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Review

Resistance to tyrosine kinase inhibitors in clear cell renal cell carcinoma: From the patient's bed to molecular mechanisms☆

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ABSTRACT

The introduction of anti-angiogenic drugs especially tyrosine kinase inhibitors (TKIs) was a breakthrough in the treatment of renal cell carcinoma (RCC). Although TKIs have significantly improved outcome in patients with metastatic disease, the majority still develop resistance over time. Because different combinations and sequences of TKIs are tested in clinical trials, resistance patterns and mechanisms underlying this phenomenon should be thoroughly investigated. From a clinical point of view, resistance occurs either as a primary phenomenon (intrinsic) or as a secondary phenomenon related to various escape/evasive mechanisms that the tumor develops in response to vascular endothelial growth factor (VEGF) inhibition. Intrinsic resistance is less common, and related to the primary redundancy of available angiogenic signals from the tumor, causing unresponsiveness to VEGF-targeted therapies. Acquired resistance in tumors is associated with activation of an angiogenic switch which leads to either upregulation of the existing VEGF pathway or recruitment of alternative factors responsible for tumor revascularization. Multiple mechanisms can be involved in different tumor settings that contribute both to evasive and intrinsic resistance, and current endeavor aims to identify these processes and assess their importance in clinical settings and design of pharmacological strategies that lead to enduring anti-angiogenic therapies.

Contents

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1. Introduction — resistance as a clinical problem in renal cell carcinoma treatment

The findings about abnormal activities of signal transduction pathways in clear cell renal cell carcinoma (ccRCC) have allowed to establish novel targeted therapies for this disease, greatly improving the treatment options and prognosis of RCC patients [1–4]. Angiogenesis is necessary to support the growth of ccRCC greater than 1 to 2 mm in diameter and its high activity is mostly mediated by mutation or epigenetic inactivation of the von Hippel Lindau (VHL) tumor suppressor gene and subsequent up-regulation of hypoxia-inducible factor (HIF) expression [5]. Overexpression of HIF protein results in an increased expression of pro-angiogenic VEGF and platelet derived growth factor (PDGF) — key players involved in ccRCC development and progression [5,6].

Clinical studies over the last few years have demonstrated that multiple agents effectively blocking the angiogenic pathway have clinical efficacy; these agents include TKIs (sunitinib, sorafenib, pazopanib, and axitinib), the anti-VEGF monoclonal antibody — bevacizumab (administered with interferon α) and mammalian target of rampamycin (mTOR) inhibitors — everolimus and temsirolimus [7,8]. Each agent offers a significant clinical benefit, determined by the rate of objective responses (OR), reduction in tumor burden (RECIST), and extension of progression-free survival (PFS) compared with the standard of care. The first approved drug in the TKI family, sorafenib, investigated in the TARGET randomized, double-blind, phase III study in patients refractory to cytokine therapy, gave a significantly prolonged PFS in comparison with placebo (5.5 *vs.* 2.8 months, $p < 0.001$) [1]. Another TKI, sunitinib, compared with INF-α in a randomized phase III trial, has shown a significant prolongation of PFS (11 *vs.* 5 months, $p < 0.001$) in previously untreated patients with ccRCC [3]. This trial also demonstrated longer OS in the sunitinib group (26.4 *vs.* 21.8 months, $p = 0.051$), as well as significantly improved objective response rate (ORR) reaching 47% for sunitinib compared with 12% for INF-α ($p < 0.001$) [9]. Clinical outcomes for pazopanib treatment, notably prolongation of PFS in cytokine-pretreated patients compared to placebo (9.2 *vs.* 4.2 months, $p < 0.001$) and treatment-naïve population (11.1 *vs.* 2.8 months, $p < 0.001$), definitely confirmed the great potential of TKIs and raised hopes for a breakthrough in ccRCC treatment [4]. Based on the results from multiple clinical trials, current clinical practice guidelines recommend the use of VEGF inhibitors, sunitinib, bevacizumab with INF-α and recently pazopanib in first-line therapy for patients with metastatic RCC (mRCC) with good or intermediate prognosis according to MSKCC [10,11]. TKIs are also currently used as standard treatment for patients after previous cytokine therapy and alternatively in sequence (with change of the TKI) in second-line treatment [12].

Despite the therapeutic progress, complete and durable responses have been noted in only a few cases [10], necessitating chronic therapy for the majority of RCC patients, which is often associated with significant toxicity [1,3]. Another issue is that the response to treatment with a specific agent differs between patients, suggesting specific molecular mechanisms promoting individual susceptibility to each TKI. A large systematic review by Park et al. analyzed clinical data from over 12 clinical centers of patients with ccRCC treated with TKIs and showed that 26% out of 1056 patients treated with sorafenib and sunitinib were primarily refractory to treatment, showing no disease stabilization nor clinical benefits. The majority of these VEGF-refractory patients exhibited a uniform poor outcome regardless of therapy received [14]. While some mRCC patients are primarily refractory to VEGF-targeted treatment, the rest who primarily respond to VEGF-targeted treatment often develop secondary (acquired) resistance to certain agents after prolonged treatment. Typically ccRCC patients develop resistance to various TKIs within a median of 6–12 months [8], at which point tumor growth resumes despite continued administration of the drug, causing progressive disease [14].

Therefore, a pressing clinical and scientific question arises about the mechanisms determining resistance to TKI therapy, and the relevant treatment approach for metastatic ccRCC patients. To date, emerging clinical and preclinical data are available to address this issue. In this review, we analyze the existing data from both fields that provide insight into clinical ccRCC treatment limitations and needs, as well as recent advances in the identification of resistance determinants on a molecular level. Filling the gap in understanding TKI resistance development is necessary to propose possible strategies for continuous and efficient treatment of patients with ccRCC and improving their dramatic prognosis.

2. Molecular mechanisms of RCC resistance

In general, tumor sensitivity to targeted agents occurs when its growth and progression depend on the constitutive activity of signaling pathways specifically targeted by these agents. On the other hand, resistance may occur 1) when targeted proteins are inaccessible for drug binding due to their structural alteration or 2) upon activation of an alternative signaling pathway(s) or 3) due to upregulation of specific molecule expression that compensates for drug-mediated inhibition [13]. Based on the results of both preclinical and clinical studies indicating drug exposure-dependent origin of resistance occurring in RCC treatment [14–16], two general models of tumor resistance to anti-angiogenic agents targeting the VEGF pathway have been postulated: an adaptive (evasive) resistance, which occurs after a prolonged application of a drug (providing a period of tumor control), and intrinsic (preexisting) non-responsiveness despite the presence of an active agent, showing no therapeutic benefit [17].

2.1. Acquired resistance

The traditional concept of drug resistance being acquired by either mutations within genes encoding a drug target or by genetic alterations in mechanisms determining drug uptake and efflux has become less probable in the light of new preclinical and clinical data [18,19]. Although experimental evidence is not yet definitive, various studies suggest that at least four distinct mechanisms mediate acquired resistance to VEGF-targeted therapies; these are 1) up- or downregulation of genes involved in the alternative signaling pathway supporting angiogenesis in the tumor environment; 2) increased pericyte coverage of tumor vessels; 3) recruitment of pro-angiogenic inflammatory cells from bone-marrow; and finally 4) increased invasiveness of tumor cells into the normal tissue, which obviates the need for neovascularization [17]. There are studies that postulate that mechanisms involved in multi-drug resistance determining RCC resistance to chemotherapy might be also involved in decreased intake of TKIs (5) [20]. Finally the study of Gotink introduced the mechanism of lysosomal sequestration as a specific cellular adaptation to the toxic TKIs concentration in TKI-resistant renal cell cancer *in vitro* models [21]. This paragraph aims to describe the role of each mechanism mentioned above in acquisition of TKI-specific resistance and explain the rationale behind them.

2.1.1. Angiogenic switch — activation of alternative pathways supporting angiogenesis

A noticeable inhibition of tumor growth followed by its restoration after prolonged treatment is commonly seen by clinicians during TKI treatment in responding RCC patients. Preclinical studies investigating this phenomenon indicate that the angiogenesis switch may be determined by both overexpression of factors involved in alternative pro-angiogenic pathways and by downregulation of angiostatic ones [22].

The evidence for the evasive resistance being mediated by an alternative signaling pathway comes primarily from the preclinical study of Casanovas et al. performed on the genetically engineered *Rip1–Tag2* mouse model of pancreatic neuroendocrine (islet cell) cancer