Review

Targeting the mTOR Signaling Pathways in Breast Cancer: More Than the Rapalogs

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Abstract: Targeted therapies were shown to improve cure rates and prolong survival in breast cancer patients. Although, therapies directed at endocrine receptors and human epidermal growth factor receptor 2 are important treatment options for patients with breast cancer; however, drug resistance and subsequent disease progression in patients with advanced disease is inevitable. It is well established that the PI3K/Akt/mTOR pathway plays a central role in various cellular processes that can contribute to malignancy. The mammalian target of rapamycin (mTOR) is proven to be centrally involved in growth, survival and metabolism. In breast cancer, mTOR is frequently hyper-activated and is a clinically validated target for drug development. Accordingly, pharmacological inhibition of the PI3K/Akt/mTOR signaling cascade has been a focus in developing targeted therapeutics. To date, agents targeting upstream receptor tyrosine kinases were the best studied and have achieved greatest clinical success. Further downstream, the Rapalogs are effective anti-cancer in certain tumor types in various preclinical models. However, both the mechanism of action and the cellular response to mTORC1 inhibition by rapamycin and related drugs may limit the effectiveness of these compounds as antitumor agents. Hence, they have been somewhat disappointing in the clinical setting. Currently, new inhibitors of PI3K, Akt, and mTORC1 and 2 are passing through early clinical trial phases, in a hope that these agents will overcome some of the drawbacks of the rapalogs leading to significant benefits for cancer patients. This review is intended to give a concise summary of recent developments in the therapy of breast cancer with a focus on new mTOR targeted therapies.

Keywords: mTOR, breast cancer, rapalogs, drug-resistance, targeted therapy

1. Introduction

Breast cancer is the most frequently diagnosed cancer and the primary cause of cancer death in females worldwide [1]. In comparison to about 500,000 cases diagnosed in 1975, more than 1 million cases of breast cancer patients are diagnosed across the world each year. This represents about 10% of all new cancer cases and 23% of all female cancers [2-3]. The aetiology of breast cancer involves environmental factors, inherited genetic susceptibility and interaction between these factors [4]. In the past years, our knowledge of the genetic changes that contribute to breast cancer development and progression has tremendously changed [5].

In this regards, our knowledge of alterations in the cancer cell have allowed us to identify the signaling pathways that when disrupted allow a cancer cell to escape from normal control mechanisms [6]. On the other hand, these malfunctioned pathways have also provided many therapeutic intervention strategies. Following the successful introduction of trastuzumab, the first human epidermal growth factor receptor (HER) targeted therapy to become widely used in breast cancer patients, other targeted agents have been developed [7]. Treatment decisions are guided by stage, tumor grade and hormone and human epidermal growth factor receptor 2 (HER2) states. Thus, targeted therapies, such as endocrine and biological therapy (e.g. trastuzumab), were developed based on rational understanding of processes underlying tumorigenesis resulting in significant improvements in treatment outcomes [8]. However, similar to traditional chemotherapy [9], drug resistance with these therapies still represent a significant problem resulting in disease recurrence and/or progression, necessitating alternative treatment strategies [10].

Other potential hallmarks of malignancy that represent a new opportunity for therapeutic targeting include abrogation of apoptosis, lack of senescence, angiogenesis, tumor invasion and metastasis [11]. Therefore, new compounds are being developed that may interfere with these hallmarks and that may prove to be effective in monotherapy or in combination with cytotoxic therapy or other targeted therapies. New optimized...
targeting agents are required because many of the current therapies have significant limitations. Among which: drug resistance, lack of target receptor expression in tumors and relatively small improvements in survival [12-15].

Thus, this review provides a focusing overview of one of the several signaling transduction pathways involved in development of breast cancer, i.e. PI3K/Akt/mTOR pathway. In addition, it focuses on recent progress with the therapeutic strategies targeting this pathway contributing to their promising success in the clinical setting. Furthermore, molecular signals of its resistance phenotype in breast cancer & their therapeutic targeting are discussed. Finally, the challenges facing the significant contribution of PI3K/Akt/mTOR targeted therapeutics in breast cancer chemotherapy are also extensively discussed.

2. Targeting breast cancer signaling pathways

The mechanisms underlying the development of breast cancer are complex and vary among individual tumors [16]. Altered patterns of gene expression are associated with corresponding variations in growth rates, cellular composition and different prognoses [17]. Given the multiple factors that affect breast cancer development, besides the use of increasingly complex genetic analysis techniques, it is expected that more refined tumor subtypes and their associated prognoses will be diagnosed [16, 18]. Advances in understanding the etiology and biology of breast cancer have identified key targets among multiple signaling pathways involved in the development and survival of breast cancer cells. Thus, optimized targeted therapies are considered the most promising novel agents for breast cancer therapy.

Signaling through the epidermal growth factor receptor family (ErbB) seems to be the most important growth stimulator for breast cancer cells although there are some data that suggest a role for insulin-like growth factor receptor signaling and other receptor tyrosine kinases as well [19]. Downstream of the ErbB receptor, signalling is transduced via 2 main pathways: Ras-Raf-MAPK and PI3K-Akt/mTOR. In this review, the PI3K-Akt/mTOR pathway will only be highlighted.

2.1. Targeting downstream PI3K/Akt/mTOR signaling pathway

The PI3K/Akt/mTOR pathway plays a central role in diverse cellular functions including proliferation, growth, survival and metabolism. In addition to their physiological role, several isoforms of the PI3K family are implicated in tumor development, including cell proliferation, cell growth, cell motility, cell survival, and angiogenesis [20]. In particular, members of class IA PI3Ks are often mutated in human cancer [21-26]. As a result of receptor tyrosine kinase RTK activation and phosphorylation, PI3K interacts with the intracellular domain of the receptors. Subsequent phosphorylation event by the mammalian target of rapamycin (mTOR)-complex is required for maximal Akt activity [27-28]. Akt, a serine/threonine kinase, is considered as the central mediator of the PI3K pathway with several downstream effectors that affect key cellular processes. Akt augments protein synthesis and cell growth by activating mTOR (as part of the mTOR-raptor or mTORC1 complex) by influencing the intermediary tuberous sclerosis (TSC) 1/2 complex. It affects cellular proliferation by deactivating cell cycle inhibitors (p27 and p21) and enhancing cell cycle proteins (c-Myc and cyclin D1), ending in enhanced cellular proliferation [29-30].

The discovery of rapamycin, a fungal macrolide isolated by a group of Wyeth scientists three decades ago [31], and the subsequent molecular elucidation of its target in yeast and mammalian cells have fundamentally impacted our knowledge of cancer biology and therapy. The mammalian target of rapamycin (mTOR) is a central regulator of growth, integrating diverse signals of growth factors, nutrients and energy sufficiency [32-33]. mTOR, an atypical serine/threonine (S/T) protein kinase, is also central regulator of cellular responses to multiple stimuli including amino acid availability and growth factor receptor signaling [34-35]. Increasing evidence indicates that mTOR acts as a ‘master switch’ of cellular anabolic and catabolic processes, thus controlling and modulating the rate of cell growth and proliferation by virtue of its ability to sense mitogen, energy and nutrient levels [36-37]. 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 [33, 38]. Thus, as a part of the mTORC1 complex, mTOR stimulates cell growth and protein synthesis through effects on mRNA translation and ribosome biogenesis [39].

mTOR resides in two distinctive multi-protein complexes, mTOR complex-1 (mTORC1) and mTOR complex-2 (mTORC2). The canonical rapamycin-sensitive mTORC1 is well known for its critical roles in the regulation of protein synthesis and growth. The PI3K/AKT pathway activates mTORC1 through phosphorylation and inactivation of the tuberous sclerosis complex (TSC), a repressor of mTORC1. The more recently discovered mTORC2 phosphatases and activates AKT, a key regulator of cell growth, metabolism and survival. The identification of mTOR as an essential downstream component of the PI3K/ AKT signaling pathway, together with the compelling evidence supporting the widespread dys-regulation of this pathway in human cancer, provides a strong rationale for targeting mTOR as cancer therapy [40].

In addition to the complexity of the PI3K/Akt /mTOR pathway, extensive crosstalk exists with other cellular signaling networks. For example, influence of Akt extends to a host of pro- and antiapoptotic proteins, such as the Bcl-2 family member Bad, limiting programmed cell death and boosting cellular survival. Moreover, mTOR exerts influence on PI3K signaling through the S6K-IRS1 feedback loop and through mTORC2 mediated Akt-Ser473 phosphorylation [28, 39]. Additionally, activated GTP-bound RAS proteins are capable of stimulating the PI3K pathway via binding directly to p110 [13]. Downstream of RAS, in the mitogen-activated protein kinase (MAPK) pathway, ERK was proven to negatively regulate TSC2 [14]. Finally, MAPK pathway activation was...
evidenced as a consequence of mTORC1 inhibition, further intercalating these two important cascades [41].

The relationship between dys-regulated PI3K activity and the onset of cancer is well documented [42]. Furthermore, dys-regulation of mTOR and other proteins in the signaling pathway often occurs in a variety of human malignant diseases and the tumor cells have shown higher susceptibility to mTOR inhibitors than normal cells. A recent immune-histochemical study performed in tissue arrays containing 124 tumors from 8 common human tumor types revealed that approximately 26% of tumors are predicted to be sensitive to mTOR inhibition [43]. These findings indicate a potential role of dys-regulated mTOR signaling in tumorigenesis and support the currently ongoing clinical development of mTOR inhibitors as a potential tumor-selective therapeutic strategy.

There is increasing evidential support that uncontrolled stimulation of the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway, achieved through numerous genetic and epigenetic alterations, contributes to human cancers development and progression, including breast cancer [44]. In breast tumors, activating mutations in PIK3A, encoding the catalytic subunit of PI3K, or loss of PTEN, the negative regulator of PI3K activity, are very frequent and contribute to constitutive pathway activation and mTOR activity. Further, they may result in resistance to upstream anti-receptor agents. For example, trastuzumab depends on intact PTEN for its action in HER2-over-expressing breast cell lines, and PTEN loss predicts for trastuzumab resistance [45].

2.2. Rationale for using mTOR inhibitors in breast cancer

Although endocrine therapy and HER2-targeted therapy are considered effective, resistance occurs limiting other treatment options. However, an accumulating body of evidence indicates that hyper-activation of the PI3K/Akt/mTOR pathway may represent a key process in the development of resistance to these treatments [46]. It has been assumed that the use of mTOR inhibitors in breast cancer may be twofold: in patients with acquired resistance, they could be used to partially regain chemo-sensitivity and provide an additional treatment period for drugs such as trastuzumab or aromatase inhibitors. On the other hand, in patients who have not previously been treated with hormonal treatments, they could be used in combination with endocrine therapies to delay the onset of resistance and prolong the treatment period [47-50].

Several studies reported the increased PI3K/Akt/mTOR signaling in trastuzumab resistance. Trastuzumab inhibits PI3K signaling through an increase in PTEN activity, and this PTEN activation is thought to contribute to the drug’s anti-proliferative effects [45]. These anti-proliferative effects, however, are decreased in cells with reduced PTEN expression, suggesting that a reduction in PTEN expression confers trastuzumab resistance [10]. These data are in accordance with previous observations in the clinical setting where PTEN-deficient breast cancers showed poor clinical response to trastuzumab [10]. Mutations leading to PTEN loss or PI3K catalytic subunit activation, both effectively increasing PI3K signaling, are also associated with trastuzumab resistance [51]. Finally, breast cancer patients screening for genes correlated with drug resistance development demonstrated that oncogenic mutations in PI3K catalytic subunit or low PTEN expression are associated with poor prognosis after trastuzumab therapy [52].

The PI3K/Akt/mTOR pathway also plays an important role in modulating responses to estrogen receptor (ER) therapy in a ligand-independent fashion, with several studies indicating that hyper-activation of Akt, and the subsequent hyper-activation of downstream mTOR, underlies resistance to endocrine therapies [53-55]. In breast cancer patients, activation of Akt is associated with a worse outcome among patients receiving endocrine therapy, with reduced clinical benefit in patients with positive expression of activated Akt [13]. Other studies have also shown an inverse correlation between Akt activation and partial response (PR) rates [56]. Expression of phosphorylated S6 kinase, a downstream marker of mTOR activation, significantly predicts overall survival in patients with hormone receptor-positive breast cancer receiving adjuvant endocrine therapy [15]. This is mimicked in vitro and in xenograft studies, where breast cancer cells with constitutive Akt activation exhibit reduced estrogen dependency and also demonstrate reduced sensitivity to anti-estrogen therapy [14]. In these studies, mTOR inhibition restored anti-estrogen sensitivity [15]. Thus, this pathway is a promising target for new cancer therapeutics, hence currently several clinical trials are underway with mTOR, PI3K and Akt inhibitors.

3. Inhibitors of the PI3K/Akt/mTOR pathway

3.1. mTOR inhibitors - the rapalogs

Rapamycin, a macrolide antibiotic, originates from Streptomyces hygroscopicus found in the soil on the island of Rapa Nui. Rapamycin (and its analogues, also known as rapalogs) acts by binding to the FKBP12 binding protein, which in turn interacts with the mTORC1 complex, inhibiting downstream signaling [57]. Rapamycin, the first defined mTOR inhibitor, specifically inhibits mTOR, resulting in inhibition of cell growth, cell cycle progression and cell proliferation [58]. However, the poor aqueous solubility and chemical stability of rapamycin restricts its application for cancer therapy [59]. The other rapalogs, synthetic derivatives of rapamycin with improved pharmaceutical properties, are temsirolimus, everolimus and ridaforolimus (formerly known as deforolimus) [59].

Though the rapalogs trace their history back to use as immunosuppressant drugs used in transplant medicine, their antiproliferative effects led to investigation of their use as anti-cancer agents [60-61]. As the first-generation mTOR inhibitors, rapamycin and its analogs (rapalogs) have proven effective in a range of preclinical models. In the clinic, rapalogs have demonstrated important clinical benefits, particularly against endometrial cancer, mantle cell lymphoma and renal cancer. Nevertheless, the overall objective response rates in major solid tumors achieved with single-agent rapalog therapy have been modest [62-64].
3.1.1. The rapalogs in combination chemotherapy

The rapalogs have been investigated as monotherapy in a host of other phase II studies in diverse tumor types, including neuroendocrine tumors, breast cancer, endometrial cancer and sarcomas [65]. Encouraging single agent clinical efficacy was observed with the use of everolimus in pretreated patients with recurrent endometrial cancer, where loss of PTEN expression was predictive of clinical benefit [66].

Despite the high expectation for their application in oncology based on sound rationale related to the presumed mechanism-of-action, the rapalogs monotherapy have only met with modest success. Most notable is their utility of these agents in combination therapy in breast cancer. The rapalogs in combination with other chemotherapeutics have shown early encouraging data. PI3K pathway activation has been found to lead to resistance to trastuzumab in HER2-overexpressing breast cancer [67]. In this regards, studies have investigated combining everolimus with trastuzumab and paclitaxel in women with prior resistance to the latter two agents. Confirmed partial responses were seen in 20% of subjects and stable disease in a further 56% in a phase II study [68].

The same strategy has been evaluated in a phase I trial of everolimus, trastuzumab and vinorelbine, achieving a disease control rate of 80% (37 of 46 evaluable patients) [69]. Recent data from two phase I trials suggest that everolimus can help overcome resistance to trastuzumab in women with HER-2+ MBC. Everolimus plus trastuzumab and weekly paclitaxel was shown to slow tumor growth in 77% of patients, and the combination of everolimus with trastuzumab and vinorelbine halted tumor growth in 62% of patients [70-71]. Although early indications suggest that targeting components of the PI3K pathway may have some activity in the treatment of metastatic breast cancer (MBC), additional data, including an understanding of combinations and patient selection, are required. However, in unselected patients with breast cancer these agents have modest anti-tumor activity in the range of around 10% [72].

mTOR targeting therapeutics such as everolimus and temsirolimus have not only been tested in combination with ErbB2 inhibitors but also with endocrine therapy. A phase III trial investigated letrozol with or without temsirolimus in ER positive, metastatic breast cancer [73]. The trial was terminated early because of increased toxicity and lack of efficacy. However, a more recent phase II trial assessing letrozol with or without everolimus in the neoadjuvant setting showed a marginally significant increase of the response rate in the combination arm [73]. More importantly yet, the phase III BOLERO-2 trial has investigated the combination of the aromatase inhibitor exemestane plus everolimus in patients with advanced breast cancer [74]. Taken together, the clinical results obtained with mTOR inhibitors are strongly dependent on the chosen concomitant therapy. This dependence on combination therapy is a feature often observed in targeted therapies.

3.1.2. Rapamycin-resistant mTOR function

Several recent studies highlight the emergence of rapamycin-resistant mTOR function in protein synthesis, cell growth, survival and metabolism [40]. The limited effectiveness of rapamycin as cancer therapy can be explained first by its biochemical mechanism as well as its complex and variable signaling responses in cancer cells. Rapamycin in complex with the 12 kDa FK506 binding protein (FKBP12) partially inhibits mTOR through allosteric binding to mTORC1. This drug mechanism does not block all mTORC1 outputs and does not directly target mTORC2-dependent AKT function [40]. Generally, the activity of rapalogs in a host of tumor types where the PI3K/Akt/mTOR pathway is frequently activated has been disappointing. As a general rule, these agents only inhibit the mTORC1 complex (although there are some cellular models where disruption of mTORC2 also occurs [39]. Therefore, there have been legitimate concerns that their efficacy may be partly limited by a failure to stop mTORC2 mediated phosphorylation and activation of Akt.

The second factor contributing to rapamycin resistance is the mTORC1 negative feedback regulation of PI3K pathway. In preclinical and clinical settings, treatment of certain tumor types with rapamycin elevates PI3K-AKT activity and counteracts the therapeutic potential of mTORC1 inhibition, a phenomenon that is undesirable for cancer therapy [40]. This phenomenon can be explained as follow: inhibiting mTORC1 releases the feedback inhibition mediated by the S6KIRS1-PI3K loop that normally acts to moderate pathway activity. Thus this inhibition may lead to a paradoxical increase in Akt activity, a consequence of both biological and therapeutic implications. Indeed, increased phosphorylated Akt has been confirmed in tumor biopsies from rapalogs treated patients [75].

The third potential explanation for the limited activity of mTOR inhibitors in breast cancer and other tumor types may be related to a ‘collateral effect’ of mTOR blockade. mTOR inhibition blocks the natural negative feed-back on IGF-1R signaling exerted on PI3K [75]. This results in an increase in PI3K and Akt activations which could significantly counteract mTOR inhibition. Thus, dual inhibition of both IGF-1 signaling, with either MAbs against the receptor or tyrosine kinase inhibitors, and mTOR results in superior anti-proliferative effect over each single strategy. In the clinical setting, there is indirect evidence that this approach may be also beneficial. Octreotide was proven to inhibit IGF-1R signaling. Although octreotide has limited activity in patients with refractory neuroendocrine tumors, it has been shown to that the combination of everolimus and octreotide has resulted in an impressive activity [76].

It is therefore warranted to identify the subset of patients that may putatively benefit from it, and optimized PI3K/Akt/mTOR-dependency genetic signatures should be developed. In this direction, it has been recently observed that a majority of locally advanced and inflammatory breast cancers over-express the translation regulatory protein 4E-BP1 and the initiation factor eIF4G, both of them are mTOR downstream targets. While additional studies are planned to further dissect this interaction, it does seem reasonable to explore the benefits of mTOR inhibitors in the treatment of locally advanced breast cancer [77].
Taken together, these data suggest that pathway activation and reactivation could be avoided by PI3K, Akt or concomitant PI3K and mTOR catalytic inhibition (that would target both mTORC1 and mTORC2). Thus, a more complete suppression of mTOR global signaling network by the new inhibitors other than the rapalogs is expected to yield a deeper and broader anti-tumor response in the clinic [40].

3.2. Dual PI3K-mTOR inhibitors

These molecules simultaneously target the ATP binding sites of mTOR and PI3K with similar potency and cannot be used to selectively inhibit mTOR-specific activities [78-81]. Thus, they are generally not considered as useful research tools to study the mTOR regulation or function. However, they may have unique advantages over single-target inhibitors in certain disease settings because they can target at least three key enzymes (PI3K, Akt, and mTOR) in the PI3K signaling pathway. Inhibition of mTORC1 activity alone by rapalogs may result in the enhanced activation of the PI3K axis because of the mTOR-S6K-IRS1 negative feedback loop [75]. Therefore, the mTOR and PI3K dual specificity double targeting inhibitors might be sufficient to avoid PI3K pathway reactivation.

As mentioned previously, agents belonging to this class target all catalytic isofoms of PI3K, mTORC1 and mTORC2. This has the theoretical advantage of efficiently shutting down the PI3K/Akt/mTOR pathway but also the possible shortcoming of enhanced toxicity. SF1126 is a small molecule prodrug of LY294002 that is conjugated to an integrin-binding component. This design promotes delivery to the tumor together with its associated vasculature where cleavage leads to the active drug release. It has shown significant anti-tumor effects in xenograft models of solid tumors including glioblastoma, breast and prostate cancer, and potent anti-angiogenic activity has also been observed, partially related to a reduction in HIF-1α levels [82]. Two dual inhibitors are under investigation: Novartis (Basel, Switzerland) – NVP-BEZ235 and NVPBGT226. NVP-BEZ235 is a novel orally available product belonging to the class of imidazoquinolines [83]. NVP-BEZ235 binds the ATP-binding clefts of PI3K and mTOR kinase, thereby inhibiting their activities [83]. Preclinical studies demonstrated anti-proliferative activity against a wide range of cancer cell lines, including HER2-overexpressing breast cancer models of trastuzumab and lapatinib resistance [67, 84]. Further, tumor growth suppression has been shown in PI3K mutated xenograft models of human cancer.

Increasing evidence showed that NVP-BEZ235 is able to effectively and specifically reverse the hyperactivation of the PI3K/mTOR pathway, resulting in potent antiproliferative and antitumor activities in a broad range of cancer cell lines and experimental tumor models [85-86]. In breast cancer cells, NVP-BEZ235 blocked the activation of the downstream effectors of mTORC1/2, including Akt, S6, and 4E-BP1 [67]. Especially, at doses higher than 500 nM, NVP-BEZ235 completely suppressed Akt phosphorylation, irrespective of exposure duration.

3.3. mTOR kinase inhibitors (selective mTOR ½ inhibitors)

Given the previous considerations with rapamycin, recently the discovery of small molecule ATP-competitive mTOR kinase inhibitors (TKIs) that bind to the active sites of mTORC1 and mTORC2, thereby targeting mTOR signaling function globally was reported [87-89]. These second-generation mTOR inhibitors bind to the ATP-binding site in the mTOR kinase catalytic domain (act as ATP-competitive inhibitors) and thereby indiscriminately inhibit both mTORC1 and mTORC2, downregulate mTOR signaling generally, and reduce the feedback activation of PI3K signaling in cancer cells. Therefore, they share more in common with the dual PI3K/mTOR inhibitors than the rapalogs in terms of their mechanism-of-action. In turn, this should mitigate the paradoxical PI3K activation consequent to de-repression of the negative feedback seen with rapalogs [87].

On the other hand, unlike PI3K/mTOR dual inhibitors, they selectively inhibit both mTORC1 and mTORC2 without inhibiting other kinases [87]. This class of agents includes PP242, PP30, Torin1, Ku-0063794, WAY-600, WYE-687 and WYE-354. Clearly, these mTOR kinase inhibitors have provided novel tools for elucidating new roles of mTOR in tumorigenesis. However, more studies are still required to understand the distinct effects and mechanisms between these pharmacological agents and rapamycin in targeting cancer cell growth and survival, and to evaluate their efficacy in the treatment of cancer and other diseases in which PI3K/Akt/mTOR pathway is hyperactivated [59].

Thus, the discovery of these specific, active-site mTOR inhibitors has opened a new era for breast cancer therapy. As discussed earlier, the development of highly potent and specific active-site inhibitors of mTOR not only provides invaluable tools for deciphering novel insights to the increasingly complex mTOR signaling network but also offers considerable new opportunities to fully exploit the therapeutic potential of mTOR targeting in cancer. Interesting preclinical data of two such agents (PP242 and PP30) suggests that they have more substantial anti-proliferative actions than rapamycin not because of the mTORC2 effects but rather because they are more effective in suppressing mTORC1 [87]. Other agents in this group include WAY-600, WYE-687, and WYE-354, the latter of which has displayed robust antitumor activity in PTEN null tumor xenografts [89]. AZD8055 (Astra Zeneca, London, UK), OSI-027 (OSI Pharmaceuticals, Melville, NY, US) and INK128 (Intellikine, La Jolla, CA, US) are the first mTOR kinase inhibitors to enter clinical trials [90].

3.4. Natural products

Increasing evidence has demonstrated that some natural products, including curcumin and resveratrol may be used as mTOR inhibitors. Recently it has been described that resveratrol activated AMPK in both ER-positive and ER-negative breast cancer cells, and consequently inhibited mTOR and its downstream 4E-BP1 signaling and mRNA translation [91]. Moreover, it was also suggested that curcumin may exert its anti-proliferative effects by inhibiting mTOR
signaling and thus may represent a new class of mTOR inhibitors. Numerous studies have shown that curcumin inhibited the growth of a variety of cancer cells and showed effectiveness as a chemopreventive agent in animal models of carcinogenesis [92-93]. In numerous cancer cell lines, it has also been suggested that curcumin may execute its anti-cancer effect primarily through blocking mTOR mediated signaling pathways [92, 94].

4. Concluding remarks and future perspectives

The era of targeted therapeutics has brought rapid progress to breast cancer treatment. Targeted therapies increase cure rates in localized and prolong survival in metastasized breast cancer. The list of targets for drug treatment has dramatically increased with a deeper understanding of the molecular pathology of breast cancer. Despite the discovery of mTOR for over 15 years, the complexity of the mTOR signaling network is just beginning to be understood. Although rapamycin has proven invaluable as a chemical probe for mTORC1-dependent signaling functions, we now recognize that this natural product only targets a subset of the intracellular activities of mTOR. These recent insights may help to explain the relatively modest impact of the rapalogs on the growth of several tumor subtypes, at least when these agents are employed in the monotherapy setting. The preliminary clinical data from phase I trials presented to date have not demonstrated significant response rates with any of the inhibitors when employed as single agent therapy. The possible reasons for this observation include poor patient selection, inadequate dosing schedules, and resistance mechanisms.

Moreover, there are some concerns that need to be addressed in the future. First, a reproducible sub-classification of breast cancer patients needs to be developed. Ideally, a novel classification should outline targets for drug treatment rather than prognosis only. Furthermore, regarding patient selection, strong preclinical work has suggested that those patients whose tumors harbor genetic aberrations that result in increased PI3K pathway activity should be most sensitive to these agents [67, 95]. However, the majority of patients with detected PTEN loss or PIK3CA mutations have not responded to monotherapy. In addition, the few confirmed clinical responses seen have occurred in both those with and those without PI3K pathway activating mutations. Nonetheless, it seems a rational strategy to enrich patient populations with those harboring such genetic changes and prospective analysis of these putative predictive biomarkers should be implemented.

Second, while we are optimistic that the second generation mTOR inhibitors will be more effective in the clinic, we might anticipate that global inhibition of mTOR will be accompanied by greater toxicity to normal tissues. Given the potential toxicity issues, it is now more important than ever before to understand which tumor types are most sensitive to the more complete mTOR inhibition by this new generation of TKIs.

Third, signaling pathways in human cancer are complex. Frequent cross-talk and feedback signal add to complexity and promote several pathways for resistance. It appears unlikely that a single pathway abrogation will be sufficient to switch off the drive for malignant development and progression in a tumor. There is much optimism that use of rationale drug combinations should overcome some of these deficiencies. This could imply any of the drug classes described here combined with either targeted therapies against RTKs or other cytotoxic agents.

Fourth, the rapidly growing body of new drugs will force us to rethink the way we do clinical trials in breast cancer research. Performing large phase III trials with every single one of these new compounds will not be possible. Selection of promising agents earlier in development will have to be more rigorous. However, development of new targeted therapies will undoubtedly continue at a quick pace and hopefully further increase both the life span and the quality of life of breast cancer patients.

Finally, researchers are continuing to devise and employ new strategies to enhance outcomes, in particular by enriching patient populations and testing a multitude of drug combinations based on sound rationale. Given the importance of the PI3K/Akt/mTOR pathway in the malignant phenotype, further optimization of the clinical use of these new compounds in the coming years is warranted and should lead to better clinical outcomes.

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