ATP-competitive inhibitors of mTOR: new perspectives in the treatment of renal cell carcinoma

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Abstract
Targeting mTOR (mammalian target of rapamycin) is an effective approach in the treatment of advanced RCC (renal cell carcinoma). Rapamycin-like drugs (rapalogues) have shown clinical activities and have been approved for the treatment of RCC. Recently, with the development of ATP-competitive inhibitors of mTOR, therapies targeting mTOR have entered a new era. Here, we discuss the biological relevance of blocking mTOR in RCC and review the mechanisms of action of rapalogues in RCC. We also advance some perspectives on the use of ATP-competitive inhibitors of mTOR in RCC.

Introduction
RCC (renal cell carcinoma) is a highly vascularized tumour that accounts for nearly 100 000 deaths per year worldwide [1]. Most symptomatic patients present with a metastatic disease that is refractory to most therapies including chemotherapy and radiotherapy. Until recently, systemic cytokine therapies were the standard of care for advanced disease resulting in response rates of nearly 15% [2]. However, since these therapies achieve only limited survival benefits in advanced RCC, considerable work has been made to understand the molecular mechanisms involved in the pathogenesis of RCC and to develop new therapeutic strategies. The identification of the role played by pVHL (von Hippel–Lindau protein) in the development of RCC has given a rationale for the use of anti-angiogenic therapies in RCC. In fact, four drugs that target the tumour vasculature by either blocking VEGF (vascular endothelial growth factor; bevacizumab) or one of its receptors (sorafenib, sunitinib and pazopanib) have been approved for the treatment of metastatic RCC as mTOR (mammalian target of rapamycin) inhibitors (temsirolimus and everolimus) have [3,4].

pVHL and RCC
A strong association exists between the inactivation of pVHL and the development of RCC. Indeed, germline inactivation of the VHL gene is associated with an increased risk of various cancers including RCC and somatic mutations of VHL is documented in up to 50% of sporadic RCC [5]. Furthermore, in experimental settings, the ability of VHL−/− renal carcinoma cells to form tumours in nude mice is blocked by the restoration of pVHL [6]. Many biological functions are controlled by pVHL, among which the regulation of HIF (hypoxia-inducible factor) plays a critical role in the development of RCC [7]. As part of an E3 ubiquitin ligase complex, pVHL participates in the degradation of the α-subunits of HIF. In the absence of oxygen or following pVHL inactivation, HIFα accumulates and dimerizes with HIFβ, resulting in the activation of pro-angiogenic genes such as VEGF [7].

Three different HIFα genes exist in humans. Although HIF1α and HIF2α activate transcription, the function of HIF3α is less clear and can act as an inhibitor of HIF1α and HIF2α [8]. Inactivation of pVHL in renal cancer cells induces the accumulation of HIF2α or both HIF1α and HIF2α. Although some of the functions of HIF1α and HIF2α are overlapping, emerging evidence shows that HIF2α is more critical than HIF1α in the pathogenesis of RCC. Indeed, silencing HIF2α blocks the ability of VHL−/− renal carcinoma cells to form tumours in vivo [9]. Furthermore, overexpression of HIF2α but not HIF1α overrides the tumour suppression function of pVHL [10].

Rapalogues and RCC
The identification of the role played by pVHL and HIFα in the pathogenesis of RCC has led to the design of therapeutical strategies that target HIFα or one of its downstream effectors, VEGF. In this context, blocking mTOR appears to be a promising approach [11]. mTOR is a protein kinase that regulates cell growth survival and metabolism as being part of two distinct protein complexes: mTORC1 and mTORC2. Whereas mTORC1 regulates translation and controls cell growth, mTORC2 phosphorylates and activates Akt (protein kinase B) [12]. Rapalogues inhibit mTOR kinase activity by forming a complex with the FK506-binding protein that binds near the catalytic site of mTOR, selectively blocking
mTORC1 but with no direct effect on mTORC2 [13]. However, in certain type of cells such as endothelial cells, prolonged exposure to rapalogues may also secondarily inhibit mTORC2 [14]. It is worth noting that mTORC1 inhibition by rapalogues also leads to the loss of feedback inhibition of PI3K (phosphoinositide 3-kinase) signalling, which counteracts the anti-tumour efficacy of rapalogues [15]. Of relevance to RCC, mTOR activation has been shown to regulate HIFα at the levels of mRNA translation as well as protein stabilization [15]. In addition, mTOR inhibition also decreases VEGF expression [16].

Targeting mTOR has shown clinical activity in patients with RCC. In a phase III clinical trial, temsirolimus improved overall survival in patients with advanced RCC and poor prognostic features compared with interferon-α [17]. Similarly, everolimus prolonged progression-free survival compared with placebo in patients with metastatic RCC who had progressed on previous targeted therapies [18]. Although rapalogues have demonstrated clinical responses in RCC, the effect is rather modest. Thus the characterization of the mechanisms of the action of rapalogues in RCC is essential to further improve their development. Emerging evidence suggests that rapalogues exert anti-tumour effects in RCC by blocking tumour angiogenesis [19]. RCC is a highly vascularized tumour and anti-angiogenic therapies such as bevacizumab have shown clinical activities, suggesting that targeting the tumour vasculature might improve the outcome of RCC [3]. Moreover, experimental reports have also shown that rapamycin inhibits angiogenesis in various cancer models through multiple mechanisms [16]. As mentioned earlier, by blocking mTOR-dependent translation of HIFα, rapamycin decreases the expression of pro-angiogenic cytokines such as VEGF [16,20]. Furthermore, rapamycin also directly affects endothelial cell proliferation, survival and migration [21]. Interestingly, treatment of endothelial cells with rapamycin inhibits both mTORC1 and mTORC2, resulting in Akt inactivation [21]. Given the importance of Akt in angiogenesis, the ability of rapamycin to block Akt in endothelial cells might therefore be an important mechanism for its anti-angiogenic effects [22].

In addition to inhibiting angiogenesis, the anticancer efficacy of rapamycin in RCC might also rely on its ability to interfere with cancer cells. Alterations of the PI3K/Akt/mTOR signalling pathway occur in many solid cancers which render cancer cells vulnerable to rapalogues. In RCC, lack of PTEN (phosphatase and tensin homologue deleted on chromosome 10) expression, which results in increased PI3K/Akt signalling, has been observed and correlates with poor patient prognosis [23]. Furthermore, several renal carcinoma cell lines exhibit high levels of Akt in vitro [24]. However, despite these observations, most of the renal carcinoma cell lines respond poorly to rapalogues or are susceptible at concentrations that are not achievable in the clinic [25]. The loss of a negative feedback loop which leads to increased PI3K/Akt signalling could limit the anticancer effects of rapalogues and may explain this lack of response [15]. Indeed, activation of Akt is frequently observed in biopsy samples of patients exposed to rapalogues [26]. Therefore the anticancer efficacy of rapalogues in RCC may not result from a direct effect on cancer cells but rather from the inhibition of angiogenesis.

ATP-competitive inhibitors of mTOR and RCC

Although the role of mTOR signalling pathway in cancer development and progression has been demonstrated, emerging evidence shows that targeting mTOR with rapalogues has limited benefits. Indeed, rapalogues only partially block mTORC1 and do not inhibit mTORC2. Furthermore, rapalogue-mediated mTORC1 inhibition can lead to the activation of PI3K/Akt signals as well as other signalling pathways that counteracts the anticancer efficacy of rapalogues [15]. To overcome these limitations, extensive work has been done to identify and develop ATP-competitive modulators of mTOR [13]. Recently, compounds that block mTOR by competing with ATP have been described and can be classified into two groups: specific mTOR inhibitors that block mTORC1 and mTORC2 or dual PI3K/mTOR inhibitors that in addition to inhibiting mTORC1 and mTORC2 also target PI3K [27,28].

Preclinical studies have shown that ATP-competitive inhibitors of mTOR significantly block tumour progression in a large variety of tumour cells [28]. Their efficacy on the mTOR signalling pathway has also been demonstrated. Particularly, ATP-competitive inhibitors are able to block Akt activity, thus avoiding the feedback activation of PI3K/Akt observed with rapalogues. In the context of RCC, the effect of NVP-BEZ235, a dual PI3K/mTOR inhibitor, has also been investigated and compared with rapamycin [25]. Overall, the tumour growth inhibition properties of NVP-BEZ235 were superior to rapamycin both in vitro and in vivo. In contrast with rapamycin, renal cancer cell lines responded significantly to NVP-BEZ235, which induced a reduction in cell proliferation and an induction of apoptosis. Interestingly, at the molecular level, NVP-BEZ235 markedly reduced the expression of HIF2α whereas rapamycin had little effect. Paradoxically, NVP-BEZ235 failed to reduce tumour angiogenesis in renal carcinoma xenografts. Consistent with this finding, an increased tumour vasculature has also been previously observed in renal carcinoma xenografts in response to PI3K inhibition [29]. Furthermore, NVP-BEZ235 failed to reduce tumour angiogenesis in pancreatic cancer xenografts whereas rapalogues did [30]. However, in contrast with these findings, it was also reported that NVP-BEZ235 inhibits angiogenesis. Indeed, NVP-BEZ235 reduced tumour angiogenesis in melanoma and glioma tumour models and blocked VEGF-induced angiogenesis in Matrigel™ [31–33]. Therefore further studies are needed to fully characterize the effect of NVP-BEZ235 on tumour angiogenesis.

In addition to NVP-BEZ235, WYE132, a specific mTOR inhibitor, has also been tested on renal carcinoma cell lines [34]. Similar to what was observed with NVP-BEZ235, the growth of renal carcinoma xenografts was more profoundly
inhibited by WYE123 than the rapalogue temsirolimus. WYE123 was also more effective than temsirolimus in blocking hypoxia-induced HIF1α and HIF2α accumulation and VEGF production.

Conclusions

Targeting mTOR with rapalogs has been a successful strategy in RCC by in part blocking HIFα/VEGF expression and thus affecting tumour angiogenesis. Although still in their early days of development, encouraging preliminary studies have shown that ATP-competitive inhibitors of mTOR reduce the growth of RCC cell lines in vitro and in vivo more effectively than rapalogs. Furthermore, their inhibitory efficacy on HIFα/VEGF expression is superior to rapalogs. The effect of these agents on tumour angiogenesis remains to be characterized and ongoing phase I clinical trials will investigate their toxicity profile. Nevertheless, the use of these agents in RCC represents a promising new cancer therapy.

Funding

This work was supported by a research grant of the Swiss National Science Foundation [grant number SCORE 32323B-123821 to O.D.].

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Received 8 November 2010
doi:10.1042/BST0390492