Kinase targets in renal-cell carcinomas: reassessing the old and discovering the new



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Renal-cell carcinoma is a heterogeneous group of tumours that arise in the adult kidneys. Irrespective of the type Lancet Oncol 2010; 11: 571-78 of renal tumour, traditional chemotherapeutic and radiation-based therapies have been largely ineffective at treating advanced tumours, with long-term survival being very low. Molecularly-targeted inhibitors of protein kinases are effective in delaying progression of advanced renal tumours. These therapies revolve around inhibition of the vascular endothelial growth factor receptor tyrosine kinase and the mammalian target of rapamycin serine or threonine kinase signalling pathways. The genetic complexity of renal tumours revealed by gene-expression profiling and other molecular-genetic technologies indicate that inhibition of additional kinase-associated pathways could also prevent renal tumour growth. In this review, we discuss the use of molecularly-targeted kinase inhibitors in the treatment of renal-cell carcinoma and identify the next generation of kinase inhibitors that show promise for treatment.

Introduction

Renal-cell carcinoma (RCC) accounts for about 209000 new cancer cases and 102000 deaths per year worldwide, making it the seventh most common type of cancer in men and the ninth in women.1 For localised disease, surgical removal of the tumour is a highly effective treatment. However, most symptomatic patients present when tumours are no longer localised. For locally invasive tumours, surgical approaches are less effective and for metastatic disease surgical approaches are not curative. In advanced cases, systematic cytokine therapies (eg, treatment with interleukin 2) have resulted in response rates of approximately 15%, with a smaller percentage exhibiting complete remission upon treatment.1 In contrast, systemic treatment with traditional cytotoxic agents (eg, fluorouracil, paclitaxel, vinblastine), radiation, and hormonal therapy have not been effective in treating renal tumours.1 The ineffectiveness of these treatments in most patients, coupled with the development of molecularly-targeted agents has changed the clinical management of RCC to a focus on management with kinase inhibitors (figure 1). Kinase inhibitors used to treat other tumour subtypes include imatinib for advanced gastrointestinal stromal tumours, and lapatinib and trastuzumab for breast cancer treatment.3,4 Here we review the rationale for treatment of the most common type of adult kidney cancer, RCC, with molecularlytargeted kinase inhibitors.

Classes of kinases

Kinases attach a phosphate group to a tyrosine, serine, or threonine residue on a target protein. Phosphorylation usually results in a change in the activity, location, or accessibility of the target protein. Serine or threonine kinases include the cyclic nucleotide-regulated (AGC), the calcium-regulated (CAMK), the casein-like (CK1), the cyclin-regulated (CMGC), and the mitogen activated (STE) kinase groups, and the atypical and lipid kinase families.5 PDK1 and Akt are examples from the AGC family, whereas the mammalian target of rapamycin (mTOR) is an example of an atypical serine or threonine kinase.

Most tyrosine kinases are represented by receptor tyrosine kinases (RTKs) and can be further divided based on the family of growth factors, that when bound to the extracellular domain, increase receptor kinase activity.6 RTKs that bind the epidermal growth factors are sometimes referred to as class I receptors, receptors that are activated by insulin (class II), platelet derived growth factors (PDGFs; class III), fibroblast growth factors (class IV), vascular endothelial growth factors (VEGFs; class V), and hepatocyte growth factors (HGFs; class VI). Once activated, the RTKs induce downstream signalling via additional kinases. For example, the PI3K-Akt-mTOR kinases and the mitogenactivated protein kinases (MAPK) are activated by various RTKs. Activation of PI3K produces phosphatidylinositol 3,4,5 triphosphates, figure 1 highlighting the role of lipid kinases. These phospholipids serve as docking sites for proteins with pleckstrin homology domains, including

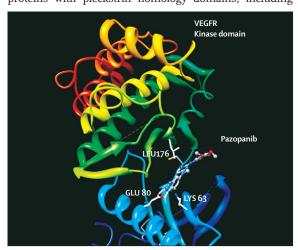


Figure 1: Structural overview of a pazopanib derivative bound to the kinase domain of the VEGFR2 receptor tyrosine kinase

Diagram of the VEGFR2 kinase domain bound to a chemical that is structurally analogous to pazopanib but without certain optimisations for P450 metabolism. The six alpha-helical structures that make up the kinase domain are shown in different colours. VEGFR2 aminoacids proximal to the pazopanib are highlighted in white. The pazopanib derivative is shown as a ball and stick model. Structural information was obtained from National Center of Biomedical Informatics Molecular Modeling Database (MMDB ID: 67164) and modelled with Chimera.

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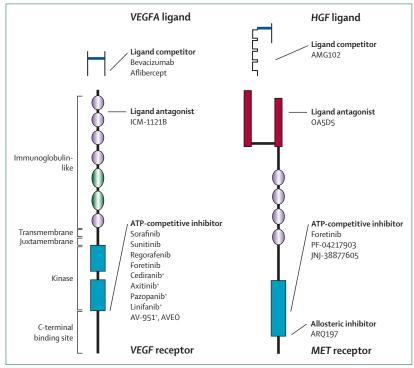


Figure 2: Modes of inhibiting receptor tyrosine kinases

Protein domain structure of the VEGFR2 and MET receptor tyrosine kinases and their associated ligands, VEGF and HGF. Second generation inhibitors that show potent in-vitro activity are indicated (+). 28-10

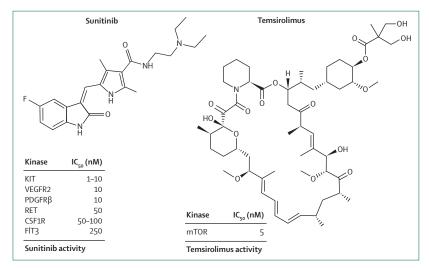


Figure 3: Chemical composition and cellular IC50 values of representative kinase inhibitors sunitinib and temsirolimus

PDK1 and Akt. PDK1 activates Akt and subsequent downstream mTOR activation.

Mechanisms of kinase inhibition

Several therapeutic avenues are available to block the activity of protein kinases (figure 2). 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 aminoacid structure of the kinase domain, ATP-competitive inhibitors can have cross-reactivity with other structurally related kinases. For example, sorafenib is an ATP-competitive inhibitor of type 2 VEGF receptor (VEGFR2). However, sorafenib also inhibits the enzymatic activity of FLT3 (another class V receptor), β -type PDGF, the KIT family of receptor tyrosine kinases, and the BRAF serine or threonine kinase. Sunitinib, another ATP-competitive inhibitor of PDGF (class III) and VEGF receptors, likewise inhibits the kinase activity of the KIT, RET, FLT3, and CSF-1R receptor tyrosine kinases (figure 3). 12

In addition to inhibitors that bind within or near the ATP binding-site, allosteric inhibitors that bind outside the kinase domain can be used to inhibit kinase activity, although this mechanism is less common than active-site kinase inhibition. Rapamycin and its analogues (temsirolimus, everolimus) bind to a domain separate from the catalytic site to block a subset of the mTOR functions (figure 3). These allosteric inhibitors are very selective for their target kinases due to the binding of non-conserved residues on the protein surface.13 However, because these inhibitors do not bind the active site, not all kinase activities are inactivated. mTOR is present in two distinct complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), with distinct downstream signalling and upstream protein targets important for cellular growth and survival.14 Rapamycin only inhibits a subset of mTORC1 substrates and does not affect mTORC2 substrates.

Additional therapeutic avenues exist for inhibiting the activation of RTKs. Monoclonal antibodies against growth factor ligands, or antibody fragments against an RTK ligand-binding domain, can prevent binding of growth factors, thus attenuating RTK activity. The class-V receptor tyrosine kinases bind VEGF types A, B, C, D, and E. Bevacizumab is an antibody that was developed to bind VEGF type A and is an example of a ligand-competitive inhibitor that prevents binding of the growth factor ligand with the receptor.¹⁵

Renal-cell carcinoma

RCC can be divided into several subtypes on the basis of differences in cellular morphology and architectural features. Clear-cell RCC is the most common form of adult kidney cancer-75-80% of all renal neoplasms.1 About 10-15% of renal neoplasms are papillary RCC, 16 which can be further subdivided into type 1 and type 2 and is the second most common adult kidney neoplasm. Other types of RCC are chromophobe (4-6%), collecting-duct (<1%), and those forms that are rare or yet to be classified (<2%). The different histological subtypes are also associated with different types of molecular defects within the tumour cells. These include differences in chromosomal abnormalities and in somatic DNA mutations. 17,18 Expression profiling with microarrays has been done for many of the renal or kidney tumour subtypes. 19,20 Wholegenome expression characteristics of the renal tumours

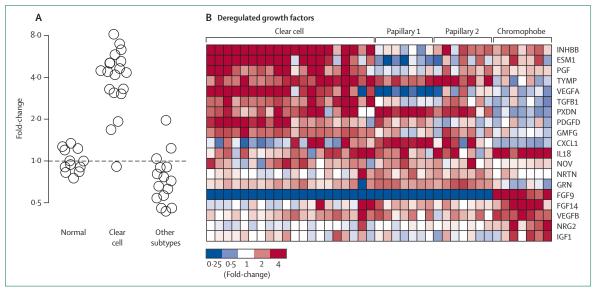


Figure 4: Expression of VEGF in renal-cell carcinomas

The expression of the VEGFA (A) and the most significantly dysregulated factors in the indicated subtypes of RCC (B). Expression of protein growth factors are established by gene expression microarray data and are plotted relative to non-diseased renal tissue. Red colour indicates over-expression of the factor and blue colour indicates under-expression of the factor. Expression data and associated publications describing the data can be obtained from National Center of Biomedical Informatics Gene Expression Omnibus (GEO ID: GSE11024, GSE14762, GSE8271, and GSE7023). The raw data were extracted from the National Center of Biomedical Informatics Gene Expression Omnibus.

activate the MET receptor tyrosine kinase. ²⁵ These additional growth factors are not as well studied in RCC as the VEGF, and the roles of these signalling pathways in renal tumour development are not fully understood.

Biomedical Informatics Gene Expression Omnibus see http:// www.ncbi.nlm.nih.gov/geo/

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mirror their histological classifications and further show that various renal tumour subtypes are genetically distinct entities. Several different mechanisms, including somatic DNA mutations, changes in kinase expression, and changes in associated-kinase regulatory proteins can lead to dysregulation of kinase signalling in RCC.

Molecular genetics of kinase activation

In the clear-cell subtype of RCC, much effort has focused on kinase activation through ligand dysregulation. Most (>80%) of patients with clear-cell RCC, have somatic DNA mutations in the von Hippel-Lindau gene, VHL.11 In an additional subset of cases, VHL can become inactivated due to promoter hypermethylation. Biallelic inactivation of VHL results from removal of the other allele of VHL through loss of the short arm of chromosome 3.22 RCCs that are devoid of VHL are unable to downregulate the hypoxia-inducible factor, a transcription factor that regulates the cellular response to oxygen deprivation. In clear-cell RCC, inactivation of VHL leads to uncontrolled concentrations of hypoxia-inducible factor and overexpression of numerous hypoxia-regulated genes, including PDGFs and VEGFs (figure 4). As a consequence, both the tumour cells and the surrounding stromal and endothelial cells display a pronounced angiogenic phenotype that is associated with hyperactivation of the family of RTKs that bind VEGFs and PDGFs.23 Other secreted growth factors the expression of which changes in response to hypoxia include, transforming growth factor TGF β1 and placental growth factor.24 Placental growth factor synergises with VEGF to activate the VEGFR1 receptor, whereas endocan, a VEGF type A-regulated factor, synergises with HGF to

Dysregulation of RTK ligands is just one mechanism by which kinases can become dysregulated in RCC. Chromosomal defects, such as DNA amplifications, can lead to dyregulated kinase activity by changing expression of kinases encoded within the amplified region. Although less common than in other tumour types, 26 several chromosomal amplifications are present in RCC tumours that are associated with dysregulated kinase expression. The deletion of the short arm of chromosome 3 is often caused by an unbalanced translocation with the long arm of chromosome 5. As such, more than 50% of clear-cell renal tumours have amplification of chromosome 5, a region where the type 3 VEGF receptor (VEGFR3) is located, with amplification of chromosome 7, 16, and 17 commonly seen in the papillary type 1 subtype.28 The MET gene, located on chromosome 7q31, is more highly expressed in the papillary subtype than in other subtypes of RCC. This high expression is commonly attributed to chromosome 7 amplification (figure 4) and is one factor that guides the application of MET inhibitors in papillary RCC.29 In papillary type 2 RCC, gains of chromosome 8q and 17 frequently occur, and in chromophobe RCC amplification of chromosome 19 is common and is associated with heightened expression of the AXL receptor tyrosine kinase.30 Other kinases that show increased expression across several of the RCC subtypes, such as the Aurora-A cell-cycle kinase, are also associated with DNA amplification.31

Although a diverse spectrum of cytogenetic mutations can lead to dysregulated kinase expression, heightened expression of kinases can occur outside of DNA amplifications. The KIT-RTK family is activated by the mast-cell growth factor, and is highly overexpressed in chromophobe RCCs when compared with other subtypes of adult tumours and to non-diseased kidney tissue.32 However, the mechanism by which this RTK becomes overexpressed in this tumour subtype remains unclear. The high expression of the KIT receptor could indicate a yet to be described genetic or epigenetic defect that is present in these cells, or could be an inherent property of the cell type from which chromophobe RCC arises. Not understanding the mechanism that leads to the high levels of the KIT receptor makes identifying the role of this receptor in tumour development more challenging. Several ATP-competitive inhibitors, including sorafenib and sunitinib, inhibit the enzymatic activity of the KIT receptor. However, reassessment of clinical trials, with an emphasis on response rates of rare RCC subtypes did not indicate notable clinical responses to these RTK inhibitors in chromophobe RCC.33 Furthermore, imatinib, a clinically effective kinase inhibitor that targets KIT in gastrointestinal stromal tumours harbouring KIT activating mutations, likewise did not show substantial effects in chromophobe RCC.34 Preclinical studies can therefore be valuable in identifying kinases that are associated with oncogenic transformation (ie, drivers) and those that are associated with cell lineage or other confounding effects (ie, passengers).

An additional mechanism by which kinases can become dysregulated is through the accumulation of somatic DNA mutations, leading to aminoacid changes either in the kinase domain or in regions surrounding the kinase domain. Although mutations in the kinase domain are the most common type of somatic mutation in cancer, somewhat surprisingly, most renal tumours do not have somatic mutations associated with a rise in kinase activity.35 The most commonly mutated kinase in RCC is the activating mutation in the MET receptor tyrosine kinase and is associated with the papillary type 1 RCC. These mutations are present in about 10% of the papillary type 1 tumour cells and in about 1% of renal tumours overall.36 Although mutations in other kinases, such as the epidermal growth factor receptor, have been reported in case studies, activating kinase mutations are a rare occurrence in RCC. 37,38

Reassessing the old

Targeting of the VEGFR-signalling axis

The inactivation of *VHL* and the associated angiogenic nature of clear-cell RCC, provides a rationale for the examination of VEGF and PDGFR signalling pathway inhibitors. Two ATP-competitive inhibitors of the class III and class V RTKs (sorafenib and sunitinib) and the ligand-competitive inhibitor of the VEGFs (bevacizumab) were initial treatments that targeted angiogenic signalling in these tumours. Each of these therapies alone, or in some

cases in combination with interferon a, significantly delayed progression-free survival in RCC.39-41 Sunitinib treatment in particular was associated with a 39% objective response and an 11 month progression-free survival in a phase 3 trial of untreated patients.³⁹ As the clinical studies have progressed, sunitinib treatment is now associated with median overall survival of more than 2 years, a duration that is a substantial extension of the 1 year overall survival rates in the years before kinase inhibitor therapy.⁴² Combination therapies that include existing multikinase inhibitors are complicated by issues of increasing toxicity and several second-generation ATP-competitive inhibitors are under investigation for RCC (figure 2). These newer inhibitors, including pazopanib, cediranib, axitinib, and linifanib, more specifically target the class-V receptors (VEGFR1, VEGFR2, and VEGFR3). Pazopanib has been approved for treatment of RCC on the basis of improved progression-free survival of treated patients as compared with untreated patients.43 Should tumour response rates of multikinase inhibitors be dominated by VEGFR signalling, then more selective inhibitors could result in lower drug doses, potentially expanding the opportunities for combination therapy. Moreover, the newer inhibitors represent compelling therapies for management of treatment refractory patients.44

Although the survival gains associated with these molecularly-targeted therapies are pronounced, renal tumours eventually become resistant to the existing RTK inhibitors. In other tumour subtypes, acquisition of a somatic mutation within the targeted kinase gene is often associated with resistance to therapy.45 Because the angiogenic inhibitors primarily target the tumour endothelium, rather than the genetically unstable tumour cells, compensatory target-kinase gene mutations might be rarer in RCC, whereas somatic kinase mutations are not associated with tumour resistance. An alternative mechanism for resistance to anti-VEGFR therapy in RCC is the activation of non-VEGFR dependent angiogenic pathways. Upregulation of an alternate pro-angiogenic pathway, including upregulation of basic fibroblast growth factor, interleukin 8, and others, have been recorded in preclinical models of resistance to kinase inhibitors.45 Parallel inhibition of these alternative pathways, or alternative treatment protocols of sequentially applied drugs that target two unique pathways, could provide a route for more durable tumour responses.

Targeting of the PI3K-mTOR-signalling axis

In RCC, there seems to be little or no somatic DNA mutations in PI3K-mTOR pathway components. However, about 4% of RCC tumours have *PTEN* mutations that activate the PI3K pathway.⁴⁶ The prevailing rationale for mTOR-targeted therapies in RCC is based on mTOR's ability to regulate the expression and actions of hypoxia-inducible factor and subsequent VEGF and PDGF expression. Rapamycin has the potential to serve a dual role through antiproliferative effects on tumour cell growth

and antiangiogenic effects on the tumour associated vasculature. Many RCC tumours have upstream activation of Akt and active S6K, an mTOR substrate. Activation of mTOR and pathway components are substantially changed in high-grade tumours and associated with poor outcome. 47,48 In May 2007, the first rapamycin analogue, temsirolimus, was approved by the US Food and Drug Administration (FDA) for advanced RCC.49 Less than 2 years later, everolimus, an orally active rapamycin analogue, was approved as a first-line treatment for patients with advanced kidney cancer after failure of either sunitinib or sorafenib.50 Rapamycin does not target the mTOR kinase domain. The two complexes mTORC1 and mTORC2 have a large amount of rapamycin resistant activity, highlighting the importance of rapid development of catalytic mTOR inhibitors for RCC.51,52 These catalytic mTOR inhibitors bind the mTOR-ATP-binding pocket and have a half maximum inhibitory concentration (IC50) in the low nanomolar range. Kidney cancer cells continue to proliferate in the presence of rapamycin, therefore the development of catalytic mTOR inhibitors should be carefully monitored as toxic effects from complete mTOR inhibition could result. 51 Rapamycin and its analogues have metabolic side-effects that result from the inhibition of the mTOR pathway. A second confounding issue is that the PI3KmTOR pathway contains negative feedback loops downstream of mTOR, activating the potent survival-kinase Akt through mTORC2-mediated phosphorylation.⁵³ additional strong negative feedback loop exists with active mTORC1 and active S6K suppressing PI3K activation. By inhibiting only mTORC1, current therapies allow for reactivation of PI3K within the tumour cell. Efforts are being made to develop dual-kinase inhibitors that target both the PI3K and mTOR kinase activity by binding to the ATP-binding cleft of these enzymes.⁵¹ The promise is that dual PI3K and mTOR catalytic inhibitors will target these two key pathway-kinases simultaneously and prevent pathway reactivation. The dual PI3K-mTOR inhibitors NVP-BEZ235 and GDC-0941 have promising activity in various preclinical models and are undergoing phase 1 clinical trials in patients with advanced solid tumours.54 Effects of these dual PI3K-mTOR inhibitors in the treatment of RCC will be of great interest.

Discovering the new

Targeting of alternative receptor tyrosine kinases

Activating mutations in the MET receptor are seen in about 10% of sporadic papillary RCC, with overexpression of the MET receptor a prominent feature in papillary type 1 and other kidney subtypes (figure 4). Foretinib is a dual-kinase inhibitor that targets both the VEGFR2 and the MET receptor (figure 2). In compelling but preliminary studies, foretinib treatment has been associated with tumour regression in most patients with papillary RCC. In particular, a daily dosing schedule of foretinib has been associated with a prolonged period of stable disease.²⁹ In view of the variety of MET inhibitors available, these initial

results suggest that inhibition of MET signalling will be a substantial advance in the treatment of papillary RCC.

Although the genetic characteristics of the papillary RCC subtype makes these tumours a rational first choice for the clinical assessment of MET inhibitors, several preclinical studies suggest that MET inhibitors could also be effective in the clear-cell subtype. Dysregulated expression of MET (figure 5) and amplification of chromosome 7 are noted in other subtypes of RCC.26 Studies that systematically examined the contribution of all known kinases in cellular growth assays revealed that MET is required for the in-vitro growth of cells without functional VHL.55 These results are consistent with earlier preclinical studies that showed that VHL-null cells have a pronounced invasive growth phenotype after addition of HGF, the growth factor ligand for the MET receptor.⁵⁶ Moreover, gene expression profiling indicates genes that are upregulated after synergistic addition of both HGF and VEGF to cultured endothelial cells are also upregulated in clear cell RCCs.^{57,58} In support of this idea, Kabbinavar and colleagues⁵⁹ showed that AMG 102, a monoclonal antibody directed against HGF, had a disease-stabilising effect in 61 (25%) of pretreated patients with RCC.59

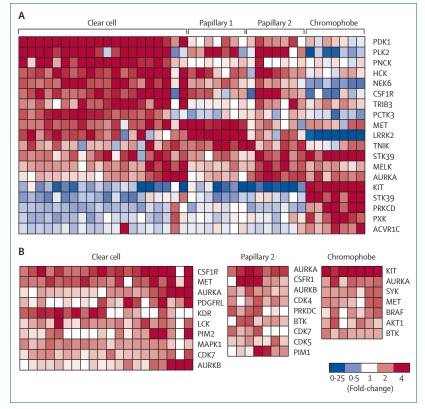


Figure 5: Expression of kinase genes in renal-cell carcinomas

The most significantly dysregulated kinases (A), and kinases that are targets of existing kinase inhibitors currently in clinical trials (B). Expression levels are plotted relative to non-diseased renal tissue. Red colour indicates overexpression of the kinase and blue colour indicates under-expression of the kinase. Expression data and associated publications describing the data can be obtained from National Center of Biomedical Informatics Gene Expression Omnibus (GEO ID: GSE11024, GSE14762, GSE8271, and GSE7023). The raw data were extracted from the National Center of Biomedical Informatics Gene Expression Omnibus.

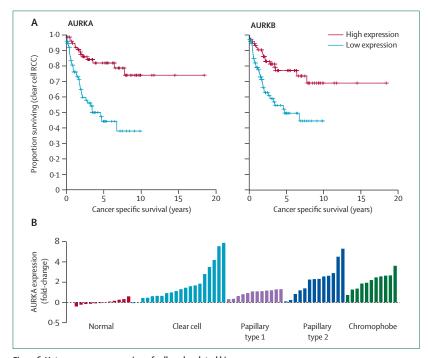


Figure 6: Heterogeneous expression of cell-cycle related kinases
(A) Survival characteristics of patients with clear-cell RCC that were separated into two groups based on median Aurora A (left) and Aurora B (right) kinase expression (unpublished), and (B) heterogeneous expression of Aurora C in several RCC subtypes.

Early work examining the Wilms' tumour suppressor gene, WT1, indicated a role for the insulin-like growth factor (IGF) signalling pathway in renal tumour development. WT1 is a zinc-finger transcription factor that represses expression of both type 1 IGF and type 2 IGF receptors. Mutations in WT1 allow for heightened expression of these factors that are associated with child-hood renal tumour development. Preclinical data suggest that the IGF signalling-axis has a role in adult renal tumour development. Expression of several IGF-axis components, including receptors, ligands, and binding proteins, are dysregulated in RCC. Additionally, VHL inactivation has been associated with IGF1 receptor up-regulation, and expression of the IGF1 receptor is an indicator of poor survival in clear-cell RCC. Additional Several ATP-competitive

Search strategy and selection criteria

Articles were selected by searches of the PubMed Medline database. The search terms "renal cell carcinoma kinase" and "renal cell carcinoma targeted therapy" were used. Only papers published in English between August, 2004, and March, 2010, were included. Relevant papers cited in articles from the original search results were also reviewed. Clinical trials were reviewed based on searches of ClinicalTrials.gov and abstracts from the most recent American Society of Clinical Oncology, the European Society for Medical Oncology, the Kidney Cancer Association, and the Symposium on Targeted Anticancer Therapies meetings were reviewed. The search terms were "kidney cancer" and "renal cell carcinoma" and "kinase". Both open and completed studies were included. Papers referencing kinase inhibitors in these clinical trials were also reviewed.

inhibitors and ligand-competitive inhibitors of the IGF signalling-axis are under active development and in various stages of clinical trials for RCC and other tumour types.⁶⁶

Targeting of the cell-cycle control kinases

Several inhibitors have been developed that target kinases regulating cell division and mitotic progression. The Aurora kinases A, B and C (AURKA, AURKB, and AURKC) are serine or threonine kinases associated with the regulation of centrosome separation and assembly of the mitotic spindle during the cell-division.⁶⁷ These mitotic kinases are overexpressed in RCC (figures 5 and 6) and are associated with chromosomal instability and clinical aggressiveness in RCC and other tumour cells, although this is not supported in all studies. 68,69 Another major class of mitotic kinases that have evidence of dysregulation in RCC are the cyclin-dependent kinases (CDKs). The CDKs, like the Aurora kinases, are serine or threonine kinases that regulate cell division. There are at least ten CDKs expressed in human cells, with kinase activity dependent upon forming a complex with a corresponding cyclin. Preclinical evidence suggest that CDKs are important regulators of cellular growth in RCC. Studies that systematically examined the contribution of all known kinases revealed that, in addition to MET, CDK6 was also required for the growth of cell-lines without functional VHL.55,70 Moreover, CDKs are regulated by CDK-inhibitors, and decreased expression of CDKN1B, a well studied CDK-inhibitor, associated with poor patient outcome.71,72 Loss of chromosome 9q is associated with recurrence and metastatic progression in RCC. 27,73 Genomic studies of RCC identified two related CDK-inhibitors, CDKN2A and CDKN2B, both inhibitors of CDK4, as residing in the 9q deletion peak.26,74 High expression of AURKA is associated with amplification of chromosome 20 and this is also associated with poor prognosis in RCC.31

These preclinical studies indicate that dysregulation of cell-cycle kinases are associated with both the development and progression of clear-cell RCC, and gene expression profiling data indicates that these cell-cycle kinases are dysregulated in all RCCs (figures 5 and 6). At least seven inhibitors of various CDKs and at least four Aurora kinase inhibitors are undergoing clinical trials in other tumour subtypes and await assessment in RCC. The key issue with these cell-cycle kinases is whether they are consequences of increased cellular proliferation (ie, passengers) or whether they contribute to the causes of renal tumour development (ie, drivers). Overexpression of cell-cycle kinases could merely indicate a high proliferative index when compared with non-cycling normal kidney cells or indicate that tumours with a higher proliferative index tend to have more aggressive clinical behaviour than tumours with a lower proliferative index. However, the molecular genetics of RCC suggest that at least in some cases, dysregulation of cell-cycle related kinases directly contribute to tumour progression. Both amplification of chromosome 20, where AURKA maps, and deletion of

chromosome 9, where *CDKN2A* and *CDKN2B* map, are associated with tumour aggressiveness. These chromosomal abnormalities supply the best evidence that dysregulation of cell-cycle kinases contribute in a direct way to tumour development and that inhibitors of these kinases would impact renal tumour growth.

Conclusion

In the near future, many inhibitors of protein kinases will be available for investigation in RCC. Preclinical data suggest that several of these inhibitors will be effective in inhibiting the growth and progression of RCC. The development of a single agent that is both effective and safe for the entire spectrum of RCCs should remain a long-term goal for the treatment of this disease. However, the genetic complexity of renal tumours and the intrinsic genetic differences between individual patients suggests that the application of many of these kinase inhibitors will benefit a more refined subset of patients in the nearer term (figure 6). Although the array of new targeted therapies for RCCs is exciting, it is not clear that a single kinase-targeted agent will wholly disrupt tumour growth. Even if as few as half a dozen kinase inhibitors show some efficacy in clinical trials, the possible selections of pairwise combinatorial therapy regimens will rise quickly. Substantial challenges remain in the selection of markers that will predict the effectiveness of a kinase inhibitor in an individual patient. Included in these challenges will be the incorporation of various biomarker-measurement technologies into routine clinical management, along with the application of appropriately designed and powered clinical trials that assess both biomarker and drug effectiveness.75,76 Substantial advancements have been made in RCC by investigating RCCs as multiple diseases rather than as a single entity. With new molecularlytargeted kinase inhibitors on the horizon, the next major advance in RCC treatment lies in the further stratification of patients, based on genetic markers that predict the effectiveness of the next wave of kinase inhibitors.

Contributors

BTT and KAF designed the review. JPM gathered and assessed data on mTOR signalling and inhibitors. KAF gathered and assessed the remaining review data and prepared the paper. All authors read and approved the final version.

Conflicts of interest

BTT has research projects sponsored by Pfizer. JPM has research projects sponsored by Novartis. KAF declared no conflicts of interest.

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