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The mTOR Pathway in Breast Cancer

Nancy E. Hynes · Anne Boulay

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Abstract There is currently a wealth of information regarding the mutations that contribute to cancer development. Most of these mutations alter the expression and activity of signal transduction proteins. The current goal in cancer therapy is to use our knowledge of the molecular alterations in a cancer cell to choose the most appropriate signal transduction inhibitor for an individual patient. The topic of this review is the mammalian target of rapamycin (mTOR) kinase signaling pathway, which is aberrantly activated in many types of human cancer. We will discuss the mTOR pathway and the potential mechanisms that contribute to its activation in cancer, together with data relating to the potential for inhibitors targeting the mTOR-signaling pathway to impact on breast cancer therapy.

Keywords mTOR · Breast cancer · Translational control · PI3K · LKB1 · AKT

Abbreviations

AMPK	AMP- activated protein kinase
CML	chronic myelogenous leukemia
eIF-4E	eukaryotic initiation factor 4E
ER	estrogen receptor
FGFR	fibroblast growth factor receptor
FKBP12	FK506-binding protein-12
FTI	farnesyltransferase inhibitor
HIF	hypoxia inducible transcription factor
HMEC	human mammary epithelial cells
IGF-1R	insulin like growth factor-1 receptor
LOH	loss of heterozygosity
mTOR	mammalian target of rapamycin
PDK1	phosphoinositide dependent protein kinase 1
PH	plekstrin homology
PIKK	phosphoinositide kinase-related kinase
PIP3	phosphatidylinositol-3,4,5 triphosphate
PI3K	phosphoinositide 3-kinase
PJS	Peutz–Jeghers syndrome
PKB	protein kinase B
PTEN	phosphatase and tensin homologue deleted in chromosome 10
RTK	receptor tyrosine kinase
STI	signal transduction inhibitor
S6K1	ribosomal S6 kinase
TKI	tyrosine kinase inhibitor
TSC	tuberous sclerosis complex
4E-BP1	eukaryotic initiation factor 4E-binding protein 1

N. E. Hynes (✉) · A. Boulay
Friedrich Miescher Institute for Biomedical Research,
Maulbeerstrasse 66,
CH-4058 Basel, Switzerland
e-mail: Hynes@fmi.ch

Introduction

The current goal in cancer therapy is to define the molecular alterations underlying the malignant phenotype. With a better description of these it should be possible to choose the appropriate signal transduction inhibitor (STI) for an individual patient. The topic of this review is the mammalian target of rapamycin (mTOR) kinase signaling pathway, which is aberrantly activated in many types of human cancer. TOR was originally identified by genetic means in the budding yeast *Saccharomyces cerevisiae* as the target of the macrolide antibiotic rapamycin [1]. Rapamycin is a potent suppressor of the immune system [2, 3] and some of its analogues are currently under intense study as anti-cancer agents (reviewed in [4, 5]). We will discuss the mTOR pathway and the potential mechanisms that contribute to its activation in cancer, together with data relating to the potential for STIs targeting the mTOR-signaling pathway to impact on cancer therapy.

mTOR

mTOR is a member of the phosphoinositide kinase-related kinase (PIKK) family consisting of large serine/threonine kinases including ATM, ATR and DNA-PK, all involved in stress checkpoint control [6]. mTOR is a central regulator of cellular responses to multiple stimuli including amino acid availability [7], energy and oxygen stresses [8, 9] and growth factor receptor signaling [10]. In cells with sufficient nutrients, mTOR relays a signal to the translational machinery leading to an enhanced translation of mRNAs encoding proteins essential for cell growth and cell cycle progression (reviewed in [4, 11]). These functions are specifically mediated by the mTOR–Raptor (also named mTORC1) complex comprising the regulatory protein GβL and Raptor; Raptor being suggested to function as an adaptor to recruit mTOR substrates [12–14].

mTOR has also been identified in a second complex containing the Rictor protein [15, 16]. The mTOR–Rictor complex controls actin cytoskeleton organization. Moreover, mTOR–Rictor has been described as a potential kinase for Ser473 in the hydrophobic regions of the Akt/PKB serine/threonine kinase [16]. mTOR–Rictor regulation and functions are still poorly understood and will not be further addressed in this review.

mTOR Effectors

The mTOR–Raptor complex signals directly to important translational regulators, the translational repressor protein eukaryotic initiation factor 4E (eIF-4E)-binding protein 1 (4E-BP1) and ribosomal S6 kinase (S6K1). Binding of 4E-BP1 to eIF-4E is controlled by mTOR dependent phosphorylation of specific serine and threonine residues [17]. Once 4E-BP1 is completely phosphorylated [18], it dissociates from eIF-4E allowing the formation of a translationally competent initiation factor complex eIF-4F [17]. eIF-4F activation results in translation of a subset of capped mRNA containing highly structured 5′-untranslated regions and encoding proteins involved in G1- to S-phase progression, such as c-myc [19] and cyclin D1 [20–22]. The second important mTOR target, S6K1, has been implicated in translational regulation of mRNAs with a 5′-terminal oligopyrimidine (TOP) tract such as those that encode ribosomal proteins, proteins involved in translation [23]. Considering the importance of the proteins that are subject to mTOR mediated translational control in cell proliferation and growth, it is not surprising that cancer cells undergo alterations that impact on mTOR activity.

Uptream Activators of mTOR

mTOR–Raptor is a component of a multisubunit complex that is controlled by inputs from two major sources, the phosphoinositide 3-kinase (PI3K) pathway, an important signaling module downstream of receptor tyrosine kinases (RTKs) [10] and the LKB1/AMP kinase (AMPK) pathway [24] (Fig. 1). PI3K activation following growth factor stimulation impacts on the Akt/PKB serine/threonine kinase, while one of the major targets of the LKB1 serine/threonine

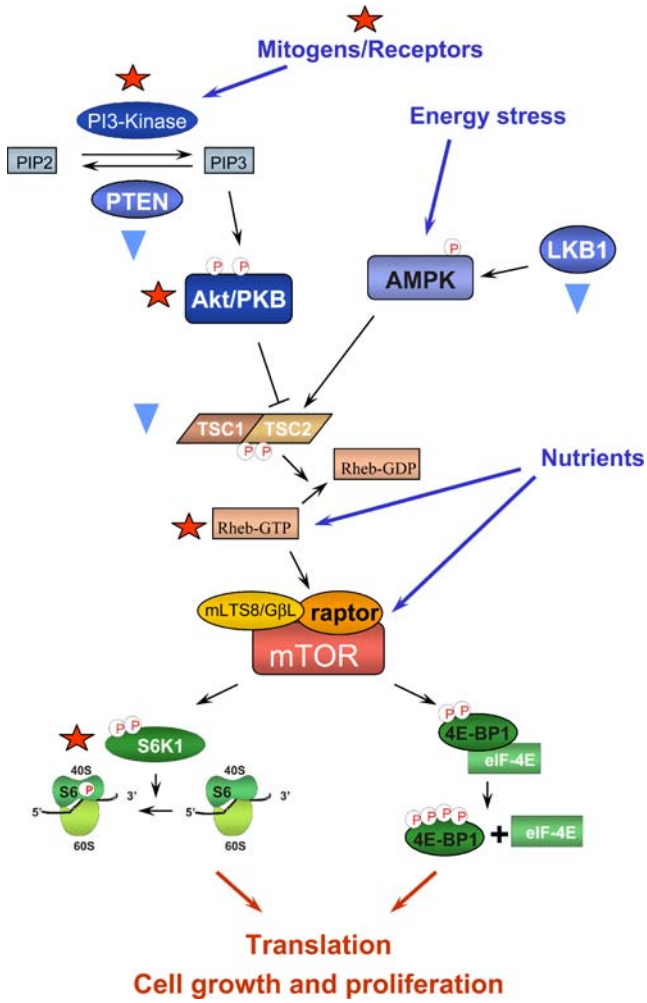

Figure 1 Model of the mTOR pathway; its regulation and cancer specific alterations. mTOR lies at the interface of two major signaling pathways, one initiated by PI3K and the other by AMPK. Mitogen signaling to receptor tyrosine kinases activates PI3K and Akt, which phosphorylates TSC2 leading to activation of Rheb GTPase and mTOR activation. AMPK is a regulator of cellular energy metabolism. In the presence of high AMP, the AMPK gamma regulatory subunit binds AMP permitting the alpha subunit to be phosphorylated and activated by LKB1 kinase. AMPK in turn phosphorylates TSC2, strengthening the ability of the TSC complex to block Rheb GTPase activity and lowering mTOR activity. The *red asterisk (*)* indicates mutations leading to pathway activation in cancer. These include constitutive activation of receptor tyrosine kinases, overexpression or mutational activation of PI3K and Akt as well as overexpression of Rheb and S6K1. The *inverted triange* (▿) indicates proteins that are lost in cancer cells. These include PTEN, the negative regulator of PI3K, TSC complex proteins hamartin and tuberin and the LKB1 kinase.

kinase is AMPK, a master sensor of cellular energy supply [25]. Control of mTOR activity is quite complex, involving inputs from multiple regulatory proteins (Fig. 1) (Reviewed in [4, 11, 26]. We will discuss mTOR–Raptor control at the interphase of the PI3K and LKB1 pathways.

PI3K catalyzes production of phosphatidylinositol-3,4,5 triphosphate (PIP3) at the cell membrane, which in turn stimulates the recruitment and activation of the serine/threonine kinase Akt/protein kinase B (PKB), a major player on the pathway. Akt is a family member of the AGC (cAMP dependent, cGMP dependent and protein kinase C) protein kinases. Binding of Akt's plekstrin homology (PH) domain to PIP3 is necessary for phosphoinositide-dependent protein kinase 1 (PDK1) phosphorylation of Thr308 in its activation loop. Complete Akt activation results from a second phosphorylation on Ser473 in the hydrophobic motif at its C-terminus [27]. At least ten protein kinases have been proposed to function as the second Akt kinase including mTOR–Rictor as mentioned above. This second kinase is often termed PDK2; this is currently an area under intense study. Nonetheless, it is likely that multiple upstream kinases can regulate Akt and other members of the AGC kinase family (reviewed in [28]). Once activated, Akt has multiple substrates, one of which is tuberin a protein directly involved in mTOR regulation.

Tuberin, also referred to as tuberous sclerosis complex (TSC) 2, together with hamartin, TSC1, form a heterodimeric complex that negatively regulates mTOR signaling [29]. Tuberin is a GTPase activating protein (GAP) for the Ras-related GTPase Rheb (Ras homologue enriched in brain). Tuberin triggers the conversion of the active GTP-bound Rheb to the inactive GDP-bound form [30, 31]. Following tuberin phosphorylation and functional inactivation by Akt [32, 33], the negative effect of TSC on Rheb function is disrupted [30], and Rheb–GTP is formed. Various lines of evidence suggested that Rheb acted upstream of mTOR [34], and it is now known that Rheb binds directly to the kinase domain of mTOR, presumably enabling a conformational change in the mTOR–Raptor complex promoting its activation [35].

Turning to the other major pathway regulating mTOR–Raptor, the serine/threonine kinase LKB1 was originally identified in 1996 by Dr. J. Nezu as a novel mammalian kinase (discussed in [24]). Interest in the kinase increased dramatically when germline inactivating mutations in the gene encoding LKB1 (also known as serine threonine kinase 11) were shown to be responsible for the Peutz–Jeghers syndrome (PJS) [36, 37]. PJS is an autosomal dominant syndrome characterized by benign hamartomatous polyps in the gastrointestinal tract [38]. The LKB1 kinase has also been implicated in sporadic cancer (discussed below).

The LKB1 kinase is part of a regulatory complex that phosphorylates and activates AMP kinase (AMPK)-related kinase family members (reviewed in [24]). AMPK, a master regulator of cellular energy metabolism [25], is a heterotrimeric complex composed of a catalytic alpha subunit and beta and gamma regulatory subunits. In cells with a high AMP to ATP ratio, AMP binds the gamma subunit and induces a conformational change converting the alpha subunit into a substrate for LKB1 that phosphorylates AMPK alpha on a threonine residue in its activation loop [39]. AMPK activation causes numerous cellular responses, one of them being a decrease in energy-consuming processes such as protein synthesis, a response that connects AMPK to mTOR. Indeed it was shown that AMPK inhibits cellular proliferation under conditions of energy starvation by phosphorylating tuberin (TSC2) and enhancing the ability of TSC to block mTOR signaling [40].

In summary, the two major regulators of mTOR–Raptor activity, the PI3K/Akt and the LKB1/AMPK pathways, both impact on TSC. Phosphorylation of tuberin by Akt blocks the ability of the complex to interfere with Rheb activity, thus stimulating mTOR activity. In contrast, phosphorylation of tuberin by AMPK strengthens its GAP activity towards Rheb, thus resulting in a blockade of mTOR activation (Fig. 1).

mTOR (dys)Regulation in Cancer

The mTOR pathway is abnormally activated in many tumors. Multiple alterations, both upstream and downstream of mTOR, leading to pathway activation have been described (Fig. 1). In the following sections we will discuss the mechanisms and mutations that lead to deregulated mTOR activity in cancer, specific data on breast cancer will be mentioned where possible.

As discussed above two of the major mTOR substrates, 4EBP1 and S6K1 are directly involved in translational control of mRNA coding for important cell cycle regulators and cell growth regulators, for example, cyclin D1 and the ribosomal proteins, respectively. Considering that cancer cells are dependent upon many of these proteins for their malignant phenotype, it is not surprising that mTOR is considered to be a potentially important target for cancer therapy. A number of mTOR inhibitors, as well as inhibitors of proteins on the mTOR pathway, are in clinical development. We will end the review with a discussion on selected pathway inhibitors.

Positive mTOR Regulators and Cancer

A major mTOR regulator, the PI3K/Akt pathway, is aberrantly activated in most human tumors. Many human cancers have alterations in RTKs that promote constitutive activation of the PI3K/Akt pathway. Aberrant activation of the insulin like

growth factor-1 receptor (IGF-1R) [41], the fibroblast growth factor receptor (FGFR) family [42, 43] and members of the epidermal growth factor receptor (EGFR)/ERBB family have been found in many human cancers. Considering breast cancer, approximately 20% of primary breast tumors overexpress the ErbB2 RTK due to gene amplification (reviewed in [44]). ErbB2-overexpressing tumors show high constitutive PI3K/Akt activity, mainly through coupling of ErbB2 to ErbB3 [45], a receptor with multiple binding sites for the p85 regulatory subunit of the PI3K [46]. Overexpression and activation of FGFR has been reported in breast cancer [42, 43] and has been linked to mTOR pathway activity [20]. The IGF-1R has also been implicated in breast cancer, which is a topic of another review in the issue (Sachdev and Yee).

Amplification and overexpression of the *PIK3CA* gene, encoding the p110α subunit of the PI3K was found mainly in ovarian cancers [47]. More recently, somatic point mutations in the *PIK3CA* gene have been described in many types of human tumors [48]. Considering breast cancer, *PIK3CA* mutations have been detected in 25% of tumors [49], making this one of the most frequently mutated genes in this cancer. The biochemical and transforming activity of several p110α mutants have been described [50, 51]. Expression vectors for two of the most common p110α mutations, Glu545Lys and His1047Arg, have been introduced into human mammary epithelial cells (HMEC); each mutant was transforming and its oncogenic potency correlated well with an enhanced level of pathway activity [51]. The impact of enhanced PI3K activity on mTOR was not described, however, it is likely that tumors expressing p110α mutants maintain mTOR activity independent from exogenous growth factor signals.

Elevated Akt1 [52] and Akt2 [53] kinase activity have been observed in various human tumors, including breast cancer. Activating Akt2 kinase domain mutations (2/180 tumors) [54] and *AKT2* gene amplification (2/146 tumors) have been described in a small percentage of colorectal cancers [54] and breast cancers (3/106 tumors), as well as a higher percentage of ovarian tumors (16/132 tumors) [55]. Thus, it appears that in breast cancer activating mutation in the catalytic sub-unit of PI3K are more prevalent than in the Akt kinase domain.

Rheb, the direct upstream activator of mTOR [34], has also been found to be overexpressed at the RNA level in many human tumors [56]. Elevated Rheb expression is likely to impact on mTOR, since it has been shown that overexpression of Rheb increases the activity of the mTOR effector protein S6K1 [34]. Moreover, the *S6K1* gene is amplified in approximately 9% of primary breast cancers [57], and elevated levels of S6K1 RNA are found in almost 40% of the tumors [58]. In summary, most of the positive regulators of mTOR activity have been found mutated and/or overexpressed in human breast cancer. The most common alteration found is activating mutations in the PI3K.

mTOR Regulation and Tumor Suppressors

There are three negative regulators of mTOR activity: phosphatase and tensin homologue deleted in chromosome 10 (PTEN), TSC and LKB1; all three have important roles in many types of human cancer. PTEN downregulates the PI3K pathway by dephosphorylating position D3 of PIP3 and thereby antagonizing PI3K function. PTEN activity is lower in many human cancers due to gene deletion, gene silencing or mutational inactivation. Mutations in PTEN are relatively rare in breast cancer (5%) [59, 60]. However, loss of heterozygosity (LOH) [59, 61] and promoter methylation of the *PTEN* gene [62], leading to low PTEN levels [63], are more common (~30%) in breast cancer. A recent immunohistochemical analysis revealed that 26% (n = 236) of primary breast cancers had low PTEN levels, which correlated with lymph node metastases and a worse prognosis [64].

Thus, in addition to activating PI3K mutations in breast cancer, PTEN, the negative regulator of the pathway is often down-regulated. Are these mutations mutually exclusive? In a recent analysis of ~150 primary breast tumors, coexistence of low PTEN levels and PI3K mutations was rare and only detected in two cases [65]. This suggests that following mutation/activation of PI3K or loss of PTEN during the genesis of breast cancer, either of which results in an increase in PIP3 levels, there is no selective advantage for mutation of the other gene. However, it should be mentioned that in a study of 66 endometrial cancers, PI3K mutations were more common in tumors with PTEN mutations (46 vs. 24%) [66]. Thus, there might be tumor type differences in the prevalence of both alterations.

TSC is a tumor suppressor syndrome with a broad spectrum of clinical manifestations, including benign hamartomas that occur in a wide variety of tissues such as brain, skin, heart and kidneys [67]. As discussed above, TSC negatively regulates mTOR activity. Hence, mTOR signaling has been shown to be overactive in primary cells derived from hamartomas in TSC patients [68] supporting the functional relationship between the hyperproliferative disease and mTOR activation. Mutations or LOH in *TSC1* and *TSC2* are associated with TSC syndrome [69]. In addition to hamartomas, TSC patients have an increased risk of developing renal cell carcinoma. Recently, expression of TSC proteins has also been studied in breast cancers [70]. RNA pooled from 120 primary tumors had ~80% decrease in hamartin specific transcripts compared to the level observed in normal breast tissue. Although there were no changes in tuberin RNA levels in the same set, the *TSC1* gene promoter was found to be heavily methylated in some breast cancer cell lines [70], suggesting that this tumor suppressor might be controlled by epigenetic mechanisms. It will be important to examine the expression levels of TSC proteins in more sets of tumors to know how frequently these proteins are targeted in breast cancer.

Germline inactivating mutations in *LKB1/STK11* encoding the LKB1/serine threonine kinase 11 are responsible for PJS [36], an autosomal dominant syndrome characterized by benign hamartomatous polyps in the gastrointestinal tract [38]. The LKB1 kinase has also been implicated in sporadic human cancer since it has been found that about 30% of lung adenocarcinomas show somatic inactivating mutations in the *LKB1* gene [71]. Patients with PJS have an increased risk for development of a variety of cancers, including breast [38, 72]; there is one report of a germline *LKB1* mutation in breast cancer [73]. Inactivating mutations in LKB1 have not been reported in a screen of 518 kinases (the kinome) in 25 sporadic breast cancers [74]. However, an examination of 140 primary breast tumors, revealed that 30% had a genomic deletion in the chromosomal region including *LKB1* (19p13.2–13.3) [75]. Although follow-up studies examining the level of LKB1 protein will be necessary, these results suggest that AMPK activation might be deregulated in breast cancer. This alteration could have an important impact on mTOR, for example, keeping it active under hypoxic conditions, when mTOR is normally down-regulated [76].

Downstream Targets of mTOR in Cancer

A number of mTOR translational targets are known to be activated and overexpressed in cancer and are involved in the transformation process as well as in drug resistance. For example, eIF-4E is overexpressed in a variety of human cancers, triggers tumor formation *in vivo* [77] and mediates Akt- and mTOR-dependent survival and drug resistance in a murine lymphoma model [78]. Cyclin D1, another mTOR target [79] is overexpressed in many primary breast tumors. These findings indicate, therefore, that targets downstream of mTOR are also worth exploiting in cancer treatment.

These results point to the importance of aberrant mTOR activity, and presumably alterations in translational control, in driving the malignant phenotype. Indeed this was demonstrated very elegantly in a study of a glioma tumor model, where total mRNA profiles or polysomal mRNA profiles were examined after the PI3K pathway was inhibited. The major effect of blocking this pathway was seen at the translational and not the transcriptional level [80]. There was a major shift in the polysome occupancy of mRNAs encoding proteins involved in proliferation and growth.

Targeting the mTOR–Raptor Pathway in Cancer

The mTOR–Raptor pathway is aberrantly activated in many human cancers. Thus, approaches to block the pathway are being actively pursued in many laboratories and pharmaceutical companies. In view of the multiple regulators of mTOR, there are a number of target proteins for which intervention would be predicted to lower mTOR activity and have an impact on cancer (Table 1).

Considering the broad experience gained in the 20 years since small molecule kinase inhibitors were described [81, 82], the pathway kinases PI3K, Akt and mTOR are appealing targets. (Inhibition of LKB1 is not desirable since this would promote mTOR activation). Furthermore, a number of inhibitors blocking the cancer promoting RTKs that contribute to PI3K pathway activation are already in the clinic [83–85]. Despite major interest in the pathway, no drugs directly targeting PI3K or Akt have entered cancer trials; however, a number are in preclinical development (reviewed in [86]). Two well characterized inhibitors of PI3K activity, LY294002 and wortmannin, have been tested in numerous models of cancer and shown to have antitumor activity both in vitro and *in vivo*. While these compounds are not suitable for human studies, these data provide compelling evidence that targeting this pathway should impact on human cancer [87].

Farnesyltransferase inhibitors (FTIs) are another interesting class of therapeutics to consider as mTOR pathway inhibitors. Substrates for farnesyltransferase are proteins with a C-terminal CAAX motif, including Ras family members. FTIs were originally designed to block the action of Ras oncoproteins, since they require a farnesyl isoprenoid membrane anchor for correct cellular activity [88]. Various lines of evidence suggest that the anti-tumor effect of FTIs is dependent upon blocking the activity of other cellular proteins. Rheb, the upstream activator of mTOR is post-translationally modified by lipids [56] and is a strong candidate for the anti-proliferative activity of FTIs in cancer models [34, 56].

Table 1 Inhibitors targeting the mTOR pathway in preclinical or clinical development.

Target	Phase	Compound	Company
mTOR	II	Everolimus (RAD001)	Novartis
	II/III	Temsirolimus (CCI-779)	Wyeth
	I/II	AP-23573	Ariad
ErbB1	Approved	Gefitinib (Iressa)	AstraZeneca
	Approved	Erlotinib (Tarceva)	Genentech
ErbB1/ErbB2/ VEGF	I	AEE778	Novartis
PI3K	Preclinical		
Akt	Preclinical		
Farnesyltransferase	II/III	lonafarnib (SCH66336)	Schering-Plough
	II/III	tipifarnib (R115777)	OrthoBiotech

Several FTIs are in clinical development (reviewed in [5]). Some of these are being tested in breast cancer models [56], and encouraging results have been obtained in a phase I clinical trial on the FTI tipifarnib when given to advanced breast cancer patients in combination with tamoxifen [89].

mTOR Inhibitors

The mTOR kinase is a target for rapamycin analogs, several of which have been approved for use in transplant patients as immunosuppressants [3]. Rapamycin and its derivatives temsirolimus (CCI-779) [90], everolimus (RAD001) [91] and AP-23573 [92], all of which are in clinical development (reviewed in [5, 92]), inhibit mTOR kinase by binding the FK506-binding protein-12 (FKBP12). The mechanism by which this complex blocks mTOR activity is still under investigation (discussed in [11]), and some experiments suggest that FKBP12-rapamycin blocks access of mTOR substrates to the complex [13]. Importantly, rapamycins inhibit the mTOR-Raptor complex functions specifically; the mTOR–rictor complex being insensitive to acute exposure to this class of inhibitors [16].

Rapamycin analogues show some clinical activity, however, not on all patients. Thus, the current clinical challenge is to determine markers that can help predict sensitivity to mTOR inhibition in order to assist in selecting the appropriate patients for therapy. In this respect, animal models of cancer might be helpful in making some predictions. In a mouse model, the prostate was shown to be particularly sensitive to PTEN levels, which dictate Akt activation and cancer development [93]. Furthermore, an Akt driven transgenic prostate tumor model was effectively treated with the mTOR inhibitor RAD001 [94]. A phase II clinical trial of the mTOR inhibitor CCI-779 in prostate cancer was initiated; however, no results have been published (discussed in [95]).

Preclinical studies suggest that breast tumor cells might be particularly sensitive to mTOR inhibitors [21, 96]. As mentioned above, PTEN is frequently altered in breast cancers, suggesting that PTEN levels might predict sensitivity to rapamycins. Although PTEN deficiency has been correlated with increased sensitivity to mTOR inhibitors in many tumor models, including glioblastoma or multiple myeloma [97, 98], a similar trend has not been observed in breast tumor cell lines [21, 96], presumably because a number of other alterations specifically driving breast cancer development are feeding into the Akt/mTOR pathway. Nevertheless, in a phase II safety trial of CCI-779 in women with advanced breast cancer three of the four patients with PTEN deficient tumors showed objective responses to treatment, while only 37% of the overall patient population (n = 28) experienced clinical benefit, supporting the idea that patients with PTEN deficient breast tumors might benefit from treatment with mTOR inhibitors.

ErbB2 is frequently overexpressed in breast cancers and is correlated with poor clinical prognosis. ErbB2 overexpression provides a strong stimulus for PI3K/Akt pathway activation and has been associated with increased phosphorylation of mTOR effectors in primary breast cancers [99, 100], indicating that ErbB2-driven tumors might be more dependent on mTOR signaling. Indeed, in the above-mentioned phase II safety trial, 2 of 3 ErbB2-overexpressing patients showed an objective response to the rapamycin analogue, indicating that ErbB2 overexpression might be a marker for choosing patients to treat with mTOR inhibitors.

The estrogen receptor (ER) has recently emerged as a potential stratification marker for an increased antitumor benefit to rapamycins in combination with endocrine therapy. The ER is an important predictive and prognostic marker in human breast cancer, being expressed in about 60% of breast cancers. It has become evident that estrogen/ER signaling exhibits pleiotropic effects through non-genomic interactions with growth factor signaling pathways. In particular, long-term estrogen-deprived breast tumor cells exhibit increased Akt/mTOR activation [101]. Moreover, the pathway has been strongly implicated in resistance to antiestrogen therapeutics (Reviewed in [102]). Thus, a number of preclinical studies combining rapamycins with endocrine therapies supported the potential for these combinations for the therapy of endocrine-dependent breast cancers [103, 104].

The rationale to evaluate the above-mentioned markers as potential predisposition markers is strong in breast cancer patients. Importantly, all these alterations and resistance mechanisms impinge on Akt signaling, eventually leading to increased dependence on mTOR for tumor cell survival and/or tumor progression. This rationale supports the possibility that tumors bearing any alterations associated with increased activated Akt levels should be particularly sensitive to mTOR inhibition.

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