Metformin and mTOR Inhibitors: Allies against Ovarian and Breast Cancers

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Abstract

Cancer is one of the leading causes of death worldwide. Every year 8.2 million people die from the disease. In this context, breast and ovarian cancer are the most incidental among women. Elucidation of cell growth pathways and the observation that these pathways are altered in human cancer have encouraged the search for specific inhibitors. The phosphatidylinositol-3-kinase (PI3K)/Protein kinase b (AKT)/Mammalian Target of Rapamycin (mTOR) pathway is an important pathway involved in cell growth, tumorigenesis, cell invasion, and resistance to therapies. This pathway is often activated in breast and ovarian cancers and the deregulation of its signaling can contribute to tumor growth, angiogenesis and metastasis. Metformin is one of the most commonly prescribed antidiabetic drugs in the world whose anticancer effects, mediated by reduced mTOR signaling, have become notable. Therefore, this review provides an overview of signaling pathway PI3K/AKT/mTOR in the ovarian and breast cancers as well as for target therapies of mTOR signaling, with an emphasis on its mechanisms, clinical applicability and future perspectives.

Keywords: Breast cancer; Ovarian cancer; mTOR pathway; mTOR inhibitors; Metformin

Introduction

The mTOR is a serine/threonine kinase ubiquitously expressed in mammalian cells being part of the PI3K/AKT pathway (Figure 1) [1,2]. In cells, mTOR is present in two distinct complexes, mTOR Complex 1 (mTORC1) and mTOR Complex 2 (mTORC2) that together regulate a variety of processes including proliferation, differentiation, metabolism, motility, survival, autophagy and angiogenesis [3-5]. Furthermore, the mTOR pathway is frequently hyperactivated in a number of human malignancies, including breast and ovarian cancer [6], therefore it is considered as a promising therapeutic target and a hotspot in cancer research.

Figure 1: Overview of PI3K/AKT/mTOR signaling pathway.

Preclinical studies have confirmed the anticancer effects of mTOR inhibitors in breast and ovarian cancer and further understanding of the molecular mechanism in PI3K/AKT/mTOR cascade is needed to develop optimized therapeutic regimens [6]. Combination therapies of mTOR inhibitor with agents such as cytotoxic chemotherapy, hormonal therapy, receptor tyrosine kinase inhibitors and vascular endothelial growth factor (VEGF) inhibitors are being intensively studied and appear to be promising.

Importantly, new players in mTOR signaling pathway have emerged with therapeutic potentials such as the antihyperglycemic drug metformin. Metformin, oral safe and well-tolerated drug, has been associated with reduced cancer risk in observational studies [7,8]. Beyond glucose lowering, metformin has shown in vitro promising results regarding reduction of cell proliferation and protein synthesis in breast and ovarian malignancies [9-11]. In this review, we provide a brief outline of our current understanding of the mTOR signaling pathway and discuss the clinical trial evidence available to date.

The mTOR Pathway

mTOR is a highly conserved intracellular serine/threonine kinase found practically in all mammalian cells. Under physiological conditions, mTOR modulates several processes, including protein translation, cell growth, proliferation, survival, metabolism, and autophagy [12-16]. mTOR is a component of two major intracellular signaling complexes, mTORC1 and mTORC2, which differ from each other in composition and functionality. mTORC1 consists of mTOR, raptor (regulatory associated protein of mTOR), PRAS40 (proline-rich AKT substrate 40 kDa), and mLST8 (mammalian lethal with sec-13); whereas mTORC2 is composed of mTOR, rictor (raptor independent companion of mTOR), mSIN1 (mammalian stress-activated protein...
Diverse environmental signals, including growth factors (e.g. insulin, insulin-like growth factor 1 (IRS-1), epidermal growth factor (EGF), nutrients (e.g. amino acids) and cellular stress, regulate mTORC1 signaling [17-19]. After activation, mTORC1 phosphorylates a range of substrates, such as the 40S ribosomal protein S6 kinase (p70S6K), 4E-binding protein 1 (4E-BP1) and UNC-51-like kinase 1 (ULK1) [1,2]. p70S6K and 4E-BP1 associate with mRNAs and regulate both mRNA translation initiation and progression, thus enhancing protein synthesis [20,21]. ULK1, in turn, modulates autophagy pathways [22,23]. On the other hand, and in contrast with the well described cellular roles of mTORC1, the function of mTORC2 is still not fully elucidated. However, it is known that mTORC2 promotes the activation of several kinases such as AKT and protein kinase C (PKC), and regulates the cytoskeleton organization [14,24-26].

It has been described that mTORC1 is activated by growth factors through the PI3K/AKT pathway. Most of the growth factors interact with receptor tyrosine kinases (RTK), upstream from the PI3K pathway, leading to the phosphorylation of phosphatidylinositol-4,5-bisphosphate (PIP2) to generate phosphatidylinositol-3,4,5-trisphosphate (PIP3). PIP3 serves as a docking site for the phosphoinositide-dependent kinase 1 (PDK1) and AKT, resulting in the activation of AKT by PDK1 [27,28]. In turn, AKT phosphorylates the tuberous sclerosis 2 (TSC2), thereby inhibiting the Ras homolog enriched in brain (Rheb) GTPase activity of the TSC1/TSC2 complex. Rheb, in its GTP-bound state, can activate mTOR [29,30]. Nonetheless, the upstream regulators of mTORC2 remain poorly characterized. Recently, in vitro kinase assays have shown that growth factors can activate mTORC2 [31]. The mTORC2 complex contributes to complete AKT activation by phosphorylating AKT at serine 473 (Ser473) [14,32].

Physiologically, some of the components of the PI3K/AKT/mTOR pathway are inhibited by proteins, such as the phosphatase and tensin homologue deleted on chromosome ten (PTEN) and the inositol polyphosphate 4-phosphatase type II (INPP4B), as well as by the TSC1/TSC2 complex [33-35]. PTEN acts on the D3 phosphorylated positions of PI3K, promoting the formation of PI2P3, thereby preventing the activation of AKT and PDK-1 [36,37]. Furthermore, INPP4B, a recently described lipid phosphatase, converts PIP2 to phosphatidylinositol monophosphate (PIP), thus regulating the PI3K activation [38,39]. Other negative regulators involved in this pathway are the PHAS1 and FKS-binding protein 8 (FKBP8), which prevent Rheb from activating mTORC1 [40,41].

Importantly, mTORC1 negatively regulates growth factor signaling in two distinct manners. When activated by mTORC1, p70S6K directly phosphorylates the insulin receptor substrate-1 (IRS1), which promotes IRS1 degradation, and leads to decreased PI3K signaling and reduced AKT Thr308 phosphorylation [42-44]. In addition, mTORC1 directly interacts with IRS1 via Raptor, and phosphorylates IRS1 at Serine 636/639 (Ser636/639), hence, interfering with its association with PI3K [45].

The Role of mTOR in Breast and Ovarian Cancer

In the last few years, significant advances have been made in understanding the role of mTOR in cancer development and progression. Increased mTOR signaling in cancers has been implicated in tumorigenesis, promotion of cell survival, angiogenesis, invasion, tumor growth, patient prognosis and resistance to standard therapies [46]. mTOR activation involves loss of PTEN expression or function, mutation or amplification of the PI3K, amplification of AKT and inactivation or mutations of AKT-associated mTOR-regulatory proteins such as TSC1/TSC2 [47]. Additionally, aberrant activation of p70S6K and elf4E has been reported in various human cancers, in which they correlate with tumor aggressiveness and poor disease prognosis [48-51]. The mTOR pathway can also be activated via exogenous oncogenes, including mutated or overexpressed RTKs, such as insulin-like growth factor 1 receptor (IGFR-1), platelet-derived growth factor receptor (PDGFR), and human epidermal growth factor receptors 1-4 (HER1-4) [52-55].

Ovarian cancer

Ovarian cancer is the most lethal gynecologic malignancy, and the fifth cause of cancer-related death among women [56,57]. According to the American Cancer Society (ACS), 22,440 new cases of ovarian cancer and 14,080 related deaths are estimated in the U.S.A. in 2017. When diagnosed at early and localized stages, the 5-year survival rate of ovarian cancer patients is approximately 94%. Nevertheless, most of the cases are detected as advanced and metastatic disease, in which cases it relapses within two years, and the patients’ survival rate decreases to 27% mainly due to chemoresistance to the platinum-taxane based chemotherapy in the adjuvant setting [57-60].

Ovarian cancer comprises a heterogeneous group of diseases classified based on morphologic and molecular-genetic features [61,62]. Type I tumors are composed of low-grade serous, low-grade endometrioid, clear cell, mucinous and transitional (Brenner) carcinomas. On the other hand, type II tumors, which are highly aggressive and almost always present in advanced stage, include high-grade serous carcinoma, undifferentiated carcinoma, and carcinosarcoma [63,64]. High grade serous ovarian cancer is the most prevalent ovarian cancer subtype, accounting for about 85% of all ovarian cancer-related deaths [65], followed by the clear cell and endometrioid subtypes that occur at similar rates, and mucinous carcinomas, the less common form of the disease [66]. Of note, genetic alterations, as somatic mutations, gene amplifications, and deletions in ovarian cancer seem to be subtype-specific, supporting the heterogeneity of the molecular, chemoresistance and clinical profiles of the multiple manifestations of the disease [67-69].

Despite the strong body of evidences pointing to specific genetic variations involved in the development and progression of ovarian cancer, there is still debate with regard to prevalent mutations and their prevalence in the disease. The analysis of 500 high grade serous ovarian tumors was conducted by the Cancer Genome Atlas (TCGA) project, revealing that 96% of all ovarian cancer subtypes present mutations in the TP53 gene [65,68]. In agreement, a systematic analysis of the TCGA Pan-Cancer cohort, which included 3,281 tumors from 12 cancer types, reported the prevalence of mutated TP53 gene in 95% of the 316 ovarian serous carcinomas included in the study [47]. Also, less frequent but yet recurrent mutations were reported in the RB1, NFI, EAT3, CSMD3, GABRA6 and CDK12 genes [47,65]. There is probable genetic specificity within ovarian cancer subtypes. In this context, whereas TP53 mutations are highly incident amongst type II tumors, they rarely occur in type I tumors [63,64]. The opposite seems to occur with respect to the rare but carcinogenic mutations in the KRAS, BRAF and ERBB2 genes that are prevalent in low-grade ovarian carcinomas in comparison to the high grade tumors [65,68]. Moreover,
ovarian clear cell tumors present mutations in the PIK3CA, TP53, KRAS, PTEN genes with a frequency of 33%, 15%, 7%, and 5%, respectively [70,71], whereas endometrioid ovarian tumors frequently have mutated CTNNB1 (encoding β-catenin), ARID1A, member of the SWI/SNF family, and PIK3CA genes [64]. The mucinous ovarian tumors present prevalent KRAS gene mutations [63,64,72].

A key controversial aspect related to the genetic variations underlying ovarian cancer development and progression, including chemo-resistance, relies on the PI3K/AKT/mTOR pathway. Bellacosa et al. [73] have documented that amplification of the AKT gene coding for all of the protein isoforms seems to occur in 15% to 20% of high grade serous ovarian carcinoma cases. Furthermore, mutations or gene amplification of the PIK3CA gene, which code for the p110α catalytic subunit of the PI3K protein, have been detected in 30.5% of all ovarian cancers [74]. Intriguingly, the recent TCGA Pan-Cancer study showed that the PIK3CA gene is the second most common mutated gene, behind the TP53 gene only, occurring frequently (>10%) in most cancer types, except ovarian serous carcinoma, kidney clear cell carcinoma, lung adenocarcinoma, and acute myeloid leukemia. Likewise, none of the ovarian serous carcinoma studied presented mutations in the AKT1 gene [47]. Notwithstanding the fact that the TCGA Pan-Cancer study has focused exclusively on gene mutations, but not on gene amplification, the data are still conflicting with previous publications [47]. Nonetheless, ongoing clinical trials support the benefit of ovarian cancer patients from the pharmacological inhibition of the PI3K/AKT/mTOR pathway, therefore reinforcing the fact that solving ovarian cancer molecular profiling by means of characterizing the heterogeneity of its subtypes and their mutational landscape likely represents an opportunity to fight the disease using target therapies effective against specific aberrations [75-77].

Breast cancer

Breast cancer (BC) is the most frequent malignancy, and the second cause of cancer-related deaths among women in the United States, where 252,710 new cases of the disease and 40,610 related deaths are expected in the year of 2017 [58]. Worldwide, the scenario is dramatic as well, with more than 1 million new cases of BC diagnosed yearly [65]. Breast cancer comprises multiple diseases harboring different genetic alterations, whose subtypes respond differently to treatment, and are associated to distinct clinical outcomes [78,79].

In 2000, Perou and colleagues proposed a more reliable method to stratify breast cancer based on gene expression profiling, resulting in four major subtypes: luminal, human epidermal growth factor receptor 2 (HER2)-enriched, normal breast and basal-like (BL). The luminal and HER2-enriched group benefit from hormonotherapy and anti-HER2 immunotherapy, respectively [80-82]. Basal-like breast cancers are often referred to as triple-negative breast cancers (TNBC) because most tumor cells lack the expression of estrogen receptor (ER), progesterone receptor (PR) and HER2. However, only 75% of TNBC are of the basal-like subtype [83]. Although chemotherapy remains the mainstay strategy to combat basal-like breast cancer, target therapy and/or novel and efficacious molecules are still unavailable, leading to poor clinical outcome and patients‘ death following short disease progression-free interval [84].

Advances in understanding the etiology and biology of breast cancer have led to the identification of key targets among multiple signaling pathways involved in the development, malignant transformation, and survival of breast cancer cells. The PI3K/AKT/mTOR pathway is commonly deregulated in breast cancer. Indeed, a systematic analysis of the TCGA Pan-Cancer cohort revealed that mutations in PIK3CA are frequent in breast cancer (33.6% of the 763 primary breast cancers included in the study), being specifically enriched in luminal, ER+ subtype tumors. Moreover, PIK3CA mutations occur in breast cancer at a relatively high average variant allele fraction, thus enabling to infer early appearance of the genetic aberrations during tumorigenesis [47]. In addition to the putative involvement of PIK3CA mutations in breast cancer initiation, there is a strong body of evidences pointing to a critical role of the abnormal expression and activity of the PI3K/AKT/mTOR pathway in drug resistance [85]. Of clinical relevance, PI3K activation has been implicated in resistance to endocrine therapy in patients with ER+ breast cancer [86]. Therefore, means to identify which ER+breast cancer patients may require PI3K/mTOR inhibition could facilitate a more accurate selection of patient populations for treatment, particularly in the adjuvant setting.

Interestingly the three main breast cancer subtypes display a remarkable difference within their mutational spectra. Mutations in the PIK3CA gene have been significantly associated with luminal breast tumors (45%). In turn, the HER2-enriched subtype has been characterized by HER2 amplification (80%), and high frequency of mutated PIK3CA (39%) and TP53 (72%) genes. In contrast, basal-like tumors have been associated to a frequency of 9% of mutations in the PIK3CA gene, and high frequency of TP53 mutated gene (80%) [65]. Intriguingly, the PI3K pathway has been described as aberrantly activated at high frequency in basal-like breast cancer, thus enabling the postulation that alternative mechanisms are elicited by these tumor cells to warrant the phenomena. This might include loss of expression of the INPP4B and PTEN genes or amplification of the PIK3CA gene [87]. Basal-like tumors also exhibit frequent amplification of the KRAS (32%), BRAF (30%), and epidermal growth factor receptor (EGFR) (23%) genes, contributing with constitutive activation of the PI3K/AKT/mTOR pathway [65].

Both breast basal-like and ovarian serous tumors are diseases related to patient’s poor clinical outcome, and share common genetic features, such as the RBI gene loss, the BRCA1 gene inactivation, the overexpression of the AKT3 gene, the MYC gene amplification, and the high frequency of TP53 gene mutation [65].

mTOR Inhibitors in Cancer Therapy

Rapamycin and its derivatives

The mTOR serine/threonine kinase is a multiprotein complex and it is directly involved in many cell signaling pathways and many aberrations of the mTOR implicated in human cancer. mTOR inhibitors studied in clinical trials for cancer treatment showed that tumor cells with mutations in p53 or PTEN are susceptible to mTOR inhibitors [88].

mTOR inhibitors could be categorized in first and second-generation-presenting a wide variety of target and mechanism . The first-generation mTOR inhibitors include rapamycin and its analogues (sirolimus, temsirolimus, everolimus) that employ allosteric mechanism to block, whereas second generation mTOR inhibitors (AZD8055, Torin1, PP242, PP30) have as target ATP binding site to impede kinase activity of both mTORC1 and mTORC2 [89].

Rapamycin, a macrocyclic lactone isolated from the soil bacterium Streptomyces hygroscopicus, first discovered in 1975, has diverse clinical applications as an anti-fungal, immunosuppressant and anti-
cancer drug [90,91]. Nevertheless, rapamycin has limited bioavailability due to its poor aqueous solubility. In an effort to improve its pharmacokinetics, several rapamycin analogues, named rapalogs, have been developed, such as the mTOR inhibitors temsirolimus (CCI-779), everolimus (RAD001) and ridaforolimus (MK-8669/AP23573) [4,92,93]. Rapamycin and its derivatives exhibit a safe toxicity profile, being the side effects skin rashes and mucositis dose-dependent [94]. Other symptoms commonly described are fatigue, nausea, anaemia, hypertiglyceridermia, hypercholesterolemia and neutropenia [95]. Furthermore, temsirolimus and sirolimus are associated with significant rate of pulmonary toxicity [94,95]. Rare side effects of the aforesaid drugs included interstitial lung disease, risk of secondary lymphoma, and reactivation of latent infections [69].

The precise mechanisms of mTOR inhibition by rapalogs are not fully understood. However, evidences point to the allosteric inhibition of mTORC1. In mammalian cells, the rapalogs associate with the intracellular receptor FK506 binding protein 12 (FKBP12). Then, this complex interacts with the FKBP12-rapamycin binding (FRB) domain, inhibiting allosterically the mTOR kinase activity. It is widely believed that rapalogs would be therapeutically effective by blocking the mTORC1’s phosphorylation activity of 4E-K and 4E-BP1 [13,100]. Secondly, the FKBP12–rapamycin complex competes with phosphatidic acid to bind to the FRB domain of mTOR, blocking mTOR kinase function [99]. Thirdly, the FKBP12–rapamycin complex bound to FKBP12 desaturates the mTOR–raptor–4E-BP1/4E-K1 scaffold complex, leading to dephosphorylation of 4E-K1 and 4E-BP1 [13,100]. However, the effects of rapamycin are dependent on cell type. This inhibitor only causes cell cycle arrest in a limited number of cell types and has modest effects on protein synthesis [101,102]. Moreover, rapamycin is a relatively poor inducer of autophagy [103]. Therefore, the clinical effects are extended for few cancers, such as cell carcinoma renal and lymphoma [96]. It may be explained, at least partly, by the fact rapamycin could not block the function of mTORC1 completely and showed little effects on mTORC2 complex in the majority of cell types [5,104]. Furthermore, PI3K pathway could be active due to feedback loop when mTORC1 is inhibited [105].

Some studies have shown that these compounds are able to disrupt the mTORC2 complex in a dose-, time- and cell type-dependent manner [14,106,107]. A possible mechanism by which rapamycin and rapalogs could inhibit mTORC2 relies on the interaction of newly synthesized mTOR molecules and rapamycin/rapalogs-FKBP12 complexes. In turn, this interaction would prevent mTOR from the interaction with RICTOR, inhibiting, thus, mTORC2. Indeed, it has been shown that prolonged exposure of cancer cells to rapamycin can promote its binding to mTOR before the assembly of the mTORC2 complex, with subsequent inhibition of the AKT-mediated signaling [14].

Two mTOR inhibitors have been approved for clinical use in cancer. Everolimus (Afinitor®), the first oral mTOR inhibitor to reach the oncology clinic, has been approved by the Food and Drug Administration (FDA) for the treatment of metastatic or unresectable pancreatic neuroendocrine tumors, advanced stage renal cell carcinoma, subependymal giant cell astrocytoma [108], progressive neuroendocrine tumors of the pancreatic origin [109], metastatic renal cell carcinoma and advanced ER+ [110], HER2 negative breast cancer [85]. Moreover, Tensirolimus (Torisel®) was also approved by the FDA in 2007 for the management of advanced stage renal cell carcinoma (FDA). Both derivative of rapamycin form a complex with FKBP-12 and this complex then binds to the FRB domain and inhibits the mTOR function [111].

Several studies have been conducted to analyze the effectiveness of rapamycin and rapalogs alone and in combination with standard chemotherapy, hormonal therapy such as EGFR and anti-VEGF inhibitors in the treatment of several types of cancers such as breast, ovarian, cervical and endometrial [112]. Phase I–II trials are now ongoing with mTOR inhibitors in patients with breast and ovarian cancer.

For example, phase II studies are ongoing in order to test everolimus in combination with chemotherapy (cisplatin and gemcitabine) in patients with metastatic triple negative breast cancer (NCT019399418 and NCT01931163). Additionally, a recent study of breast cancer (BOLERO-3) demonstrated that the combination of everolimus with trastuzumab and vinorelbine significantly prolongs progression-free survival (PFS) in patients with trastuzumab-refractory and taxane-pretreated, HER2+, advanced breast cancer [113]. Moreover, BOLERO-1 is an ongoing phase III, randomized, double-blind, placebo-controlled trial that will evaluate 717 patients with untreated metastatic HER2+ breast cancer randomly assigned to receive paclitaxel and trastuzumab with or without everolimus as first-line therapy [114]. A randomized placebo-controlled phase III trial (BOLORO-2) evaluated everolimus in combination with the aromatase inhibitor, exemestane, in postmenopausal women with HR+/HER2– advanced breast cancer that progressed after previous letrozole or anastrozole therapy. This study showed a significant increase in PFS (10.6 versus 4.1 months) and led the approval of everolimus in combination with exemestane by FDA in 2012 [115].

Clinical studies have evaluated the aromatase inhibitor letrozole in combination with everolimus in patients with metastatic endometrial carcinoma (NCT01068249). Phase II study showed that patients with endometrioid histology and CTNNB1 mutations responded well for the treatment [116]. In another phase II study in patients with breast cancer (NCT00107016), everolimus significantly increased letrozole efficacy in neoadjuvant therapy with ER+ patients [117]. Studies have been conducted using aromatase inhibitor anastrozole with everolimus in patients with ER and/or PR+ breast and gynecologic tumors as ovarian and endometrial cancer and the combination prolonged periods. Also, patients with multiple molecular alterations still benefited from therapy [118].

A phase II trial of ridaforolimus (AP2357) had been conducted in patients with advanced endometrial cancer and clinical benefit response was reported in 33% of the patients [119]. Another phase II study using oral ridaforolimus in patients with advanced or recurrent endometrial cancer also showed partial response in 7.7% patients [120]. Also, temsirolimus is being evaluated with bevacizumab and in combination with chemotherapeutic agents in endometrial cancer cell lines, and results showed that it increases progesterone mRNA expression and inhibits ER mRNA expression [121]. Preliminary a phase II study conducted by Tinker and colleagues, 2013 using temsirolimus in patients with metastatic cervical cancer showed that 3.0% of patients had a partial response lasting 7.2 months, and 57.6% had stable disease with a median duration of 6.5 months [122].
Promising results also have been found in a study conducted by Campone et al. (2009) [123] to assess the safety and the pharmacokinetic interactions combining everolimus and paclitaxel in patients with breast cancer and ovarian carcinoma. Another phase II clinical study (GOG0268) that evaluates additional effects of the temsirolimus combined with paclitaxel/carboplatin therapy has been conducted in patients with stages III/IV clear cell adenocarcinoma [112,124]. However, some studies failed to show the efficiency of temsirolimus in patients with persistent/recurrent epithelial ovarian cancer/primary peritoneal cancer showing a modest activity of this mTOR inhibitor and the results were insufficient to justify further study in a phase III [125].

Although clinically promising, the efficacy of rapalogs is partially limited by the negative feedback loops in the mTOR pathway. With this regard, the exclusive inhibition of the mTORC1 complex by the rapalogs compromises the p70S6K-mediated feedback loop towards IRS-1, resulting in the activation of both the PI3K/AKT and MAPK/ERK pathways, hence promoting compensatory cell survival, and the acquisition of chemoresistant phenotype [5,44,93]. Efforts have been made to overcome the aforesaid clinical limitation by means of developing new generation mTOR inhibitors, which inhibit the catalytic activity of both mTORC1 and mTORC2 complexes.

ATP-competitive inhibitors

Although rapamycin is a potent allosteric mTORC1 inhibitor with clinical applications, a second generation ATP-competitive inhibitor has been developed, including Torin1, Torin2, PP242, PP30, KU0063794, WAY-600, WYE-687, WYE-354, XL-388, INK-128, AZD-2014, AZD8055 and OSI-027 [126-132]. The ATP-competitive inhibitors of mTOR directly inhibit the mTOR kinase activity, affecting both the mTORC1 and mTORC2 complexes. In comparison with rapamycin and rapalogs, ATP-competitive inhibitor is more potent and efficacious against cancer not only because of the complete inhibition of mTORC1 but also due to the additional inhibition of mTORC2, consequently preventing Akt phosphorylation at Ser473 [104,130,133].

Studies which have been conducted with PP242 in colon cancer cells in vitro and in vivo showed decrease cell growth alone or in combination with MEK inhibitors [134]. Another ATP competitive inhibitor, Torin2, was developed to overcome the pharmacological limitations of Torin1 and is also a potent inhibitor of ATR, ATM and DNA-PK [135,136]. Lung cancer cell treatment with Torin2 resulted in a prolonged block in negative feedback and consequent Thr308 phosphorylation on AKT. These effects were associated with strong growth inhibition in vitro [137].

At present, there are several clinical trials focused on the examination of new agents, such as AZD-8055, OSI-027, WYE125132 and INK128, in a variety of human hematological malignancies and solid tumors, including breast cancers [112]. Also some studies were conducted using GSK795 in patients with advanced platinum resistant ovarian and showed interesting results as tumor regressions and CA125 decreases [138]. Phase I study is ongoing in order to evaluate the safety and toxicity profile of AZD2014 in combination with paclitaxel in patients with ovarian cancer (NCT02193633).

In spite of the clinical improvements observed with the ATP-competitive inhibitor when compared to the rapalogs, the literature still acknowledges significant limitations that outcome from compensatory cellular events. With this regard, it has been found that loss of the feedback on PI3K results in compensatory activation of the MAPK/ERK cascade by mTOR downstream effectors, such as 4E-BP1/ eIF4E, then, maintaining cell proliferation [139]. Furthermore, it has been shown that chronic inhibition of mTORC2 induces the activation of AKT by its phosphorylation on the residue Thr308 mediated by PDK-1, even in the absence of the priming Ser473 phosphorylation. Altogether, the referred mechanisms ultimately drive the acquisition of the resistant phenotype by the cancer cells [140,141].

Dual mTOR/ PI3K inhibitors

Scientists have explored to shed light on strategies to overcome the limitations by concomitantly targeting two molecules in the PI3K/AKT/mTOR pathway, PI3K and mTOR, whereas the resistance mTOR inhibitors cloud arise via feedback PI3K activation. This molecular knowledge has stimulated the development of news inhibitors termed dual PI3K-mTOR inhibitors that include NVP-BEZ235, XL765, BGT226, PI-103, PF-04691502, PKI-587 and GDC-0980 [142-148]. Comparing with the other types of PI3K pathway inhibitors, dual PI3K/mTOR inhibitors have the possible advantage of inhibiting all PI3K catalytic isoforms, mTORC1 and mTORC2 [6]. The catalytic sites of PI3K and mTOR share a high degree of sequence homology, thus enabling the abrogation of the catalytic activity of both PI3K and mTOR, consequently blocking downstream signaling related to cell proliferation, survival, and angiogenesis [142-145]. Therefore, these inhibitors may effectively turn off this pathway completely and display best efficacy in feedback inhibition normally observed with mTORC1 inhibitors [149]. However, it is not clear that dual PI3K-mTOR inhibitors will be tolerable at doses that effectively inhibit all p110 isoforms and mTOR [6].

The potential clinical value of the dual PI3K/mTOR inhibitors has been demonstrated by their significant inhibition of cell growth, and the induction of apoptosis and/or autophagy in a variety of tumor cancer cells [150-152], and these inhibitors have shown powerful effects in xenograft models of breast cancer [153], pancreatic cancer [154], melanoma [155], multiple myeloma [156], glioma [157], RCC [158], and acute myeloid leukemia (AML) [159].

In agreement, dual PI3K/mTOR inhibitors have entered clinical trials either as monotherapy as BEZ235/NVP-BEZ235, Novartis (NCT00620594) and BGT226, Novartis (NCT00600275 and NCT00742105) in advanced solid tumors and breast cancer, GDC-0980 (Genentech) (NCT00854126 and NCT00854152) in non-Hodgkin lymphoma, PF-04691502, Pfizer (NCT00927823) and GSK2126458, GlaxoSmithKline (NCT00972686) in solid tumors or in combination with other therapeutic agents, for example XL765 (Exelixis) associated with erlotinib (NCT00777699), letrozole (NCT01082068) and temozolomide (NCT00704080) in non–small cell lung cancer, breast cancer and gliomas, respectively [112]. Both BEZ235 and XL765 have shown good tolerability, with adverse effects including diarrhea, anorexia and nausea [160]. Furthermore, the combined therapy using rapamycin and dual PI3K/mTOR kinase inhibitor (PI-103) has been shown to be efficacious against human ovarian cells in vitro [161].

Metformin

Metformin, a biguanide derivative, is a widely prescribed antihyperglycemic drug and is prescribed as the first-line therapy for type 2 diabetes mellitus (T2D), and insulin resistance syndromes [162].
It is a relatively safe drug, with known pharmacokinetics and a favorable safety profile. Importantly, metformin does not affect insulin secretion nor induces hypoglycemia in normal patients [163], aside from the fact that no teratogenic effects have been reported in the newborn of drug users. The main limited side effect of metformin is gastrointestinal discomfort, such as nausea and diarrhea, which are usually self-limited [164]. Rarely, the serious adverse effect of lactic acidosis is documented, which has been primarily restricted to patients with concomitant renal and hepatic disorders [165]. Metformin may also cause B12 deficiency in long-term therapy [166].

Several in vitro studies have shown that metformin treatment inhibits cell growth, induces apoptosis, and reduces invasion in a variety of human cancer cell lines, including breast and ovarian cancers [167-172]. These preclinical studies propose that metformin exerts its antineoplastic effects through multiple direct and indirect pathways; however, the exact mechanism by which metformin acts remains poorly understood.

The indirect effects of metformin are related to the suppression of the transcription of key gluconeogenesis genes in the liver, and the increase of glucose uptake by skeletal muscle, thus leading to declines in circulating insulin and glucose levels [173]. Besides lowering insulin levels, metformin can indirectly increase insulin-like growth factor-binding protein 1 (IGFBP1) production, ultimately decreasing the bioavailability of IGFI [174]. Insulin and IGFs are key regulators of metabolism and growth, promoting the development of cancer through the activation of the IRS/P3K/AKT/mTOR and IRS/AMPK axis [43,175].

In fact, the direct effects of metformin against cancer cells are mostly mediated by the activation of AMP-activated protein kinase (AMPK), and subsequent inhibition of mTOR, hence modulating cell metabolism and protein synthesis. Metformin must be actively transported into cells by the transmembrane protein organic cation transporter-1/2 (OCT1/OCT2) [176,177]. Once inside the cells, metformin inhibits the complex I of the mitochondrial respiratory chain, which leads to the disruption of the mitochondrial function, and to an increased ratio of adenosine monophosphate/adenosine triphosphate (AMP/ATP), mimicking cellular energy stress [178,179]. AMPK exists as heterotrimers composed of a catalytic subunit, and two regulatory subunits [180]. Each subunit is encoded by different genes, and has a unique role in the regulation and activation of AMPK. The subunit contains the activating phosphorylation site Threonine 172 (Thr172) [181], the subunit has the function of docking the protein to membranes [182], and the subunit binds to AMP or ATP [183]. AMPK can be allosterically activated by AMP, leading to the dephosphorylation of the catalytic subunit by protein phosphatases. Moreover, AMPK activation requires the phosphorylation of the (Thr172) by upstream kinases [184-186]. The upstream master kinase that regulates AMPK activation is LKB1, a tumor suppressor gene with relevance in many types of neoplasias, including breast cancer [187,188]. Once activated, AMPK regulates several effectors proteins, thus governing the activation of catabolic pathways (lipolysis and glycolysis), and the inhibition of anabolic pathways (gluconeogenesis, lipid and protein synthesis) [189-192].

As aforesaid, tumor cells often display alterations in P3K/AKT/mTOR pathway, which is a key signaling mechanism towards cellular growth and proliferation. Protein synthesis consumes a high proportion of ATP in the cell, thus mTOR is a major target of AMPK under conditions of metabolic stress. AMPK inhibits mTOR signaling through two distinct mechanisms: phosphorylation of TSC2, converting Rheb to its inactive GDP-bound form [193], and direct phosphorylation of Raptor, a subunit of the mTORC1 complex [194]. In addition, metformin inhibits the mTOR activity in the absence of TSC1/TSC2 and AMPK by suppressing RAG GTGases, which are involved in mTOR activation [195,196].

The interest in metformin as anticancer drug emerged from numerous retrospective, population-based studies in diabetic patients. Studies have suggested that metformin reduces cancer incidence and/or mortality among T2D patients, compared to those taking other antidiabetic medications [7,8,197-202]. Prospective data concerning metformin use in non-diabetic cancer patients are beginning to emerge. Hadad et al. [203] performed a pilot study on a small cohort of patients with breast cancer revealing that the use of metformin (500-1000 mg/day) decreased tumor cell proliferation, estimated by Ki-67 staining and altered the expression of various genes including those involved in inflammation, metabolism and mTOR signaling. Similarly, metformin treatment has been associated with beneficial effects in non-diabetic breast cancer women, concerning Ki67 staining, and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) scores [204]. However, Bonani et al. [205] have failed to demonstrate clinical benefit of metformin in breast cancer neoadjuvant setting through the evaluation of Ki67 expression. Nevertheless a different effect of metformin according to insulin resistance (homeostasis model assessment (HOMA) index) was noted, particularly in luminal B tumors, with a trend to a decreased proliferation in women with elevated HOMA index. Taken in conjunction, the state of the art knowledge about metformin and breast cancer has enabled authors to hypothesize that insulin resistance modulates both breast cancer biology and the antineoplastic actions of metformin [206,207].

There are more than 50 ongoing or upcoming clinical studies investigating metformin in cancer patients as monotherapy or in combined therapy with other antineoplastic agents [112]. For example, two clinical trials (NCT015299593/ NCT021455590) have been initiated aiming the evaluation of the treatment of advanced cancers, including breast cancer, with temsirolimus/sirolimus and metformin. In addition, an ongoing phase II study proposes to evaluate the combination of metformin, everolimus and letrozole in postmenopausal overweight or obese women with advanced receptor-positive breast cancer (NCT01627067).

**Conclusion**

Both highly lethal malignancies comprise deregulated and anomalously activated P3K/AKT/mTOR pathways, hence emerging as eligible diseases to be fought with common targeted therapeutic strategies. In this context, early trials of mTOR inhibitors have shown some clinical benefit and, combinations of mTOR inhibitors with other treatment modalities have demonstrated clinical results without significant additional toxicity. Furthermore, the combination seems to be promising due to the fact that metformin inhibits mTOR, even in the absence of AMPK, through the decreased activation of IGFR1/ MAPK, an important pathway related with resistance to mTOR inhibitors. Find below a summary of the main inhibitors mentioned in this paper.
References


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