol 3-kinase; PDK1: phosphoinositide dependent protein kinase 1; PTEN: phosphatase and tensin homolog; mTOR: mammalian target of rapamycin; TSC: tuberous sclerosis complex; mLST8: mammalian lethal with sec 13 protein 8; PRAS40: prolin-rich AKT substrate 40 kDa; IRS-1: insulin receptor substrate-1; VHL: von Hippel-Lindau; HIF: hypoxia inducible factor; TCF; T cell factor; HGF: hepatocyte growth factor; CSL: CBF1, Suppressor of hairless, Lag-1; HES: hairy enhancer of split; Hey: hairy enhancer of split related with YRPW. Inhibitory arrows (┤) show clinically available or in development therapeutic targets for the treatment of RCC and mRCC.

mTOR inhibitors were originally developed as immunosuppressants for patients undergoing transplantation with rapamycin (also known as sirolimus or Rapamune®) being the first mTOR inhibitor identified. Clinical experience and subsequent trials identified the anti-proliferative (Schmelzle & Hall, 2000) and anti-angiogenic (Del Bufalo et al., 2006) properties of these agents in various cancer types, including RCC. This is of particular clinical importance as RCC demonstrates significant uncontrolled angiogenesis. More specifically mTORC1 activity is inhibited by rapamycin and associated analogs (temsirolimus, everolimus and ridaforolimus) which are collectively termed rapalogs. mTORC2, however, is largely insensitive to rapalogs, although prolonged treatment may be able to reduce mTORC2 activity in some cell types (Sarbassov et al., 2006).

6.1.1 mTOR inhibitors-rapamycin

Rapamycin is a naturally occurring macrolide triene antibiotic that acts as a specific, allosteric inhibitor of mTORC1 (Hay & Sonenberg, 2004). Rapamycin either associates with the immunophilin FKBP12 (FK 506-binding protein of 12 kDa) and the resulting complex interacts with the FRB (FKBP12-rapamycin binding) domain located in the C-terminus of mTOR or directly to FRB. This binding prevents the binding of mTORC1 to raptor which is thought to uncouple it from its substrates 4E-BPs and S6Ks (Oshiro et al., 2004). The ability of rapamycin to suppress both cellular proliferation and growth through its interaction with mTORC1 indicated that it could be used as an anti-cancer agent (Faivre et al., 2006). This lead to the development of rapamycin analogs (rapalogs) which display the same pharmacodynamics as the parent drug but have improved pharmacokinetic properties.

6.1.2 Temsirolimus

Temsirolimus (Torisel®), also known as CCI-779, is a macrocyclic lactone and a water-soluble ester prodrug of rapamycin. It is administered by i.v. injection, is rapidly cleared from the plasma and is converted by CYP4503A4/5 into rapamycin. It binds with high affinity to the immunophilin FKBP12 and selectively inhibits mTORC1 with no effect on mTORC2 (Le Tourneau et al., 2008). Inhibition of mTORC1 kinase activity results in decreased phosphorylation of S6K and 4E-BP1. In addition by inhibiting mTORC1 it has been shown to reduce expression of HIF-1α and -2α which leads to decreased VEGF and PDGF expression (Thomas et al., 2006). Thus, the clinical efficacy of temsirolimus reflects bimodal phamacodynamics resulting in null signalling of RTK cascades and inhibition of protein synthesis can result in inhibition of cell cycle and tumourigenesis. Temsirolimus was approved as first-line therapy for patients with mRCC in the US and Europe in May 2007 demonstrating improved efficacy in poor-prognosis patients in comparison with IFN-α (Hudes et al., 2009). The efficacy of temsirolimus in the second-line setting remains unclear. However, recently it has demonstrated disease control rate of 70% and overall median time to progression of four months in intermediate to poor-prognosis patients with VEGF-refractory mRCC (Mackenzie et al., 2011). Side effects of the drug include diarrhoea, asthenia stomatitis, rash, nausea, anorexia, hypertension, dyspnea, hyperglycaemia, hypercholesterolemia and anemia.

6.1.3 Temsirolimus plus immunotherapy combination

Temsirolimus was first investigated as combination therapy with IFN-α in phase I/II study (Motzer et al., 2007b). This study revealed that the combination of the two had an accepted

safety profile and displayed anti-tumour activity in patients with mRCC. A pivotal phase III trial was also carried out comparing temsirolimus or temsirolimus plus IFN-α with IFN-α alone in patients with mRCC. In summary, the median OS time was significantly longer with temsirolimus alone than with IFN-α alone (10.9 months versus 7.3 months, respectively), and combination therapy with temsirolimus and IFN-α did not lead to a significantly longer median OS time than with IFN-α alone (8.4 months versus 7.3 months, respectively) (Hudes et al., 2007).

6.1.4 Temsirolimus plus anti-angiogenics combination

Phase I studies examining the efficacy of temsirolimus with sunitinib have not shown sufficient safety to-date. Trials using temsirolimus in combination with sunitinib and temsirolimus in combination with sorafenib were discontinued owing to significant toxicity (Patel et al., 2009). The efficacy of temsirolimus plus bevacizumab has also been studied but again based on toxicity profiles a phase II trial has indicated that this combination cannot be recommended for patients with mRCC (Negrier et al., 2011). In summary, the combined usage of temsirolimus and anti-angiogenic agents has proved disappointing to date, phase III trials are still continuing whose results may shed light on possible best practice for combination therapy in the near future.

6.2 Everolimus

Everolimus (Afinitor®) is an orally bioavailable hydroxyethyl ester of rapamycin. Like temsirolimus it is an inhibitor of mTORC1 and was approved on March 30th, 2009 for patients suffering from advanced RCC following failure when treated with previous TKI therapy (de Reijke et al., 2009). It has now become the standard second-line agent after the approved first-line drugs sunitinib and/or sorafenib (Soulieres, 2009).

6.2.1 Everolimus plus anti-angiogenics combination

The combination of everolimus and the VEGFR TKIs are showing promise in initial studies. A phase II study of everolimus plus sorafenib has been prompted following successful completion of a phase I trial with the combination of both demonstrating acceptable toxicity and evidence of anti-tumour activity in patients with previously untreated mRCC (Harzstark et al., 2011). Similarly, a phase II trial of everolimus plus sunitinib is warranted following successful maximum-tolerated dose of everolimus plus sunitinib in patients with mRCC (Kroog et al., 2009). In contrast, everolimus plus imatinib is not recommended for future studies following results from a phase II study in previously treated patients with mRCC as the combination did not demonstrate a three month PFS rate of 49%, which did not meet the specified criteria for continuation (Ryan et al., 2011). Finally, in a phase II trial with two different mRCC patient cohorts, one with and one without prior TKI treatment, everolimus plus bevacizumab was active and well tolerated (Hainsworth et al., 2010). This regimen which uses full doses of each agent, is being evaluated as first-line therapy in a phase II study, RECORD (Renal Cell Cancer Treatment With Oral RAD 001 Given Daily)-2.

6.3 Ridaforolimus

The mTOR inhibitor, ridaforolimus (formerly deforolimus), is yet another promising rapamycin analog in RCC treatment but not yet approved. Ridaforolimus (also known as AP23573), a non-prodrug of rapamycin, has demonstrated prominent anti-proliferative

activity against a range of cancers (Hartford et al., 2009). The most common side effects associated with ridaforolimus to-date include stomatitis, fatigue, diarrhoea and thrombocytopenia. Important additional information such as OS and the safety profile of ridaforolimus has yet to be identified.

6.4 Combination therapy

At present there are numerous ongoing and planned studies evaluating the efficacy of both temsirolimus and everolimus with other targeted therapies including VEGF ligand competitors, VEGFR inhibitors, AKT inhibitors, p70S6R inhibitors, tubulin inhibitors, IGF-1R antagonists and Bcr-ABL antagonists (Pal & Figlin, 2011). Information pertaining to the successes associated with these exploratory trials is limited at present.

6.5 New mTOR inhibitors

Presently, temsirolimus and everolimus are approved for the treatment of mRCC. Despite their efficacy there are some drawbacks including resistance but also the fact that they are both specific mTORC1 inhibitors that lack activity against mTORC2. This allows the latter to activate AKT. Indeed, increased activation i.e. phosphorylation of AKT has been documented in tumour biopsies isolated from patients treated with rapalogs (O'Reilly et al., 2006). In addition the crosstalk with other pathways such as MEK/ERK on AKT could limit the efficacy of mTOR inhibitors. There are reports of newer drugs targeting mTORC2 as well as MAPK interacting pathways. New mTOR inhibitors are not rapalogs but are small molecule inhibitors resembling TKIs. They bind competitively and reversibly to the mTOR-ATP binding pocket blocking the enzymatic activity of the kinase. Compounds such as PP242, Torin1, WYE-354, WYE-125132 (WYE-132) and Ku-006379 suppress both mTORC1 and mTORC2 displaying more dramatic effects on cell growth, proliferation and cell cycle than rapamycin. This has been attributed to suppression of mTORC2 mediated AKT phosphorylation at Ser 473 and greater inhibition of 4E-BP1 phosphorylation (Thoreen et al., 2009). Active site mTOR inhibitors have the potential to be potent anti-cancer drugs as they inhibit mTORC2 activity which rapamycin and its analogs do not but also because they counteract the activation of AKT which can occur as a result of rapamycin-mediated disruption of the mTOR/S6K/IRS-1 negative feedback loop. To-date these potentially effective cancer therapeutic agents have yet to be investigated in patients with RCC or mRCC.

7. Targeting mTOR upstream moieties – PI3K

PI3K is a lipid kinase that converts phosphatidylinositol bisphosphate to PIP3. PI3K further recruits PDK1 and AKT to the cell membrane where PDK1 activates AKT. Because of its location upstream of mTOR, it has become another attractive target for treatment of patients with RCC to be used solely or in combination with existing mTOR inhibitors. Recently, activation of the PI3K pathway has been shown to be directly linked to adverse clinical outcomes in patients with RCC (Merseburger et al., 2008). The PI3K inhibitor prototypes wortmannin and LY294002 have been shown to decrease AKT activation and significantly reduce cell growth *in vitro* particularly in PTEN-null or PI3K-overexpressing RCCs with the latter also demonstrating *in vivo* tumour regression (Sourbier et al., 2006). Given that PI3K is highly expressed in RCC metastases, which are themselves radioresistant, newer generation PI3K inhibitors such as PX-866, with better bioavailability and less toxicity, may show utility

as radiosensitizers in RCC metastases. Another chemotherapeutic drug, PI-103, has recently been shown to independently inhibit both PI3Kα and mTOR (Fan et al., 2006) thereby overcoming a potential disadvantage of rapamycin in the treatment of AKT-dependent tumors. In fact GDC-0941 which is derived from PI-103 has demonstrated improved bioavailability and partial response in breast and ovarian cancer patients. Ongoing studies using PI3K and dual PI3K/mTOR inhibitors such as SAR245408, SAR245408, NVP-BKM120 and NVP-BEZ235 modified for clinical use are ongoing (Maira et al., 2008). These have yet to be tested in patients with RCC.

7.1 PDK1

Similar to PI3K, a key mediator of AKT activation, PDK1 is poised to respond to targeted inhibition with blockade of AKT signalling (Figure 2). Both highly specific inhibitors, such as AR-12 (Arno), and inhibitors with dual function on PDK1/PI3K or PDK1/AKT are in development (Najafov et al., 2010). These targets present another potentially robust way to render the AKT pathway completely inhibited, mitigating the confounding issues of inhibition of each of the AKT family members.

7.2 AKT

AKT, also known as protein kinase B (PKB), can be activated by a number of mechanisms including PIP3 activation of PDK1 at Thr308 and at Ser473 by mTORC2 (Figure 2). Decreased expression of the inhibitory PTEN can also activate AKT (Hara et al., 2005). Upon phosphorylation (p-)AKT is known to interact with a large set of substrates, including mTOR and through inactivation of the TSC impacts many key cellular processes such as cell cycle progression and apoptosis, both of which play a vital role in oncogenesis. Recently, p-AKT expression was shown to be correlated with pathologic variables and survival, with higher levels of cytoplasmic p-AKT expression compared with nuclear p-AKT in primary RCC (Pantuck et al., 2007). Recently, a specific p-AKT (S473) inhibitor, WYE-132, has been tested on RCC cell lines and achieved complete regression of A498 large tumours when administered with bevacizumab (Yu et al., 2010).

8. Targeting downstream moieties-HIF-1α

RCC is closely linked to mutations in the von Hippel-Lindau (*VHL*) gene. A deletion of one allele of *VHL* has been identified in >90% of patients with sporadic clear cell RCC (Gnarra et al., 1994). The remaining *VHL* allele is also commonly inactivated by a deletion event or altered methylation (Nickerson et al., 2008). In normal cell conditions, the VHL protein is a direct inhibitor of the activity of a key regulator of responses to hypoxia i.e. HIF. HIFs are heterodimers and contain an α and a β subunit. VHL targets the HIF-α isoform for proteasomal degradation. This prevents it from translocating to the nucleus and binding to HIF-β which would result in the induction of over 200 target genes that contain hypoxia-response elements in their promoters. The best described of the numerous HIF targets are growth factors which promote angiogenesis and proliferation (Figures 1 and 2). Under hypoxic conditions, VHL itself is degraded. This stabilizes HIF-α within the cell and allows it to accumulate in the nucleus. Here it initiates the expression of genes which promote cell survival and growth. The effects of biallelic inactivation of the *VHL* gene in clear cell RCC cells mirror those which result from VHL degradation in response to hypoxia. Expression of HIF targets such as VEGF, PDGF, and TGF-β are upregulated.

The *VHL* gene product, pVHL, is an E3 ubiquitin ligase that promotes the proteasomal degradation of HIF-1α, -2α and -3α. Consequently, renal carcinomas with mutations in *VHL* have high steady-state levels of HIF expression. Functional studies show that HIF is sufficient for transformation caused by loss of *VHL*, thereby establishing HIF as the primary oncogenic driver in kidney cancers (Maranchie et al., 2002). mTOR increases the translation of HIF-1 and HIF-2 which in turn can drive the expression of angiogenic factors such as VEGF, PDGF-β, bFGF and TGF-α. Thus, much interest has recently focused on targeting one or both HIF factor signals for cancer therapy. EZN-2968 is an RNA antagonist that specifically binds and inhibits HIF-1α mRNA *in vitro* and *in vivo* (Greenberger et al., 2008). In this study it proved to be a potent, selective, and durable antagonist of HIF-1 mRNA and protein expression resulting in reduced prostate and glioblastoma tumor cell growth. Its efficacy in RCC has not yet been investigated. In contrast, targeting heat shock protein 90(HSP90) with 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) which reduces levels of HIF-1α in the setting of *VHL* loss has shown promising results in clinical trials including patients with RCC (Kummar et al., 2010; Ronnen et al., 2006). Furthermore, YC-1, 3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole (an agent originally developed for circulatory disorders) and YC-1 analogs, 1, 3-disubstituted selenolo[3,2-c]pyrazole derivatives have now been found to repress HIF-1 activity and inhibit renal cancer tumour growth (Chou et al., 2010). Lastly, PX-478 (S-2-amino-3-[4'-N,N,-bis(2-chloroethyl)amino]phenyl propionic acid N-oxide dihydrochloride) an inhibitor of constitutive and hypoxia-induced HIF-1α levels and thus HIF-1 activity has proven efficacy *in vitro* with different RCC cell lines (Koh et al., 2008).

8.1 HIF-2α

HIF-2α, also referred to as endothelial PAS domain protein 1 shares 48% homology with HIF-1α. Current knowledge pertaining to the regulation of HIF-2α is somewhat lacking when compared with HIF-1α. However, the tumourigenic role of HIF-2α has been studied most extensively in RCC. Both *in vitro* and *in vivo* studies with human kidney tumours suggest that HIF-2α is more oncogenic than HIF-1α (Maranchie et al., 2002; Raval et al., 2005). RCC tumours express either HIF-1α and HIF-2α or HIF-2α alone, leaving HIF-2α expression as a common point of *VHL* mutated cancers. Moreover, consistent with this data, HIF-1α expression has been shown to decrease in advanced lesions as HIF-2α expression increases (Mandriota et al., 2002). Resulting from these observations HIF-2α is now being studied as the more important isoform for therapeutic intervention of RCC. Although several potentially important drugs targeting HIF-1α have been described (as outlined above) reports of HIF-2α are few. One potentially effective drug that needs further investigation is emetine, a protein synthesis inhibitor that blocks the translocation of peptidyl-tRNA from the acceptor site to the donor site on the riboosome. Emetine is not a novel drug (in fact it has been around for almost a century) but has been used for the treatment of bacterial, viral and amoeba *Entamoeba histolytica* infections as well as being used as an antiemetic (Zhou et al., 2005). It has been shown to induce HIF-2α downregulation in the setting of *VHL* loss in RCC cell lines. Further analysis of this drug is necessary for its efficacy in the *in vivo* setting of RCC.

9. HIF targets-Ror2

Regulated receptor tyrosine kinase-like orphan receptor 2 (Ror2) is a member of a family of orphan RTKs. Ror2 is found heavily phosphorylated in the kidney of RCC patients and is

expressed highly in human RCC cell lines indicating a role for Ror2 in the pathology of RCC (Wright et al., 2009). In fact suppressed expression of Ror2 results in reduced expression of matrix metalloproteinase (MMP)-2, whose upregulation correlates with advanced stages of RCCs (Slaton et al., 2001). Thus, Ror2 represents a promising therapeutic target for patients with RCC. Although the direct target of Ror2 kinase activity has yet to be deciphered, it does appear to act as a mediator of Wnt signals in the further activation of tumour cell signalling events (Figure 2). There are currently no clinically available drugs targeting Ror2 for the treatment of RCC.

9.1 Carbonic anhydrase IX

Carbonic anhydrase IX (CAIX), a hypoxia-induced protein, is unique in that it is a cell surface protein that is present in human tumours but absent from normal tissue (Figure 2). It is found to be highly expressed in clear cell RCC and is associated with grade (Genega et al., 2010). Currently, CAIX is being pursued as a prognostic indicator, diagnostic tool and a future potential targeted therapy for the treatment of RCC. Presently, there exists a chimeric antibody (cG250) used for its localisation, but also for direct antibody-dependent cellular cytotoxicity (ADCC). Thus, it has progressed through phase 1 (Davis et al., 2007), phase 2 (Bleumer et al., 2004), and is presently undergoing a phase 3 trial (NCT00087022) in patients with RCC. In addition, cG250 accumulation in RCC lesions is extremely high and so is being investigated as a strategy to deliver tumour-sterilising radiation doses with cG250 as a carrier molecule (Divgi et al., 2004). Furthermore, preliminary research with dendritic cells loaded with CAIX-derived peptides have shown to activate the cellular and humoral immune system in patients with cytokine refractory RCC (Uemura et al., 2006). Large prospective trials, however, are required to establish dendritic cell vaccination with CAIX–derived peptides or indeed direct vaccination with these peptides.

9.2 Angiopoietins and Tie-2

Angiopoietin/Tie-2 signalling pathways are important together with VEGF/VEGFR in the process of vascular endothelial growth for angiogenesis (Figure 1). Due to the highly vascular nature of RCC identifying new anti-angiogenic agents is highly desirable in an effort to try and treat this largely refractory cancer. One such target is Tie-2, an endothelium-specific tyrosine kinase, which serves as a receptor for the family of angiopoietin ligands, angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2), pro-angiogenic targets of HIF transcriptional activity. Binding of the former induces autophosphorylation of Tie-2, the latter antagonizes the actions of Ang-1 by competitively binding to Tie-2 without activating it. Ang-1 plays an important role in the assembly of newly formed vasculature and in the maintenance of vascular integrity. The role of Ang-2 in angiogenesis is highly dependent on the presence of other angiogenic factors, particularly VEGF. Tie-2 expression has been shown to correlate with angiopoietin-2 expression in RCC tumours (Liu et al., 2008).

Development of therapeutic drugs targeting the Angiopoietin/Tie-2 pathway differ somewhat. They may be direct inhibitors of the tyrosine kinase i.e. to target Tie-2, selective and non-selective traps to target Ang-1 or -2, or systemic delivery of angiopoietins to induce their anti-tumour effects (Huang et al., 2010). Tie-2 kinase inhibitors include CEP-11981 and CE-245677, the former is currently being evaluated in an open label phase 1 trial in patients with advanced solid tumours. The latter tested in a phase I trial has been discontinued owing to unacceptable side effects. There are currently two angiopoietin traps in clinical

development. AMG-386 is an anti-angiopoietin peptibody which inhibits the interaction between ligands Ang-1 and -2 with the Tie-2 receptor. It is currently undergoing phase II trials in combination with sorafenib and sunitinib for the treatment of RCC (Huang et al., 2010). The second compound PF-4856884 (also known as CVX-060) selectively targets Ang-2 and in a phase I study demonstrated a significant reduction in RCC tumour blood flow. The mild side effect profile of both of these drugs provides an attractive basis for their combination with other anti-angiogenic or chemotherapeutic agents.

9.3 Notch

Notch signalling controls a variety of processes, involving cell fate specification, differentiation, proliferation, and survival (Artavanis-Tsakonas et al., 1999). Mammals have four Notch proteins, namely Notch 1-4, that function as receptors for five Notch ligands – Jagged1 and 2, and the delta-like ligands (DLL)-1, -3 and -4. Ligand binding leads to at least three subsequent proteolytic cleavages that release the intracellular domain (ICD) of Notch. Notch ICD subsequently translocates into the nucleus, where it forms a complex with essential cofactors such as c-promoter binding factor 1 (CBF1), mastermind-like 1 (MAML1) and the transcription factor CSL. This complex mediates the transcription of target genes such as that encoding Deltex, genes in the hairy enhancer of split (HES) and HES-related families of basic helix-loop-helix transcription factors, and others (Figure 2). Aberrant signalling within this pathway has previously been reported in multiple malignancies (Miele et al., 2006) including RCC with elevated Notch 1, 3 and Jagged-1 mRNAs (Rae et al., 2000; Sjolund et al., 2008). Elevated expression of DLL-4 has also been reported in RCCs with reduction of such basal expression having considerable effects on endothelial function important in tumour angiogenesis (Patel et al., 2005). Interestingly, Notch signalling can lead to either tumour progression or suppression depending on the cellular context (Nicolas et al., 2003, Xia et al., 2001). However, in the context of RCC, Notch signalling inhibition has lead to inhibition of RCC growth thus indicating a potential novel therapeutic pathway in RCC. Presently, there is strong evidence for signalling crosstalk between Notch and HIF-1α. Cleaved Notch ICD and HIF-1α appear to play an important point between the two signalling cascades (Gustafsson et al., 2005). Moreover, there is recent evidence to support Notch signalling linking AKT and hypoxia in melanomas (Bedogni et al., 2008). Whether this interaction is pertinent to RCC has yet to be investigated. Inhibition of Notch signalling as a strategy for cancer treatment has been proposed in numerous studies (Nickoloff et al., 2003). Two approaches have been identified; selective strategies involving antisense, mAbs and RNA interference; nonselective strategies involving soluble or cell-associated Notch decoys, γ-secretase inhibitors, intracellular MAML1 decoys and Ras signalling inhibitors (Miele et al., 2006). Currently it is too early to evaluate the true efficacy of these strategies and of the different drugs involved but what is known from present findings is that Notch inhibition in cancer deserves a thorough investigation including in patients with RCC.

9.4 Wnt/β-catenin signalling

Wnts are a family of secreted glycoproteins that regulate a wide range of cellular functions such as growth, differentiation, migration and polarity (Moon et al., 2004). Wnt signalling is controlled by the transcriptional coactivator β-catenin, which is emerging as a key molecule in the pathogenesis of renal cancer. In normal quiescent cells, β-catenin is bound to casein kinase 1, glycogen synthase kinase 3β (GSK3), adenomatosis polyposis coli protein and axin. This complex controls β-catenin phosphorylation targeting it for proteosomal degradation.

Wnt positively regulates β-catenin, preventing its phosphorylation, ubiquitination and degradation. Thus, upon Wnt pathway activation hypophosphorylated β-catenin translocates into the nucleus and forms a complex with the DNA binding protein T cell factor (TCF) (Figure 2). The β-catenin/TCF complex activates transcription of a wide range of genes including growth promoting genes such as VEGF (Easwaran et al., 2003) as well as the *MYC* oncogene which shows copy number amplification in RCC (Beroukhim et al., 2009). Wnt can also mediate its effect on cell growth and tumour promotion by activating the mTOR pathway (Inoki et al., 2006). TSC2 is phosphorylated by GSK3 for its activation and subsequent inhibition of mTOR. Wnt activates mTOR pathway by inhibiting GSK3. There are several papers outlining the involvement of Wnt signalling in RCC. Overexpression of β-catenin can induce renal tumours in mice (Sansom et al., 2005). In some RCC tumours the *APC* gene promoter is aberrantly hypermethylated providing a mechanism by which β-catenin is liberated (Battagli et al., 2003). β-catenin is also degraded by the E3-ubiquitin ligase activity of VHL and so loss of VHL in RCC has been shown to enable HGF-driven oncogenic β-catenin signalling (Peruzzi et al., 2006). In a recent article further evidence for the activation of Wnt signalling in RCC was outlined when a deletion of *CXXC4* a gene coding for Idax, an inhibitor of this pathway, was identified in aggressive RCC (Kojima et al., 2009). In addition, various Wnt antagonists such as secreted-Frizzled receptor proteins, Dickkopf 2 and Wnt inhibitory factor 1 were found to be hypermethylated and thus silenced in RCC (Awakura et al., 2008; Hirata et al., 2009; Kawakami et al., 2009). It is also possible that loss of *VHL* could lead to the combined de-repression of HIFs and β-catenin which could also lead to RCC (Linehan et al., 2009). Finally, Jade-1 (gene for apoptosis and differentiation in epithelia) has been identified as a *VHL*-interacting protein which brings about β-catenin degradation. Thus, Jade-1 is thought to function as a renal tumour suppressor (Chitalia et al., 2008). Loss of *VHL* can bring about a reduction in Jade-1 levels with subsequent increases in β-catenin levels, providing another caveat by which loss of *VHL* can promote renal tumourigenesis. In summary Wnt signalling has a dual role in the pathogenesis of RCC. It induces transcription through activation of β-catenin but also activates mTOR signalling driving cellular growth. Thus, Wnt signalling and β-catenin provide attractive targets for therapeutic intervention in RCC. At present there are no clinically available drugs targeting this pathway in RCC but may become available in the near future.

10. HGF/MET signalling

Kidney tissue is an abundant source of hepatocyte growth factor (HGF) a stromal cell-derived cytokine involved in cell proliferation, tissue regeneration, tumour growth and tumour invasion through the HGF/scatter factor (SF):c-MET pathway (Cantley et al., 1994). HGF binding to MET leads to its phosphorylation and subsequent recruitment of adapter proteins such as Gab1, Gab2, SHC, STAT3 and PI3K with downstream activation of Ras/MAPK and PI3K/AKT pathways (Figure 2) resulting in RCC growth and metastasis (Eder et al., 2009). Different studies have shown that HGF and its receptor c-MET are overexpressed in RCC and play a significant role in the progression of RCC (Horie et al., 1999; Natali et al., 1996). Moreover, *VHL*-negative RCC cells exhibit cell invasion and branching morphogenesis in response to HGF (Koochekpour et al., 1999). c-MET has also been shown to be constitutively phosphorylated in RCC and high levels of serum HGF/SF in RCC patients are associated with

reduced survival (Tanimoto et al., 2008). Presently, MET is being targeted in clinical trials for the treatment of RCC (Giubellino et al., 2009). Strategies involve preventing c-MET autophosphorylation; prevention of HGF binding to c-MET and lastly targeting the signalling cascade of activated c-MET (Toschi & Janne, 2008). One such investigational drug is AMG 102, a fully human mAb that blocks HGF/SF binding to c-EMT and blocks signalling events driving tumour proliferation, migration, invasion and survival (Figure 2). Reports of a phase II trial with AMG 102 identified that although the drug brought about tumour burden reduction and long-term disease stability, it was unclear from the study whether this drug is capable of tumour growth inhibition in a histologically diverse population of patients with mRCC (Schoffski et al., 2010). Other drugs currently in development include foretinib (GSK1363089) an oral multi kinase inhibitor of MET and VEGFRs (Kataoka et al., 2011), ARQ 197, a selective non-ATP competitive inhibitor of MET (Adjei et al., 2011) and a range of orally available c-MET kinase inhibitors namely (R)-3-[1-(2,6-dichloro-3-fluoro-phenyl)-ethoxy]-5-(1-piperidin-4-yl-1H-pyrazol-4-yl)-pyridin-2-ylamine (PF02341066) and 2-[4-(3-quinolin-6-ylmethyl-3H-[1,2,3]triazolo[4,5-b]pyrazin-5-yl)-pyrazol-1-yl]-ethanol (PF04217903) (Yamazaki et al., 2011). The efficacy of these drugs have yet to be investigated in patients with RCC. Thus, further investigations of these potentially effective therapeutic drugs is warranted at this time.

11. Conclusions and future directions

RCC is similar to other cancer types in that it is asymptomatic initially with a lack of early warning signs. This results in a high percentage of patients presenting with metastasis at diagnosis or indeed relapse following nephrectomy. RCC is also known for its unpredictable clinical behaviour. Historically RCC and mRCC has been associated with treatment resistance and poor prognosis. However, with ever increasing knowledge of angiogenesis and aberrant signalling pathways in patients with RCC recent basic and clinical developments has exerted a substantial impact on outcomes for patients with mRCC.

Throughout the past fifteen years there has been an increased understanding of the tumour biology of RCC. There is now a myriad of treatment options available making the treatment of RCC and mRCC immensely complex. Sequential therapy with targeted agents is currently the standard of care while combination therapies are still under active investigation. Combination therapies can provide additive or synergistic effects resulting from more complete blockade of the many aberrant signalling cascades outlined above. This approach can also prevent or delay the development of resistance that would eventually arise with single-agent therapy owing to signalling pathway redundancy. Despite recent advancements, however, current chemotherapeutics can only increase the overall survival of patients from weeks to months and unfortunately cannot cure RCC. Combination regimes have many drawbacks and in many instances have not proven beneficial in terms of inferior efficacy and excessive toxicity. It is also clear from the multitude of described and ongoing clinical trials that patient response to targeted agents is not universal. Thus, we have reached the stage where there is compelling need to identify new combinations with the goal of providing maximal efficacy with manageable toxicity. Increased knowledge of mechanisms of disease, drug resistance, new targets and new targeted agents may eventually provide optimal strategies for patients with RCC and mRCC.

Another area of note to improve the treatment of patients with RCC and mRCC is the identification of genetic and epigenetic markers as promising biomarkers (Pal et al., 2010; Vickers & Heng 2010) . This may indicate the suitability of a patient to treatment with one

agent above another and also the optimal sequential or combination of therapies. Many RCC biomarkers have been examined over the past decade and include *VHL* gene mutation, plasma VEGF, tissue and plasma VEGFRs, tissue HIF and tissue and serum CAIX as outlined already in this report. Others include B7-H1 a cell surface glycoprotein that acts as a negative regulator of anti-tumoural T cell-mediated immunity and whose high levels of expression in patients with RCC has been associated with shorter survival (Vickers & Heng, 2010). Another prognostic biomarker includes neutrophil gelatinase-associated lipocalin (NGAL). NGAL is elevated in a number of cancers and has been linked to MMP-9, which is involved in the degradation of the extracellular matrix and therefore invasion and metastasis. Thus, lower levels of NGAL is associated with longer PFS in RCC patients (Vickers & Heng, 2010). Despite growing research in this area, however, there are currently no biomarkers used in the clinical management of patients with RCC. Future studies such as the NIH funded Cancer Genome Atlas project may provide further insight into the genome, mRNA and micro RNA transcriptome and methylome of RCC revealing the pathways and networking that is aberrant in RCC and thus aid in the identification of new biomarkers and therapeutic agents. Furthermore, the use of increasingly sophisticated integrative multivariate models which incorporate both molecular and genetic information will ultimately aid in the development of curative, non-toxic personalised therapies. Thus, research is ongoing and newer improved technologies hold promise in the expansion of our knowledge of pathogenesis and determinants of outcome. In summary, in the future researchers and clinicians alike will have to unite and develop a workable cohesive strategy to optimise use of available agents as well as those undergoing clinical trials and identify optimal strategies for the successful treatment of patients with RCC.

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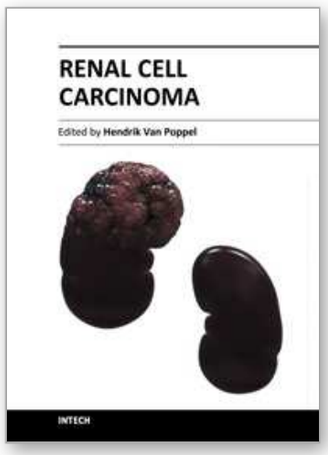

Renal Cell Carcinoma

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Surgical and medical oncologists have been unable to decrease renal cell carcinoma mortality for uncertain reasons, although a lot of progress has been made in diagnosis and imaging, recognition of different genetic and pathological entities, management of localized disease and in the research on new drug treatments for advanced stages of the disease, potentially combined with surgery. The purpose of this book, which tackles a number of separate interesting topics, is to provide further insight into the disease and the management of early and advanced renal cell carcinoma. The volume is divided into different parts; the first part covers the characterization of renal masses and the second part covers rare distinct pathological entity. In the management section, active surveillance, partial nephrectomy and radiofrequency ablation are presented. A separate chapter reviews the management of Von Hippel Lindau disease, and finally, conventional and aberrant signaling pathways are explored.

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