Chapter 12
PI3-kinase, Akt, and mTOR Inhibitors in RCC

Daniel C. Cho and James W. Mier

Molecular Biology of the PI3-K/Akt/mTOR Pathway

The kinase mTOR is regulated in large part through the activity of phophatidylinositol 3-kinase (PI3-K) and Akt (protein kinase B). The PI3-K pathway regulates critical aspects of cell growth, metabolism, survival, and proliferation. In human malignancy, this pathway is one of the most frequently altered and plays a critical role in tumor cell growth, invasiveness, and metastatic behavior [1, 2].

Activation of PI3-K in RCC

Other than the clinical activity of mTOR inhibitors, one of the primary reasons for the interest in the PI3-K/Akt pathway in RCC is that it appears to be activated in a large percentage of RCC tumor specimens, and this activation is correlated with higher histologic grade and worse clinical outcomes [3]. Class IA PI3-K, the most relevant of the three classes of PI3-K to human cancer, are heterodimeric kinases consisting of a p85 regulatory subunit and a p110 catalytic subunit. There are three class IA p110 isoforms (α, β, and δ) encoded by three genes (*PIK3CA*, *PIK3CB*,

D.C. Cho, M.D. (✉)
Department of Hematology and Oncology, NYU Cancer Center,
160 East 34th Street, Rm 829, New York, NY 10016, USA
e-mail: Daniel.cho@nyumc.org

J.W. Mier, M.D.
Division of Hematology and Oncology, Beth Israel Deaconess
Medical Center, Boston, MA, USA

R.M. Bukowski et al. (eds.), *Renal Cell Carcinoma*,
DOI 10.1007/978-1-4939-1622-1_12

and *PIK3CD*, respectively) and one related class IB p110 isoform (γ). Of these, the α and β isoforms are believed to be expressed ubiquitously, whereas the δ and γ isoforms are expressed only in the hematopoietic lineage [4]. While mutations in both the p110α subunit (*PIK3CA*) and p85 regulatory subunit have been described and can lead to constitutive activation of PI3-K, *PIK3CA* mutations are far more common [5, 6]. In renal tumors, *PIK3CA* mutations are quite rare [7], suggesting that the PI3-K activity observed in renal cell carcinoma (RCC) is due to alternate mechanisms.

The class IA PI3-K phosphorylate phosphatidylinositol-4,5-biphosphate (PIP2) generates phosphatidylinositol-3,4,5-triphosphate (PIP3). This activity is directly opposed by the tumor suppressor phosphatase and tensin homologue (*PTEN*), which dephosphorylates PIP3 to PIP2, reversing the activity of PI3-K. Therefore, another mechanism by which the PI3-K pathway can also be activated is by the loss of *PTEN* [8]. While somatic mutations in *PTEN* appear rare in RCC [9, 10], the expression of PTEN appears to be frequently downmodulated in RCC relative to normal renal tissue [11]. The molecular basis for this downmodulation is currently unknown. Regardless of mechanism, however, the relative absence of PTEN is thought to contribute to the PI3-K activity observed in these tumors.

Class IA PI3-K can also be activated through upstream signaling by receptor tyrosine kinases (RTK). Activation of RTKs, most commonly through growth factor signaling, results in the phosphorylation of the Y-X-X-M motif present in the cytoplasmic tail of the RTK, which then binds to the Src homology (SH2) domain of the p85 regulatory subunit of PI3-K. This results in a functional dissociation of the p85 subunit from the p110 subunit, augmenting kinase activity of the latter. Mutations in RTKs are exceedingly rare in RCC. However, it is likely that some RTKs may play a role in the basal PI3-K activity observed. For example, epidermal growth factor receptor (EGFR) is activated by transforming growth factor (TGF)-α and other EGFR ligands produced by the tumor cells in a hypoxia-inducible factor (HIF)-dependent manner [12]. It is likely that this autocrine loop contributes to the PI3-K activation frequently observed in RCC.

Activation of mTOR in RCC

mTOR exists in two functionally distinct complexes, TORC1 and TORC2, distinguished by their relative sensitivity to rapamycin. TORC1, a complex including mTOR and Raptor (regulatory-associated protein of mTOR), is sensitive to rapamycin and regulates many of the functions canonically associated with mTOR such as growth, proliferation, cap-dependent translation, and protein synthesis. The activity of TORC1 responds to numerous environmental signals, including the availability of oxygen, nutrients, ATP, and amino acids, some of which are transmitted through PI3-K. PI3-K mediates the activation of mTOR through its downstream effector, Akt, which phosphorylates tuberous sclerosis complex (TSC) 2 at multiple sites, causing it to disassociate from binding partner TSC1. The TSC1/TSC2 complex

functions as a GTPase-activating protein (GAP) for the G protein Rheb. PI3-K/Akt activation reduces the GAP activity of TSC1/TSC2 toward Rheb, allowing it remain in a GTP-bound state capable and activating TORC1. The activity of TSC1/TSC2 complex can be affected by numerous other pathways besides PI3-K/Akt including the AMP-LKB1 pathway and MAP-K [13, 14]. TORC1 activity is also regulated by TSC-independent inputs such as amino acid availability which modulates mTOR activity through the RAG GTPases [15]. Although mutations in various members of the mTOR regulatory pathways have been traditionally felt to be uncommon in RCC, data emerging from the Cancer Genome Atlas (TCGA) project will more accurately profile the frequency of these mutations in RCC tumor specimens. Indeed, the presence of activating mutations in mTOR and inactivating mutations in TSC1 and TSC2 has already been associated with prolonged responses to rapalogue therapy in patients with advanced RCC [16].

Biologic Consequences of PI3-K/Akt/mTOR Activation

Regardless of the mechanism by which it is activated, PI3-K signals to a vast network of kinases, transcription factors, and other proteins which promote cellular growth and proliferation. While the best described effector PI3-K is Akt (protein kinase B), there are several other PI3-K dependent pathways including those possibly relevant to cancer, such as serum and glucocorticoid kinases (SGKs) and Bruton tyrosine kinase (BTK) [17, 18]. However, Akt has traditionally been regarded as the primary executer of PI3-K and regulates the function of a broad array of proteins involved in cell growth, proliferation, motility, adhesion, neovascularization, and apoptosis [19]. Akt enhances cellular resistance to apoptosis by directly phosphorylating and inactivating several proapoptotic proteins, including procaspase 9, the bcl-2 family member BAD, and apoptosis signal-regulating kinase-1 (ASK1) [20–22]. Akt also differentially regulates transcriptional factors controlling expression of apoptotic genes, negatively regulating factors promoting expression of death associated genes (e.g., forkhead family members [FOXO]) and positively regulating genes promoting survival (NF-κB) [23, 24].

In addition to its pro-survival effects, Akt also promotes tumor proliferation by enhancing progression through the cell cycle. Several Akt-regulated proteins appear to modulate the activity of cyclin-dependent kinases (CDKs) which in turn inactivate retinoblastoma protein (RB) and allow progression through the G1-S checkpoint. Perhaps the most important example of this cell cycle promoting activity of Akt is the modulation of cyclin D1 levels, which are elevated in many human cancers. Akt enhances cyclin D1 levels through suppression of glycogen synthase kinase 3 (GSK3β) [25, 26]. GSK3β is also known to phosphorylate and promote the degradation of other cell cycle regulatory proteins such as c-Myc and cyclin E1 as well as transcription factors governing cell fate such as c-Jun, β-catenin, GLI, and Notch.

As discussed earlier, mTOR is activated downstream of Akt and executes its biologic functions in two distinct complexes, TORC1 and TORC2. TORC2, which

includes mTOR and Rictor (rapamycin-insensitive companion of TOR), is relatively insensitive to rapamycin and functions to enhance Akt activity by mediating its phosphorylation on the Ser473 residue. TORC1 executes most of the biologic functions traditionally attributed to mTOR, acting through its downstream effectors, the eukaryotic translation initiation factor 4E-binding protein (4E-BP) and the 40S ribosomal protein p70 S6 kinase (S6K), to stimulate protein synthesis and entrance into G1 phase of the cell cycle. The activation of S6K by mTOR is critical for ribosomal biogenesis, cell growth, anti-apoptosis, and translation of structured 5′UTR containing mRNA species, while the phosphorylation (and inactivation) of 4E-BP1 promotes cap-dependent translation of nuclear mRNA by releasing the inhibition of eukaryotic translation initiation factor 4E (eIF4E).

In addition to stimulation of growth and proliferation, activation of the mTOR pathway may be of particular relevance to RCC because of its role in the regulation of the expression of both HIF-1α and HIF-2α. Inappropriate accumulation of HIF-1α and HIF-2α as a result of biallelic alterations in the von Hippel-Lindau (VHL) gene observed in the majority of clear cell RCC is believed to be a critical step in RCC tumorigenesis [27, 28]. It has recently been suggested that the expression of HIF-1α is dependent upon the activity of both TORC1 and TORC2, while the expression of HIF-2α is dependent upon TORC2 activity alone [29]. While the overlap between the roles of HIF-1α and HIF-2α is poorly understood, it is generally accepted that HIF-2α is the more relevant HIF with respect to the development and progression of RCC [30, 31]. In fact, recent studies suggest that HIF-1α may function as a tumor suppressor in clear cell RCC [32]. Another recent study segregating *VHL*-deficient sporadic RCC into two subtypes, those expressing both HIF-1α and HIF-2α and those expressing HIF-2α alone, found no specimens expressing HIF-1α alone [33]. Thus the differential activation of TORC1 and TORC2 might play a critical role in RCC tumorigenesis and progression.

Clinical Results with mTOR Inhibitors

The rapalogues temsirolimus and everolimus have both demonstrated clinical efficacy in large randomized phase III trials in patients with advanced RCC. Temsirolimus is an intravenously administered analog of rapamycin. After showing promising activity in a phase II trial randomizing patients with metastatic RCC to three different doses [34], temsirolimus was assessed in a randomized three-arm phase III trial comparing temsirolimus alone versus IFN-α alone versus the combination [35]. As the phase II study suggested potentially unique efficacy in patients with poor prognostic features in a retrospective analysis, the phase III study enrolled only patients with metastatic RCC and ≥3 of 6 risk factors (5 MSKCC risk factors [Karnofsky PS <80, time from diagnosis to randomization <12 months, serum LDH >1.5 ULN, hemoglobin <LLN, corrected serum calcium >10 mg/dl] + >1 metastatic site). Overall, 626 previously untreated patients were enrolled and randomized in a 1:1:1 fashion to receive IFN-α alone (3 million units three times weekly), temsirolimus alone (25 mg IV weekly), or the combination (temsirolimus 15 mg weekly and

6 million units IFN-α three times weekly). The overall survival of patients treated with temsirolimus alone was statistically longer than those treated with IFN-α alone (7.3 versus 10.9 months; 0.73 hazard ratio, $p = 0.0069$). There was no statistical difference between patients treated with IFN-α alone and the combination of IFN-α and temsirolimus. Overall, temsirolimus was well tolerated with the most common adverse effects being asthenia, rash, anemia, nausea, peripheral edema, hyperlipidemia, and hyperglycemia. Based on these findings, temsirolimus was approved by the FDA for therapy in advanced RCC on May 30, 2007, and is now considered a standard therapeutic option in the first-line setting for patients with poor prognosis features.

Everolimus is an orally administered rapalogue and was assessed in a randomized, double-blind, placebo-controlled phase III trial in patients with advanced RCC who had failed prior treatment with either sorafenib, sunitinib, or both (other prior therapy also allowed) within the preceding 6 months (*RE*nal Cell cancer treatment with *O*ral *R*AD001 given *D*aily-1 [RECORD-1]) [36]. Overall, 416 patients were enrolled and randomized in a 2:1 fashion to receive either everolimus ($n = 277$) or placebo ($n = 139$) each together with best supportive care. The primary end point was PFS as randomization was unblinded at time of progression, and patients on placebo were allowed to crossover to open-label everolimus, confounding any potential differences in overall survival. The trial was halted at the second interim analysis after 191 progression events had been observed. At the final central radiology assessment, the median PFS for patients treated with everolimus was 4.88 months as compared with 1.87 months in the placebo group (hazard ratio 0.33, [95 % CI 0.25–0.43] $p < 0.0001$) [37]. Five patients (2 %) in the everolimus group experienced partial responses versus none in the placebo group. Similar to temsirolimus, the side effect profile of everolimus was favorable with most common adverse events with everolimus being stomatitis (40 %), rash (25 %), fatigue (20 %), hypercholesterolemia (76 %), hypertriglyceridemia (71 %), and hyperglycemia (50 %). Pneumonitis was observed in 22 patients (8 %) compared with 0 in the placebo group. Based on these findings, everolimus was approved by the FDA in March 2009 for the treatment of patients with advanced RCC who failed either sorafenib, sunitinib, or both and is now considered a standard second-line therapeutic option following the failure of VEGF-targeted TKI.

Special mention should be made of the potential efficacy of the rapalogues in non-clear cell RCC. Of the molecularly targeted agents, only temsirolimus has been studied in a randomized phase III trial allowing patients with non-clear cell histology [35]. Upon sub-analysis of this phase III trial, among the 73 patients with non-clear cell histology (75 % of which had the papillary subtype) randomized to receive either temsirolimus ($n = 36$) or IFN ($n = 37$), the median overall survival of patients was 11.6 months in the temsirolimus group versus 4.3 months in the IFN group [38]. For this reason, temsirolimus is the only agent given a category 1 recommendation by the National Comprehensive Cancer Institute (NCCN) for the treatment of patients with metastatic non-clear cell RCC. Studies comparing the efficacy of the rapalogues in comparison to VEGFR-TKI in patients with non-clear cell RCC are underway and should better characterize the efficacy of these agents in this group of patients.

Predictive Biomarkers

Unfortunately, only a subset of patients experience substantial clinical benefit from treatment with rapalogues. Therefore, the therapeutic index of this class of agents might be enhanced by the development of patient selection strategies to direct these drugs to the patients most likely to benefit. As with other molecularly targeted agents, however, there are currently no clinically validated predictive clinicopathologic features or biomarkers of benefit from therapy with mTOR inhibitors. Although temsirolimus has demonstrated specific efficacy in patients with poor-risk MSKCC features, the same finding has yet to be observed with everolimus, raising questions as to whether this is a class effect of all mTOR inhibitors. Several lines of evidence suggest, however, that treatment outcome is likely to be determined by the particular genetic alterations and signaling pathways activated in individual tumors.

Many studies have suggested the familiar paradigm that the pretreatment activation status of PI3-K/Akt/mTOR signaling may be a predictor of the likelihood of response to agents targeting this pathway [39]. For example, in a small study carried out in parallel with a recent phase II trial of temsirolimus in patients with RCC, a correlation between tumor cell Akt and S6 phosphorylation as defined by immunohistochemistry and clinical response was demonstrated [40]. The significance of this study is limited because of its retrospective nature and the small number of tumors examined. This study was also limited by the reliance on immunohistochemistry which is associated with an inherent subjectivity in interpretation and also dependent upon the availability of reliable antibodies against the substrates of interest. Many investigators are now moving toward genetic predictors of mTOR pathway activation as a more objectively determined biomarker. In a recent study reported by Voss et al., specimens from six patients treated with rapalogues who were felt to be robust responders were analyzed by directed sequencing [16]. It was subsequently found that two cases had mutations in TSC1, one case with a mutation in TSC2, and one case with a mutation in mTOR. Data from The Cancer Genome Atlas (TCGA) project will soon be emerging and will provide valuable information on the expected frequency of mutations expected to result in constitutive mTOR activation in RCC and shed light on the feasibility of the correlation of such mutations to clinical response in a broader sample of patients.

Novel Inhibitors of PI3-K, Akt, and mTOR

In addition to identifying predictive biomarkers, efforts to improve upon the therapeutic index of rapalogues have also focused on developing more effective drugs targeting this pathway. As discussed earlier, although mTOR is a validated therapeutic target in RCC, it is but one of many kinases governed by PI3-K and Akt, which activate several other downstream signaling pathways essential for energy generation, protein synthesis, proliferation, and cell survival. It is clear that there are several mechanisms by which TORC1 inhibition is felt to potentially result in the feedback activation of PI3-K and Akt, including via release a feedback loop involving

the IGF-1 receptor and derepression of TORC2 resulting in TORC2-mediated phosphorylation of Akt on Ser^{473} [41, 42]. The feedback activation of PI3-K may directly undermine the efficacy of TORC1 inhibitors by promoting the phosphorylation of eIF4E by Mnk1, thereby enhancing its affinity for the mRNA cap structure and activating cap-dependent translation [43]. Therefore, inhibition of PI3-K or Akt has emerged as a therapeutic strategy that may negate activation of this feedback loop and more effectively suppress the translation of critical mRNAs.

Another pharmacologic approach worthy of investigation is the direct inhibition of the catalytic domain of mTOR. Such an approach has the advantage of inhibiting the kinase activity of mTOR regardless of whether it is in a complex with Raptor (TORC1) or Rictor (TORC2). As noted earlier, the expression of HIF-2α (the dominant HIF in RCC) is largely dependent upon the activity of TORC2 and independent of TORC1 activity. As such, this therapeutic approach may have advantages to the allosteric inhibition of TORC1 alone and have particular relevance to RCC.

Many agents are currently in development which are pan-isoform inhibitors of PI3-K, inhibitors of Akt (both catalytic and allosteric), and dual inhibitors of PI3-K and mTOR. Preclinical studies with PI3-kinase inhibitors in RCC have supported the hypothesis that these agents may have activity in RCC. Inhibition of PI3-K/Akt signaling by PI3-K inhibitors LY294002 and wortmannin resulted in significant reduction in cell proliferation and induction of tumor cell apoptosis by both TUNEL and propidium iodide staining in RCC cell lines (786-O) [44]. Treatment of nude mice bearing RCC xenografts derived from the 786-O cells with LY294002 resulted in up to 50 % reduction in tumor size. Similarly, the treatment of nude beige mice bearing RCC xenografts with NVP-BEZ235, a dual inhibitor of PI3-K/mTOR, resulted in significantly greater suppression of tumor growth compared with either rapamycin or vehicle [45]. This suppression of tumor growth was correlated with reduced markers of proliferation (Ki67 staining) and modest induction of markers of apoptosis (cleaved caspase 3 staining), as well as suppression of the expression of HIF-2α and cap-dependent gene products such as cyclin D1. Together, these preclinical studies have suggested that PI3-K/Akt may be a relevant therapeutic target in RCC and provided the rationale for the clinical assessment of novel agents targeting this pathway.

One of the earliest such agents to be assessed in RCC was perifosine, an orally available alkylphospholipid which prevents Akt activation by blocking its pleckstrin homology domain-dependent recruitment to the cell membrane. Perifosine was recently assessed in two independent phase II trials in patients with advanced RCC who had failed prior targeted therapy [46]. In Perifosine 228, 24 patients with advanced RCC who had progressed after prior therapy with VEGF-targeted agents and/or cytokines were enrolled and treated with perifosine at 100 mg once daily. In Perifosine 231, 50 patients with advanced RCC were enrolled into two groups and treated with perifosine at a dose of 100 mg once daily. Group A included patients who failed a VEGFR-TKI but not on an mTOR inhibitor, whereas group B included patients who failed both targeted agents. In the combined analysis of 74 patients on both trials, six patients experienced a partial response (ORR 8 %), and the median PFS was 14 weeks [95 % CI (12.8, 20.0)]. The most common toxicities were fatigue, musculoskeletal pain, diarrhea, and nausea. Although perifosine had

Table 12.1 Ongoing trials with novel PI3-K/Akt inhibitors in RCC

Agent	Mechanism of action	Trial
MK2206	Allosteric inhibitor of Akt	A randomized phase 2 study of MK-2206 in comparison with everolimus in refractory renal cell carcinoma (NCT01239342)
GDC-0980	Pan-isoform inhibitor of PI3-K and catalytic inhibitor of mTOR	A phase II, open label, randomized study of GDC-0980 versus everolimus in patients with metastatic renal cell carcinoma who have progressed on or following VEGF-targeted therapy (NCT01442090)
BEZ-235	Pan-isoform inhibitor of PI3-K and catalytic inhibitor of mTOR	A phase 1b/2 study of BEZ235 in patients with advanced renal cell carcinoma (RCC) (NCT01453595)

clear clinical activity in RCC, it was felt that this activity was not superior to currently available agents, and this agent was not worthy of further development as a single agent in RCC.

The lack of robust clinical activity seen with perifosine has not muted the enthusiasm for PI3-K/Akt as a therapeutic target in RCC, however. Perifosine is an indirect inhibitor of Akt. As mentioned earlier, more reliable inhibition of this pathway may be achieved with the catalytic inhibitors of PI3-K/mTOR or with direct inhibitors of Akt (both catalytic and allosteric). Not surprisingly, several clinical trials with novel inhibitors of PI3-K/mTOR and Akt are underway as shown in Table 12.1. It is hoped that the results from these clinical trials may provide further validation of the PI3-K/Akt pathway as a therapeutic target in RCC.

Conclusion

The clear clinical activity of the rapalogues has established the relevance of the mTOR pathway in renal cell carcinoma. Enhanced understanding of the biology of this pathway has facilitated the identification of both potential predictive biomarkers of response and novel therapeutic strategies. Efforts must remain focused on identifying the subset of patients who derive the most clinical benefit from agents targeting this pathway. At the same time, agents that might prove superior to rapalogues are in active clinical development in RCC. The hope remains that the concurrent development of both patient selection strategies and better drugs will result in improved clinical outcomes for patients with advanced RCC.

References

1. Luo J, Manning BD, Cantley LC. Targeting the PI3K-Akt pathway in human cancer: rationale and promise. Cancer Cell. 2003;4(4):257–62.
2. Bunney TD, Katan M. Phosphoinositide signaling in cancer: beyond PI3K and PTEN. Nat Rev Cancer. 2010;10(5):342–52.

3. Horiguchi A, Oya M, Uchida A, Marumo K, Murai M. Elevated Akt activation and its impact on clinicopathologic features of renal cell carcinoma. J Urol. 2003;169(2):710–3.
4. Engelman JA. Targeting PI3K signaling in cancer: opportunities, challenges and limitations. Nat Rev Cancer. 2009;9(8):50–62.
5. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the *PIK3CA* gene in human cancer. Science. 2004;304(5670):554.
6. Jaiswal BS, Janakiraman V, Kljavin NM, et al. Somatic mutations in p85-alpha promote tumorigenesis through class IA PI3K activation. Cancer Cell. 2009;16(6):463–74.
7. Qian CN, Furge KA, Knol J, et al. Activation of the PI3K/Akt pathway induces urothelial carcinoma of the renal pelvis: identification in human tumors and confirmation in animal models. Cancer Res. 2009;69(21):8256–64.
8. Hollander MC, Blumenthal GM, Dennis PA. PTEN loss in the continuum of common cancers, rare syndromes and mouse models. Nat Rev Cancer. 2011;11(6):289–301.
9. Alimov A, Li C, Gizatullin R, et al. Somatic mutation and homozygous deletion of PTEN/MMAC1 gene of 10q23 in renal cell carcinoma. Anticancer Res. 1999;19(5B):3841–6.
10. Kondo K, Yao M, Kobayashi K, et al. PTEN/MMAC1/TEP1 mutations in human primary renal cell carcinomas and renal carcinoma cell lines. Int J Cancer. 2001;91(2):219–24.
11. Brenner W, Farber G, Herget T, Lehr HA, Hengstler JG, Thuroff JW. Loss of tumor suppressor protein PTEN during renal carcinogenesis. Int J Cancer. 2002;99(1):53–7.
12. Gunaratnam L, Morley M, Franovic A, et al. Hypoxia inducible factor activates the transforming growth factor-alpha/epidermal growth factor receptor growth stimulatory pathway in $VHL^{(-/-)}$ renal cell carcinoma cells. J Biol Chem. 2003;278(45):44966–74.
13. Shaw RJ, Bardeesy N, Manning BD, et al. The LKB1 tumor suppressor negatively regulates mTOR signaling. Cancer Cell. 2004;6(1):91–9.
14. Ma L, Chen Z, Erdjument-Bromage H, Tempst P, Pandolfi PP. Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis. Cell. 2005;121(2):179–93.
15. Kim E, Goraksha-Hicks P, Li L, Neufield TP, Guan KL. Regulation of TORC1 by Rag GTPases in nutrient response. Nat Cell Biol. 2008;10(8):935–45.
16. Voss MH, Hakimi AA, Brannon AR, et al. Next-generation sequencing reveals genomic determinants of long-term response to mTOR inhibitors in patients with advanced RCC. BJU Int. 2012;110 suppl 2:11.
17. Qiu Y, Kung HJ. Signaling network of Btk family kinases. Oncogene. 2000;19(49):5651–61.
18. Vasudevan KM, Barbie DA, Davies MA, et al. Akt-independent signaling downstream of oncogenic PIK3CA mutations in human cancer. Cancer Cell. 2009;16(1):21–32.
19. Nicholson KM, Anderson NG. The protein kinase B/Akt signaling pathway in human malignancy. Cell Signal. 2002;14(5):381–95.
20. del Peso L, Gonzales G-M, Page C, Herrera R, Nunez G. Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. Science. 1997;278(5338):687–9.
21. Cardone MH, Roy N, Stennicke HR, et al. Regulation of cell death protease caspase-9 by phosphorylation. Science. 1998;282(5392):1318–21.
22. Kim AH, Khursigara G, Sun X, Franke TF, Chao MV. Akt phosphorylates and negatively regulates apoptosis signal-regulating kinase 1. Mol Cell Biol. 2001;21(3):893–901.
23. Brunet A, Bonni A, Zigmond MJ, et al. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell. 1999;96(6):857–68.
24. Romashkova JA, Makarov SS. NF-kappaB is a target of AKT in anti-apoptotic PDGF signalling. Nature. 1999;401(6748):86–90.
25. Ferkey DM, Kimelman D. GSK-3: new thoughts on an old enzyme. Dev Biol. 2000;225(2):471–9.
26. Diehl JA, Cheng M, Roussel MF, Sherr CJ. Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. Genes Dev. 1998;12(22):3499–511.
27. Iliopoulos O, Kibel A, Gray S, Kaelin Jr WG. Tumour suppression by the human von Hippel-Lindau gene product. Nat Med. 1995;1(8):822–6.
28. de Paulsen N, Brychzy A, Fournier MC, et al. Role of transforming growth factor-alpha in von Hippel-Lindau (VHL)(-/-) clear cell renal carcinoma cell proliferation: a possible mechanism

coupling VHL tumor suppressor inactivation and tumorigenesis. Proc Natl Acad Sci U S A. 2000;98(4):1387–92.
29. Toschi A, Lee E, Gadir N, Ohh M, Foster DA. Differential dependence of hypoxia-inducible factors 1 alpha and 2 alpha on mTORC1 and mTORC2. J Biol Chem. 2008;283(50):34495–9.
30. Kondo K, Klco J, Nakamura E, Lechpammer M, Kaelin WG. Inhibition of HIF is necessary for tumor suppression by the von Hippel-Lindau protein. Cancer Cell. 2002;1(3):237–46.
31. Kondo K, Kim WY, Lechpammer M, Kaelin WG. Inhibition of HIF2alpha is sufficient to suppress pVHL-defective tumor growth. PLoS Biol. 2003;1(3):439–44.
32. Shen C, Beroukhim R, Schumacher SE, et al. Genetic and functional studies implicate HIF1α as a 14q kidney cancer suppressor gene. Cancer Discov. 2011;1(3):222–35.
33. Gordan JD, Lal P, Dondeti VR, et al. HIF-alpha effects on c-Myc distinguish two subtypes of sporadic VHL-deficient clear cell renal carcinoma. Cancer Cell. 2008;14(6):435–46.
34. Atkins MB, Hidalgo M, Stadler WM, et al. Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. J Clin Oncol. 2004;22(5):909–18.
35. Hudes G, Carducci M, Tomczak P, et al. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med. 2007;356(22):2271–81.
36. Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, Grunwald V, Thompson JA, Figlin RA, Hollaender N, et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomized, placebo-controlled phase III trial. Lancet. 2008;372(9637):449–58.
37. Motzer RJ, Escudier B, Oudard S, et al. Phase 3 trial of everolimus for metastatic renal cell carcinoma: final results and analysis of prognostic factors. Cancer. 2010;116(18):4256–65.
38. Dutcher JP, de Souza P, McDermott D, et al. Effect of temsirolimus versus interferon-alpha on outcome of patients with advanced renal cell carcinoma of different tumor histologies. Med Oncol. 2009;26(2):202–9.
39. Gera JF, Mellinghoff IK, Shi Y, et al. AKT activity determines sensitivity to mammalian target of rapamycin (mTOR) inhibitors by regulating cyclin D and c-myc expression. J Biol Chem. 2004;279(4):2737–46.
40. Cho D, Signoretti S, Dabora S, et al. Potential histologic and molecular predictors of response to temsirolimus in patients with advanced renal cell carcinoma. Clin Genitourin Cancer. 2007;5(6):379–85.
41. Wan X, Harkavy B, Shen N, Grohar P, Helman LJ. Rapamycin induces feedback activation of Akt signaling through an IGF-1R-dependent mechanism. Oncogene. 2007;26(13):1932–40.
42. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science. 2005;307(5712):1098–101.
43. Wang X, Yue P, Chan CB, et al. Inhibition of mammalian target of rapamycin induces phosphatidylinositol 3-kinase-dependent and mnk-mediated eukaryotic translation initiation factor 4E phosphorylation. Mol Cell Biol. 2007;27(21):7405–13.
44. Sourbier C, Lindner V, Lang H, et al. The phosphoinositide 3-kinase/Akt pathway: a new target in human renal cell carcinoma therapy. Cancer Res. 2006;66(10):5130–42.
45. Cho DC, Cohen MB, Panka DJ, et al. Efficacy of the novel dual PI3-Kinase/mTOR inhibitor NVP-BEZ235 compared to rapamycin in renal cell carcinoma. Clin Cancer Res. 2010; 16(14):3628–38.
46. Cho DC, Hudson TE, Samlowski W, Sportelli P, Somer B, Richards P, Sosman JA, Puzanov I, Michaelson MD, Flaherty KT, Figlin RA, Vogelzang NJ. Two phase II trials of the novel Akt inhibitor perifosine in patients with advanced renal cell carcinoma following failure to VEGF-targeted therapy. Cancer. 2012;118(24):6055–62.