Chapter 8

Resistence of Renal Cell Carcinoma to Targeted Therapy

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Abstract

Targeted therapy has replaced immunotherapy as the standard of care for metastatic renal cell carcinoma (RCC). Currently available targeted agents include multi-tyrosine kinase inhibitors (axitinib, sunitinib, sorafenib and pazopanib), mammalian target of rapamycin (mTOR) kinase inhibitors (temsirolimus and everolimus) and bevacizumab.
humanized monoclonal antibody against vascular endothelial growth factor (VEGF). Despite the beneficial clinical outcomes, resistance to therapy is emerging as a new challenge. Resistance to therapy can be either intrinsic or extrinsic (acquired). About 30% of patients have intrinsic resistant and almost all patients who respond initially will develop acquired resistance within 12 months of treatment. In this chapter, we summarize the current challenges of drug resistance in the effective treatment of metastatic RCC with particular emphasis on targeted therapies.

**Keywords:** Renal cell carcinoma, resistance, targeted therapy, multi-tyrosine kinase, mTOR

### Introduction

Renal cell carcinoma (RCC), thought to arise from the epithelial cells of the renal tubules [1], are a heterogeneous group of diseases. They are highly metastatic, and account for about 3% of all adult malignancies [2, 3]. Clear cell carcinoma is the predominant subtype (70-80%), followed by papillary (10-15%), chromophobe (5%) and collecting duct (<1%) carcinomas [1, 4]. Metastatic RCC is highly resistant to conventional radio and immunotherapies. The field of RCC study has undergone a revolution in the past decade, largely because of our understanding of the role of the von-Hippel Lindau (VHL) gene in RCC biology. The VHL gene mutations are the most important risk factors for the development of RCC. A functional VHL degrades hypoxia inducible factor-1 alpha (HIF-1α). In the absence of a functional VHL, either through mutations or hyper-methylation, HIF-1α is stabilized, leading to the transcriptional activation of over 300 genes, especially pro-angiogenic factors such as vascular endothelial growth factor (VEGF), its tyrosine kinase receptors (VEGF-R1-3) and platelet-derived growth factor (PDGF) (Figure 1) [5, 6].

VEGF binds to VEGF-R1 and VEGF-R2, and promotes angiogenesis through endothelial cell proliferation, migration and vascular permeability [7]. Elucidation of these molecular pathways has led to the development of VEGF and multi-tyrosine kinase inhibitors as summarized in Figure 2 and Table 1.
Mammalian Target of Rapamycin (mTOR) Inhibitors

Mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase that regulates cell growth, proliferation, motility and survival [5]. It is composed of two molecular complexes: mTORC1 and mTORC2 [30]. mTORC1 can be activated by many factors including VEGFR, PDGFR, EGFR and IGFR through the PI3K/Akt pathway [31].

Activated mTORC1 regulates cyclin D, m-Myc and other proteins involved in cell proliferation through the disassociation of 4E binding protein-1 (4E-BP1) and eukaryotic initiation factor-4 subunit E (eIF-4E) [32, 33]. mTORC2, through protein kinase Cα, regulates cell morphology and adhesion [8, 34] (Figure 3). mTOR activity is enhanced in RCC [35-37]. The two major mTOR inhibitors in clinical practice are temsirolimus and everolimus. Their mode of action is summarized in Figure 3 and Table 2 [36].
Resistance to Targeted Therapy

Despite the availability of the novel targeted therapeutics, and the promising clinical outcomes, nearly all patients with metastases will develop resistance to treatment.

Figure 2. An overview of the VHL-HIF-VEGF pathway in RCC and the mode of action of targeted therapy. A functional VHL gene targets hypoxia-inducible factor (HIF)-α for proteolysis. When the VHL gene is inactivated, either through mutations, hyper-methylations or loss of heterozygosity, HIF is stabilised, activated and translocated to the nucleus, where it binds to HIF-responsive elements leading to the transcription of many hypoxia-inducible genes, including VEGF, mTOR and platelet-derived growth factor (PDGF). The binding sites of the targeted therapies on VEGF, mTOR and TKI pathways are emphasized. FKBP, FK binding protein; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; mTORC1/2, mammalian target of rapamycin complex 1 or 2; mLST8, mammalian lethal with SEC13 protein 8; 4E-BP1, 4E binding protein-1; eIF-4E, eukaryotic initiation factor-4 subunit E; VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet-derived growth factor receptor; Pro, proline; P, phosphorous; Ub, Ubiquitin; FGF, fibroblast growth factor; IL-8, interleukin-8; PTEN, phosphatase and tensin homologue; P70S6K, P70S6 kinase, TKI, tyrosine kinase inhibitor. Modified from (8).

Approximately 30% of patients are intrinsically resistant to therapy [42] and almost all patients who initially respond to therapy will eventually develop extrinsic or acquired resistance. In intrinsic or primary resistance, tumors do not respond to targeted therapy from the beginning of the treatment. Extrinsic
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Resistance is the result of adaptive mechanisms to the action of angiogenesis inhibitors leading to the re-emergence of tumor angiogenesis [5, 43].

**Table 1. Summary of currently available VEGF inhibitors and TKI for the treatment of metastatic RCC**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>Vascular endothelial growth factor (VEGF) monoclonal antibody</td>
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<tr>
<td>Bevacizumab (Avastin®, Genentech Inc.)</td>
<td>Binds and neutralizes all biologically active isoforms of VEGF [2, 9, 10]</td>
<td>Predominantly used in combination with interferon-alpha (IFN-α); median overall survival 18.3–23.3 months; overall response rate, 25.5% [11-13]</td>
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<tr>
<td>Multi-tyrosine kinase inhibitors</td>
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<tr>
<td>Sunitinib (SUTENT®, Pfizer Inc.)</td>
<td>Inhibits PDGFR-α and β, c-KIT, FLT-3, VEGFR1-3, CSF-1R and neurotropic factor receptor [14-16]</td>
<td>Standard first-line of therapy; median overall survival, 26.4 months; objective response rate, 31-47% [11, 17, 18]</td>
</tr>
<tr>
<td>Sorafenib (Nexavar®, Bayer HealthCare/Onyx Pharmaceuticals)</td>
<td>Inhibits VEGFR 1-3, PDGFRα &amp; β and Raf kinase, RET [19]</td>
<td>First or second line of therapy; overall survival 12.5-17.8 months; response rate 40%; stable disease 80% [11, 20, 21, 22]</td>
</tr>
<tr>
<td>Axitinib (Pfizer Inc.)</td>
<td>Inhibits VEGFRs 1 and 2, PDGFRβ and c-KIT [26, 27]</td>
<td>Overall response rate 44.2%; median overall survival 20.1-29.9 months; [11, 28, 29]</td>
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While the mechanisms of intrinsic resistance are largely elusive, the understanding of the extrinsic or acquired resistance is rapidly expanding. In a nutshell, the acquired resistance to targeted therapy can be summarized under four categories: activated alternative angiogenic pathways, inadequate target inhibition, up-regulation of HIF, and faulty mTOR pathway as illustrated in Figure 4 [7, 8, 42].

**Alternative Pro-Angiogenic Pathways in Drug Resistance**

One of the mechanisms of resistance to targeted therapy is the activation of VEGF-independent alternate angiogenic pathways. The emerging consensus is that these alternate angiogenic pathways activate a variety of pro-angiogenic
factors, which would compensate for the inhibited VEGF signalling and promote angiogenesis [5, 42].

Figure 3. Overview of the role of mTOR in RCC progression [7]. PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; mTORC1/2, mammalian target of rapamycin complex 1 or 2; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; IGF, Insulin-like growth factor; EGF, Epidermal growth factor.

For example, the proposed mechanism of sunitinib resistance includes the activation or up-regulation of FGF-s, ephrins and angiopoietins, and for sorafenib, the recruitment of pro-angiogenic bone marrow-derived cells and monocytes to the tumor site [5, 42, 43]. The recruitment of pericytes that help to maintain vessels permeable and functional, and prevent endothelial cells from being affected by anti-angiogenic therapies, is the proposed mechanisms of resistance to pazopanib [44]. For bevacizumab, the increased potential of tumor cells to invade without the need of neovascularization is thought to be the mechanism [7, 43].

It is well-known that interleukin-8 (IL-8) is a potent pro-angiogenic factor and a prognostic factor for RCC. Emerging data show that IL-8 is also a key player in the development of resistance to sunitinib.
Table 2. mTOR inhibitors in clinical practice

<table>
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<tr>
<th>Drug</th>
<th>Mechanism</th>
<th>Outcome</th>
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<tr>
<td>Temsirolimus (Torisel®, Pfizer Inc.)</td>
<td>Inhibits VHL/HIF/VEGF and PI3K/AKT/mTOR pathways [32, 38] [5, 39].</td>
<td>Second line of treatment after sunitinib [40, 41]; overall survival 10.9 months; median progression-free survival 3.8-5.5 months; objective response rate 8.6%; stable disease 32.1% [11, 22, 35]</td>
</tr>
<tr>
<td>Everolimus (Afinitor®, Novartis Pharmaceuticals)</td>
<td>Inhibits mTOR serine-threonine kinase [37]</td>
<td>Second line of treatment after sunitinib or sorafenib; median progression free survival 4-4.9 months; median overall survival 14.8 months; stable disease 66.8% [11, 36, 37]</td>
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</table>

In a xenograft model of RCC, increased IL-8 secretion was associated with reactivation of tumor angiogenesis and subsequent resistance to sunitinib [36, 45]. Administration of IL-8 neutralizing antibody re-sensitized the tumors to sunitinib. Furthermore, elevated IL-8 expression was found in RCC patients who did not respond to sunitinib [36, 45].

Angiopoietin-2 (Ang-2) and sphingosine kinase are potent pro-angiogenic agents. It is emerging that they also play crucial roles in conferring resistance to sunitinib. Ang-2 and SIP are decreased at the initiation of sunitinib treatment. However, with the development of resistance, their expression and activation are significantly increased [42]. Neutralizing antibodies against sphingosine kinase have been shown to reverse resistance in animal models of RCC [42]. Down-regulation of angiostatic factors is another mechanism of resistance to TKIs. Treatment with sunitinib and sorafenib decreases the expression of several IFN-γ inducible molecules including the angiostatic chemokines CXCL 10 and CXCL 11 [42, 46, 47]. Furthermore, recruitment of bone marrow-derived cells, especially CD11b + GR1 + myeloid cells, to the tumour site, enables the development of resistance to sunitinib [42]. In other instances invasion of tumour cells into normal tissue and the recruitment by these invaded tumour cells of normal tissue vasculature protect them from anti-angiogenic therapy [46-48].
Inadequate Target Inhibition and Resistance to Therapy

Although research into this aspect of drug resistance is still in its infancy, there is emerging data that inadequate target inhibition could be a player in resistance to axitinib, sunitinib and sorafenib [49-51]. Inadequate target inhibition can be either due to enhanced receptor signalling or reduced intracellular drug levels (Figure 4). Over time, physiological changes can lead to reduced plasma concentrations of the drugs that in turn lead to resistance through inadequate inhibition of the VEGFR signalling. These data, although support the concept of physiological resistance, fail to identify dose-escalation as the best clinical strategy for overcoming resistance.
Therapy-Mediated VEGF Up-Regulation in Drug Resistance

VEGFR inhibition in clear cell RCC patients leads to a significant increase in serum VEGF levels within the first few weeks of treatment as seen with bevacizumab. However it has been shown that the amount of available unbound bevacizumab is sufficient to adsorb this excess VEGF [52]. It has been hypothesised that this increase reflects effective VEGFR blockade and that an intracellular or intercellular feedback loop leads to greater HIF activity and more VEGF production. If this increase reflects the degree to which VEGFR are inhibited, as opposed to a macroscopic effect on tumour blood vessel architecture leading to increased tumor hypoxia and HIF activation, then one might expect greater increases in VEGF would associate with improved clinical outcome [53, 54]. However, this does not appear to be the case. The emerging consensus regarding the significant rise in VEGF levels following VEGFR inhibition is that tumor progression might in time be mediated by overcoming incomplete receptor inhibition or stimulation of lower affinity VEGFR. Although experimental evidence may be lacking, animal models of tumor angiogenesis (not in RCC) show that VEGF or VEGFR inhibition leads to increased production of PDGF and FGF by tumours. PDGF up-regulation may be addressed by the cross reactivity to PDGFRβ inherent with sorafenib and sunitinib but pro-angiogenic growth factors including basic bFGF, transforming growth factor β (TGFβ), hepatocyte growth factor (HGF) Ang, and ephrins may mediate tumor escape in the face of VEGF and VEGFR inhibition [9, 54, 55].

Faulty Mammalian Target of Rapamycin (mTOR) Pathway in Drug Resistance

mTOR plays a critical role in tumour angiogenesis [32, 56-59]. Temsirolimus, exerts its inhibitory effects on both the tumor and endothelial cells [35]. The mechanisms of resistance to mTOR inhibition may be quite distinct compared to that observed with VEGF-targeted therapy [32, 60]. It has been proposed that acquired resistance is the result of activation of feedback loops that promotes cell survival through the activation of mTORC2, IGF and extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) signaling pathway [43, 61]. The rapamycin analogs inhibit only
one of the two signaling complexes of which mTOR is a part. TORC1 is inhibited by temsirolimus and other rapamycin analog mTOR inhibitors, whereas TORC2 is not [38]. As a result, part of the downstream mTOR activation is unopposed. It has been demonstrated in preclinical studies that mTOR inhibition with rapamycin analogs results in feedback up-regulation of the PI3K pathway, assessed by activation of Akt, which is more proximal in the PI3K pathway than mTOR. This is analogous to VEGF up-regulation in the setting of VEGF receptor blockade. Because Akt can activate the TORC2 complex, which is not inhibited in rapamycin analogs, it is possible that the up-regulation of this pathway compensates for TORC1 inhibition in tumors that are refractory to temsirolimus [42, 43, 60, 62]. Other proposed mechanisms of resistance to mTOR are redundant signaling pathways including KRAS or BRAF mutations and loss of PTEN [5, 42].

**Advances in Overcoming Resistance to Targeted Therapy**

The approaches that have been employed to overcome resistance to targeted therapy are summarized in Table 3. In a nutshell, the available evidence suggests that the use of a second TKI after the failure of the first could ‘reset’ the tumor microenvironment and overcome resistance [43]. Apart from the approaches described in Table 3, there is emerging evidence that a third line of treatment may restore the sensitivity to the initial treatment [5-7, 63-65]. That is, re-challenging with VEGF-TKI as a third line of treatment after the failures of VEGF-TKI as a first line and mTOR inhibitors as a second line. [7]. Ferrari and team compared the administration of mTOR inhibitors everolimus or temsirolimus as a third line therapy, and illustrated them to be promising treatment options [66]. Di Lorenzo et al. showed that sorafenib could be considered as a third line of treatment for metastatic RCC after the failure on sunitinib and mTOR inhibitor, however, with adverse events such as hand-foot syndrome, anaemia, fatigue, diarrhoea and neutropenia [63].

**Future Directions**

Currently, patients with metastatic RCC can obtain benefit from multiple lines of targeted therapy. Resistance to TKI and mTOR inhibitors seem to be
at least partially reversible. Re-challenging with the inhibitors from the same group in subsequent lines of therapy may be a therapeutic option if toxicity does not limit it. Tailoring treatment to a particular patient is of utmost importance [5, 6, 64].

**Table 3. Current approaches to overcome RCC resistance to targeted therapies**

<table>
<thead>
<tr>
<th>Approach</th>
<th>Description</th>
<th>References</th>
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<tr>
<td>Alternating dose</td>
<td>Scheduling 4 weeks on drug and 2 weeks off drug (e.g. sunitinib); re-challenging the same drug after a discontinuation period (e.g. sorafenib)</td>
<td>[36, 42]</td>
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<tr>
<td>Combined TKI and immunotherapy</td>
<td>Sunitinib and sorafenib with or without IFN-α</td>
<td>[67, 68]</td>
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<td>New agents</td>
<td>Dovitinib alone or in combination with VEGF-TKI/mTOR inhibitors; Tivozanib</td>
<td>[69, 70]</td>
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<tr>
<td>Combination therapy</td>
<td>Combination of temsirolimus and sunitinib</td>
<td>[39, 42, 43]</td>
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<tr>
<td>– Two TKIs together</td>
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<tr>
<td>– mTOR inhibitor after first line TKI</td>
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<tr>
<td>– TKIs after first line anti VEGF</td>
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<td>– Second line VEGF TKI after first line VEGF-TKI</td>
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Below are some possible interventions that could improve treatment outcome for metastatic RCCs.
Fourth-Line Therapy Regimens

Even though no clinical guidelines exist for a fourth-line targeted therapy, some reports suggest that patients may gain clinical benefit of sequential targeted agents with different mechanisms of action. Up to 48 months of progression-free survival was achieved in metastatic RCC patients treated with four lines of targeted therapies (sunitinib, everolimus, sorafenib, and temsirolimus), advocating use of the sunitinib, everolimus, sorafenib and temsirolimus for better treatment outcome and overcoming resistance [7, 51, 73].

New Agents

Novel agents that modulate c-MET and Ang/Tie-2 may be effective in overcoming drug resistance. c-MET is a significant player in papillary RCC [74, 75] and c-MET inhibitors such as tivantinib and cabozantinib hold potential as either first or second line therapy [76-79]. Ang/Tie 2 signaling axis is a well-known promoter of angiogenesis. Metastatic RCC is positively associated with circulating Ang-2, making it an interesting therapeutic target [80]. For example, the Ang-2 inhibitors AMG386, CVX060 and CVX241 have been shown to be effective in pre-clinical models of RCC (81-83). The PI3K pathway is related with poor prognosis and serine/threonine Akt kinase is a critical player downstream from PI3K and crucial for cell survival. BKM-120 is a pan-class I PI3K inhibitor, and has undergone phase I trial in combination with bevacizumab (84). Moreover the Akt inhibitor MK2206 is also in phase II clinical trial [85].

Conclusion

Despite the immense advancement, resistance to targeted therapy is emerging as a major hurdle for the treatment of metastatic RCC. Defining the mechanisms and pathways of resistance to therapy will help develop methods to overcome therapy resistance. Many issues remain unanswered and open in this emerging field. Further research will shed more light into the mechanisms of drug resistance, and enable the development of better therapeutics for the treatment of metastatic RCC.
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References


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