REVIEW

The mTOR Signalling Pathway in Cancer and the Potential mTOR Inhibitory Activities of Natural Phytochemicals

Heng Kean Tan[1], Ahmed Ismail Hassan Moad[2], Mei Lan Tan[1,2]*

Abstract

The mammalian target of rapamycin (mTOR) kinase plays an important role in regulating cell growth and cell cycle progression in response to cellular signals. It is a key regulator of cell proliferation and many upstream activators and downstream effectors of mTOR are known to be deregulated in various types of cancers. Since the mTOR signalling pathway is commonly activated in human cancers, many researchers are actively developing inhibitors that target key components in the pathway and some of these drugs are already on the market. Numerous preclinical investigations have also suggested that some herbs and natural phytochemicals, such as curcumin, resveratrol, timosaponin III, gallic acid, diosgenin, pomegranate, epigallocatechin gallate (EGCC), genistein and 3,3'-diindolylmethane inhibit the mTOR pathway either directly or indirectly. Some of these natural compounds are also in the clinical trial stage. In this review, the potential anti-cancer and chemopreventive activities and the current status of clinical trials of these phytochemicals are discussed.

Keywords: mTOR signalling pathway - PI3K/Akt/mTOR - natural compounds - mTOR inhibitors

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Introduction

The mammalian target of rapamycin (mTOR) kinase is a conserved serine/threonine protein kinase that plays an important role in regulating many fundamental molecules mediating cell growth and cell cycle progression in response to cellular signals in eukaryotes (Liu et al., 2009b; Houghton, 2010). The mTOR signalling pathway has a central role in cellular processes such as cell survival, cell growth and proliferation, cell death, and tumor angiogenesis. This pathway is frequently hyper-activated in several human malignancies and therefore is considered to be an interesting and attractive therapeutic target for anti-cancer therapy.

The mTOR is also known as FKBP12-rapamycin associated protein (FRAP), or rapamycin and FKBP12 target (RAFT), or rapamycin target (RAPT), or sirolimus effector protein (SEP). The mTOR gene is located on human chromosome 1 in location 1p36.2 (Huang and Houghton, 2003). It is identified in mammalian cells as a 289 kDa serine/threonine kinase consisting of 2549 amino acids and the structural domains of mTOR, are evolutionarily conserved, comprising of six functional domains (Sabatini et al., 1994; Sabers et al., 1995; Abraham, 1998). The domains comprise of (1) HEAT (Huntingtin elongation factor 3, a subunit of protein phosphatase 2A and TOR1) domain, which mediates protein-protein interactions; (2) FAT (FRAP-ATM-TRAPP) domain; (3) FRB (FKBP12-rapamycin binding) domain, which mediates the inhibitory action of rapamycin on Raptor-bound mTOR; (4) PIKK (PI3-kinase-related kinase) domain, serine phosphorylation sites (S2035 and S2481); (5) RD (Repressor domain); and (6) the carboxy-terminal FATC domain (Kirken and Wang, 2003; Asnaghi et al., 2004).

The mTOR kinase plays a crucial role in regulating cell growth, cell proliferation, cell survival, protein synthesis and autophagy. It regulates and controls the transcription of ribosomal proteins and the synthesis of rRNA and tRNA (Hardwick et al., 1999; Powers and Walter, 1999). In general, the activity of mTOR is regulated by insulin and other growth factors via the phosphatidylinositol 3-kinase (PI3K)–Akt pathway (Kadowaki and Kanazawa, 2003).

In eukaryotic cells, mTOR exists as two different complexes: mTORC1; a rapamycin-sensitive complex defined by its interaction with Raptor (regulatory-associated protein of mTOR) and mTORC2; a rapamycin-insensitive complex defined by its interaction with Rictor (rapamycin-insensitive companion of mTOR) (Bharti and Aggarwal, 2002; Loewith et al., 2002; Sarbassov et al., 2004). Raptor is the first protein shown to bind directly to mTOR that is required to mediate mTOR regulation of p70 ribosomal S6 kinase (p70S6K) and the binding protein of eukaryotic translation initiation factor 4E (4E-BP1) activities (Bharti and Aggarwal, 2002; Kim et al., 2002a). On the other hand, PRAS40 and Deptor are identified as

*[1]Malaysian Institute of Pharmaceuticals and Nutraceuticals, Ministry of Science, Technology and Innovation (MOSTI), Halaman Bukit Gambir, [2]Advanced Medical and Dental Institute, Universiti Sains Malaysia, Pulau Pinang, Malaysia *For correspondence: tanml@usm.my, drtanmelan@yahoo.com*

distinct negative regulators of mTORC1 (Sancak et al., 2007; Peterson et al., 2009).

In the rapamycin-sensitive mTOR signalling pathway, rapamycin binds to FK506-binding protein of 12 kDa (FKBP12), and subsequently, the complex binds to the FRB domain of mTORC1. This weakens the interaction between mTOR and Raptor and subsequently inhibits the mTORC1 functions (Kirken and Wang, 2003; Guertin et al., 2004; Hay and Sonenberg, 2004). However, the mechanisms on how rapamycin and several rapamycin derivatives bind to FKBP12 to inhibit mTORC1 signalling remain poorly defined (Dowling et al., 2010). Starvation or lack of nutrients such as amino acids and/or glucose appears to mimic rapamycin treatment which causes rapid inactivation of p70S6K and hypophosphorylation of the 4E-BP1 (Proud, 2002).

The activity of mTOR is regulated by various growth factors such as insulin, insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and platelet-derived growth factor (PDGF) (Gomez-Pinillos and Ferrari, 2012). Growth factor-induced activation of mTOR is mediated by Class I PI3K which has the unique ability to generate oncogenic phosphatidylinositol-3,4,5-triphosphate (PIP3). Class II and Class III PI3Ks lack this ability and therefore have not been linked to cancer (Vogt et al., 2010). Class I PI3Ks are further divided into Class IA PI3Ks and Class IB PI3K. Class IA PI3Ks are heterodimers consisting of a p85 regulatory subunit that associates with p110α, β or δ catalytic subunit and are involved primarily in the pathogenesis of human cancer (Rodon et al., 2013).

Following growth factor binding to its cognate receptor tyrosine kinase (RTK), Class IA PI3Ks are recruited to the cell membrane by direct interaction of the p85 subunit with the activated receptors or by interaction with adaptor proteins associated with the receptors. Binding removes the inhibitory effect of p85 on p110, resulting in activation of p110 catalytic subunit. The activated p110 subunit catalyses the phosphorylation of phosphatidylinositol-4,5-bisphosphate (PIP2) to PIP3 at the membrane. PIP3 is an important second messenger in the cell and is the predominant mediator of PI3K activity. PIP3 acts as docking sites for signalling proteins that have pleckstrin homology (PH) domain, including Akt and 3-phosphoinositide-dependent kinase 1 (PDK1) (Vogt et al., 2010; Baselga, 2011). Figure 1 illustrates the mTOR signalling pathway in general.

The serine/threonine protein kinase Akt, also known as protein kinase B (PKB), a downstream effector of PI3K, is a critical mediator of mTOR activity (Hay and Sonenberg, 2004). Akt activation is initiated by translocation to the plasma membrane, which is mediated by docking of Akt to PIP3 on the membrane. Akt is then phosphorylated on Thr308 by PDK1 and on Ser473 by putative PDK2. A number of potential PDK2s have been identified, including integrin-linked kinase (ILK), protein kinase C β2, DNA-dependent protein kinase (DNA-PK), ataxia telangiectasia mutated (ATM), Akt itself and mTORC2. Both phosphorylation events are required for full activation of Akt. Once Akt has been phosphorylated and activated, it phosphorylates many other proteins, thereby regulating a wide range of cellular processes involved in protein synthesis, cell survival, proliferation and metabolism. Akt activates mTOR either by direct phosphorylation of mTOR at Ser2448 (Nave et al., 1999) or by indirect phosphorylation and inhibition of tuberous sclerosis complex 2 (TSC2) (Inoki et al., 2002). Akt phosphorylation of TSC2 represses GTPase-activating protein (GAP) activity, thereby allowing GTP-bound

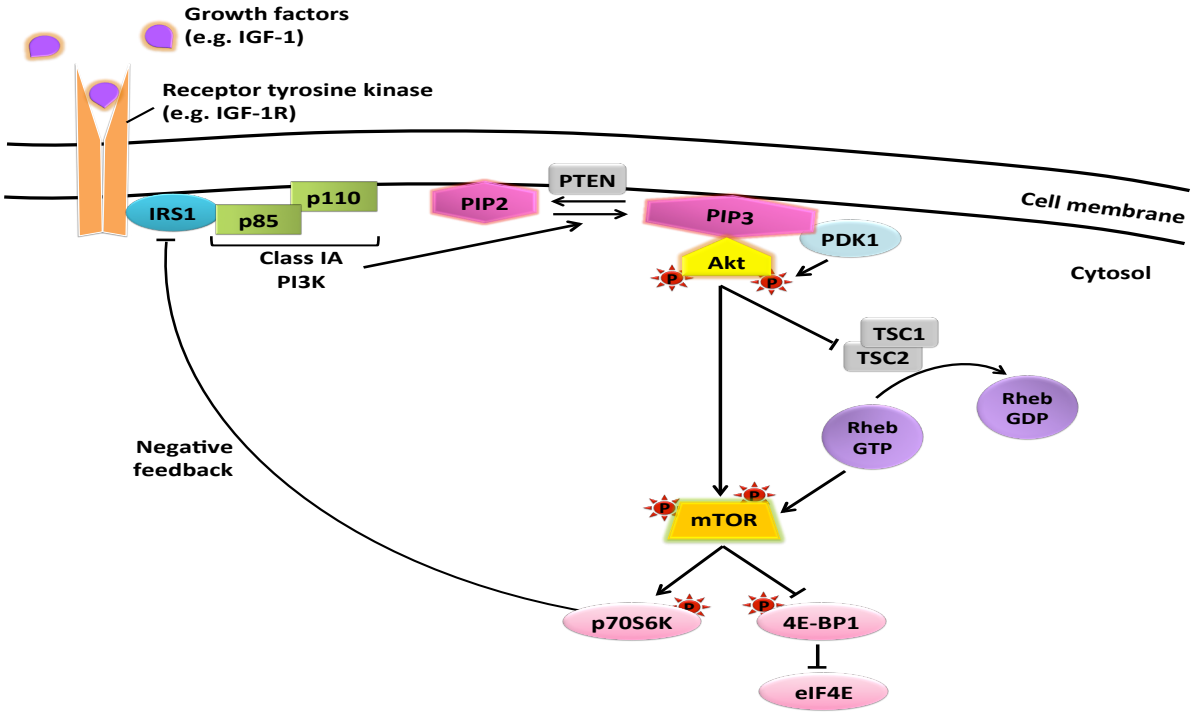

Figure 1. The mTOR Signalling Pathway and Regulatory Feedback Loop

active Ras homolog enriched in brain (Rheb) to activate mTOR (Plas and Thompson, 2005). Phosphorylation of mTOR at Ser2481 (an autophosphorylation site) correlates to the activation of mTOR catalytic activity (Caron et al., 2010; Soliman et al., 2010).

When conditions are favourable for cell growth, activated mTORC1 phosphorylates several substrates to promote anabolic processes (such as ribosome biogenesis, translation and the synthesis of lipids and nucleotides) and suppress catabolic processes (such as autophagy) (Fruman and Rommel, 2014). The mTORC1 regulates protein synthesis through the phosphorylation and inactivation of the repressor of mRNA translation, 4E-BP1 and through the phosphorylation and activation of p70S6K. Phosphorylation of 4E-BP1 releases eukaryotic translation initiation factor 4E (eIF4E), allowing it to interact with eIF4G to initiate cap-dependent translation. Activated p70S6K regulates cell growth via increased translation of 5'TOP (terminal oligopyrimidine tract) mRNAs, which encode components of the translation machinery, such as ribosomal proteins and elongation factors. Through the phosphorylation of several other effectors, mTORC1 promotes lipid biogenesis and metabolism, and suppresses autophagy (Hay and Sonenberg, 2004; Gomez-Pinillos and Ferrari, 2012; Laplante and Sabatini, 2013). In contrast, mTORC2 does not have direct role in regulating protein translation. However, mTORC2 is found to phosphorylate serum and glucocorticoid-regulated kinase 1 (SGK1), protein kinase C (PKC), and also Akt at Ser473, which in turn regulates cell cycle progression, cell survival, metabolism and cytoskeletal organization (Gomez-Pinillos and Ferrari, 2012; Laplante and Sabatini, 2012).

The tumour suppressor phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is the most important negative regulator of the PI3K signalling pathway. PTEN is a phosphatidylinositol-3 phosphatase that antagonizes PI3K activity by dephosphorylating PIP3 that is generated by PI3K (Abdulkareem and Blair, 2013). Loss of PTEN results in an unrestrained signalling of the PI3K pathway, leading to the formation of cancer. It is also associated with many types of cancers, including breast cancer (Vivanco and Sawyers, 2002; Sansal and Sellers, 2004). Another important protein involved in the regulation of mTORC1 activity is the tuberous sclerosis complex (TSC), which is a heterodimer of two proteins, TSC1 (also known as hamartin) and TSC2 (also known as tuberin) (Hay and Sonenberg, 2004). TSC1 and TSC2 functions as a GAP that negatively regulates a small GTPase called Rheb, transforming Rheb into its inactive GDP-bound state which subsequently unable to activate mTOR (Hay and Sonenberg, 2004). Finally, regulatory feedback loop exists as an intrinsic mechanism of self-control to refrain further activation of mTOR pathway. Following mTOR phosphorylation, activated p70S6K phosphorylates and destabilizes insulin receptor substrate 1 (IRS1), thereby inhibiting PI3K activation and blocking upstream overstimulation of the PI3K/Akt/mTOR cascade (Gomez-Pinillos and Ferrari, 2012; Shimobayashi and Hall, 2014) (Figure 1).

One of most studied and important pathways involved in the regulation of autophagy is the PI3K/Akt/mTOR signalling pathway. Inhibition of mTOR by nutrient-depletion, starvation or rapamycin leads to the induction of autophagy. Increased levels of the mTOR kinase are found to inhibit the autophagy process, resulting in excessive cell growth and tumor development. Studies have shown that mTORC1 controls autophagy through the regulation of a protein complex composed of ULK1 (unc-51-like kinases), mAtg13 and FIP200 (Ganley et al., 2009; Hosokawa et al., 2009; Jung et al., 2009). ULK kinase complex is directly controlled by mTOR, of which maintains the hyperphosphorylation state of mAtg13 and suppresses the induction of autophagy (Galluzzi et al., 2008). Inhibition of mTOR by rapamycin leads to dephosphorylation of ULK1, ULK2, and mAtg13 and activates ULK to phosphorylate FIP200, which suggests that ULK-Atg13-FIP200 complexes are direct targets of mTOR and important regulators of autophagy in response to mTOR signalling (Jung et al., 2009).

In contrast to mTORC1, relatively little is known regarding the regulatory pathway of mTORC2. The mTOR-Rictor complex, unlike mTOR-Raptor, does not bind to FRB domain and is insensitive to rapamycin treatment (Loewith et al., 2002; Sarbassov et al., 2004). The mTORC2 complex promotes cell signalling through phosphorylation and activation of the pro-survival and pro-proliferative kinase Akt, which positively regulates cell survival, proliferation and metabolism (Sarbassov et al., 2006; Manning and Cantley, 2007). The molecular mechanism by which mTORC2 regulates cytoskeletal organization has not been clearly defined, although many different studies have reported that knocking down mTORC2 components affects actin polymerization and disrupts cell morphology (Jacinto et al., 2004; Sarbassov et al., 2004). In another study, depletion of mTOR and Rictor, but not Raptor, impairs actin polymerization in neutrophils stimulated with chemoattractants and that small Rho GTPases Rac and Cdc42 serve as downstream effectors of Rictor to regulate actin assembly and organization in neutrophils (He et al., 2013).

The mTOR Signalling Pathway and Cancer

The mTOR pathway is a key regulator of cell proliferation and several upstream activators and downstream effectors of mTOR are known to be deregulated in some cancers such as renal cell carcinoma, non-small cell lung cancer, breast cancer, sarcomas, colorectal and gastrointestinal tumors (Law, 2005; Tokunaga et al., 2008; Li et al., 2013; Takahashi et al., 2014; Wang and Zhang, 2014). The mTOR signalling is constitutively activated in many tumor types, suggesting that mTOR is an attractive target for cancer drug development and therapy (Yu et al., 2001; Chan, 2004; Shor et al., 2009; Han et al., 2013; Pandurangan, 2013). The mTOR signalling network consists of a number of tumor suppressor genes and proto-oncogenes, thereby explains that aberrant activities of these genes will promote the formation of cancerous cells.

The signalling network defined by PI3K, Akt and mTOR controls most hallmarks of cancer, including cell cycle, survival, metabolism, motility and genomic

instability. Cancer genetic studies suggest that the PI3K pathway is the most frequently altered pathway in human tumours, where the PIK3CA gene (which encodes the PI3K p110α catalytic isoform) is the second most frequently mutated oncogene, and PTEN is among the most frequently mutated tumour suppressor genes (Fruman and Rommel, 2014). Therefore, PI3K pathway is probably one of the most important pathways in cancer metabolism and growth, and has been identified as an important target in breast cancer research (Baselga, 2011).

The p110α and p110β isoforms of Class I PI3Ks are expressed in almost all tissues and cell types, both of which play important roles in regulating cell growth and metabolism (Vogt et al., 2010). The p110α isoform is the most important subunit in PI3K as it is important for the growth and maintenance of numerous tumours that feature PI3K activation. Ablation of p110α resulted in substantially reduced Akt phosphorylation in response to stimulation by various growth factors (Zhao et al., 2006; Pal and Mandal, 2012). Of the four Class I PI3K catalytic isoforms, only PIK3CA (encoding p110α) is frequently mutated in human cancer. Mutations in Class I PI3K regulatory subunit genes are also found in cancer cells and cause increased PI3K activity (Fruman and Rommel, 2014). PIK3CA and PIK3R1 (which encodes p85 regulatory subunit) are mutated at frequencies ranging from 5%-25% in several common cancers, including cancers of the breast, endometrium and large intestine (Vogt et al., 2010). Overall, 20%-25% of breast tumors exhibit PIK3CA mutation (Baselga, 2011). PIK3CA mutation has been shown to increase PIP3 level, activate Akt signalling and promote oncogenic transformation (Baselga, 2011).

Akt is frequently and constitutively active in many types of human cancer. Constitutive Akt activation can occur as a result of amplification of Akt genes or due to mutations in components of the signalling pathway that activate Akt. Constitutive Akt signalling is believed to promote proliferation and increase cell survival, thereby contributing to cancer progression (Nicholson and Anderson, 2002). Amplification of Akt1, Akt2 and Akt3 has been reported in breast, ovarian, pancreatic and gastric cancers (Rodon et al., 2013). Activating mutation in Akt1, which results in growth factor-independent membrane translocation of Akt and increased Akt phosphorylation, was identified in breast, melanoma, colorectal and ovarian cancers. Phosphorylation of Akt at Ser473 has been associated with poor prognosis in human cancers, including breast cancer (LoPiccolo et al., 2008). Transgenic mice generated by expressing myristoylated-Akt1 (myr-Akt1) under the control of the MMTV-LTR promoter revealed that expression of myr-Akt1 in mammary glands alone did not increase the frequency of tumor formation. However, there was an increased susceptibility of forming mammary tumors induced by DMBA in the transgenic mice, especially in post-lactation mice, indicating that Akt1 accelerates carcinogen-induced tumorigenesis (Wu et al., 2014). Interestingly, although mutations in PDK1 are rarely found in human cancer, amplification or overexpression of PDK1 was found in ~20% of breast cancers (Liu et al., 2009a).

Aberrant activation of mTOR has been implicated in certain cancers. Activation of mTOR provides tumour cells with a growth advantage by promoting protein synthesis and contributes to the genesis of cancer through its effect on cell cycle progression (Fingar et al., 2004). The effects of mTOR on cell cycle progression is mediated, at least in part, by the increased translation of positive regulators of cell cycle progression, such as cyclin D1 and Myc, and by decreased translation of negative regulators thereof, such as p27kip1 (Gera et al., 2004; Hay and Sonenberg, 2004). On the other hand, tumor suppressor PTEN is frequently mutated in advanced stages of human cancers, particularly glioblastoma, endometrial and prostate cancers. Germline mutations in the PTEN gene give rise to Cowden's disease, which is associated with an increased risk of developing breast cancer and other cancers (Nicholson and Anderson, 2002). Somatic loss of PTEN by gene mutation or deletion frequently occurs in human cancers. PTEN is deleted or mutated in approximately 45% of uterine endometrial cancers, 30% of glioblastomas and spinal tumors, and less commonly in cancers of the prostate, bladder, adrenal glands, thyroid, breast, skin (melanomas) and colon (Abdulkareem and Blair, 2013).

Clinical Development of PI3K/Akt/mTOR (PAM) Inhibitors

Since mTOR signalling pathway is one of the most commonly activated signalling networks in human cancers and that kinases are amenable to pharmacological intervention, many pharmaceutical companies and academic laboratories are actively developing inhibitors that target key components in the pathway (Moschetta et al., 2014). Many of the agents developed and evaluated in early stage clinical trials have been shown to be safe, well tolerated and effective in multiple tumor types. Current PAM inhibitors in early development include reversible ATP-competitive inhibitors of the four p110 isoforms of Class I PI3K (also known as pan-PI3K inhibitors), the irreversible pan-PI3K inhibitors, p110 isoform-specific inhibitors, dual pan-PI3K-mTOR inhibitors, Akt inhibitors and mTOR inhibitors (Rodon et al., 2013; Porta et al., 2014).

Wortmannin and LY294002 are two well known, first generation pan-PI3K inhibitors. Wortmannin and LY294002 are effective inhibitors of PI3K and have shown anti-proliferative and apoptotic effects *in vitro* and *in vivo*. However, the use of these two compounds is limited to the preclinical level due to their instability in aqueous solutions, toxic side effects, poor pharmaceutical properties and lack of selectivity for individual PI3K p110 isoforms (Pal and Mandal, 2012). Isoform-specific inhibitors are of particular interest because agents that target single isoform may produce fewer side effects and less toxicity to the immune system due to the fact that p110α and p110β play important roles in multiple cellular processes while p110γ and δ isoforms are important in the immune system. Some inhibitors of Akt are being tested clinically, although the development of Akt-specific and isozyme-selective inhibitors was predicted to be difficult due to high degree of homology in the ATP binding pocket

between Akt, protein kinase A (PKA) and PKC (Rodon et al., 2013).

Rapamycin, also known as sirolimus, is a prototypical mTOR inhibitor. It is an antibiotic macrolide derived from bacterium *Streptomyces hygroscopius*, and first isolated in 1975 (Sehgal et al., 1975; Vezina et al., 1975). Rapamycin was first developed as immunosuppressant by Wyeth pharmaceutical company in 1997 and more recently presented as anti-cancer agents in the form of various analogues (Liu et al., 2009b). Rapamycin binds to its intracellular receptor FKBP12, and subsequently attaches to the mTORC1 and suppresses mTOR-mediated phosphorylation of p70S6K and 4E-BP1. Rapamycin has been precluded from clinical development due to its poor aqueous solubility and chemical instability (Hidalgo and Rowinsky, 2000; Mita et al., 2003). Rapamycin analogues (also known as rapalogues) inhibit mTOR through the same mechanism as rapamycin, but have better pharmacological properties for clinical use in cancer. In general, the therapeutic effects of rapamycin analogues are similar to rapamycin (Tsang et al., 2007). Rapamycin analogues with improved stability and pharmacological properties have been significantly tolerated by patients in Phase I trials, and the agents have shown promising antitumor effect in many types of cancers including breast cancer (Noh et al., 2004).

Temsirolimus (CCI-779) and everolimus (RAD001) are two rapamycin analogues that have been developed as anti-cancer drugs (Hasskarl, 2014). Temsirolimus is the first mTOR inhibitor approved by FDA, USA for the treatment of advanced renal cell carcinoma in 2007. This is followed by the approval of everolimus for the treatment of adults with advanced and recurrent renal cell carcinoma (2009); adults with progressive neuroendocrine tumors of pancreatic origin (2011); adults with tuberous sclerosis complex (TSC) who have renal angiomyolipomas not requiring immediate surgery (2012); children with TSC who have a rare brain tumor called subependymal giant cell astrocytoma (2012); and for use in combination with exemestane to treat certain postmenopausal women with advanced hormone receptor positive, HER2-negative breast cancer (2012) (Hasskarl, 2014).

Nevertheless, rapalogues are not broadly effective as single agents, although they have been approved for the treatment of a few tumour types for which modest therapeutic effects can be achieved (Fruman and Rommel, 2014). Preclinical studies demonstrated that Akt activation was triggered after blockade of mTORC1 by rapamycin and rapalogues (Sun et al., 2005; O'Reilly et al., 2006; Wan et al., 2007). Clinically, upon mTOR blockade with everolimus, Akt phosphorylation was upregulated in 50% of the treated tumors (Tabernero et al., 2008). The increased Akt activity can ultimately enhance tumour growth. This limited anti-tumour activity of mTOR inhibitors is suspected to be related to the fact that these agents only inhibit the mTORC1 complex. The blockade of mTOR and the resulting inhibition of p70S6K relieves regulatory feedback loop, which results in IGF-1R-mediated feedback activation of Akt (Baselga, 2011; Rodon et al., 2013). Therefore, agents targeting both mTORC1 and mTORC2, and dual pan-class I PI3K-mTOR inhibitors are being developed (Rodon et al., 2013). In addition, preclinical models have shown that combining mTOR inhibitors and IGF-1R antibodies/inhibitors result in blockage of mTOR inhibitor-induced Akt activation (Wan et al., 2007), and this combination is currently being explored in clinical trials (Chen and Sharon, 2013). In the pre-clinical and clinical studies, the inhibitors targeting the different members of mTOR pathway have been used alone or in combination with other targeted agents for the treatment of breast cancer (Ghayad and Cohen, 2010).

Although the mTOR-targeting therapy was based on the premise that an essential PI3K effector Akt activates the rapamycin-sensitive mTORC1 pathway, new data suggests that rapamycin-insensitive mTORC2 phosphorylates Akt on a key activation site, providing some knowledge that the relationship between mTOR and PI3K signalling is complex (Guertin and Sabatini, 2009). Inhibitors that target both mTORC1 and mTORC2 would be expected to block activation of the PI3K pathway more effectively than rapamycin and its analogues (Liu et al., 2009b). Current evidences from the analyses of some solid tumors also suggests that dual PI3K/mTOR inhibitors, which bind to and inactivate both PI3K and mTOR, may achieve better outcomes among resistant cancers (Tang and Ling, 2014). Currently, OSI-027 (OSI Pharmaceuticals, USA), AZD8055 (Astra Zeneca, UK), and INK128 (Intellikine, USA) are the first three ATP-competitive mTOR inhibitors to enter clinical trials in patients with advanced solid tumors and lymphoma (Liu et al., 2009a; Garcia-Echeverria, 2010; Houghton, 2010). OSI-027 is the first orally bioavailable small-molecule mTORC1/mTORC2 inhibitor, a semi-synthetic compound with the ability of eliciting both tumor cell apoptosis and autophagy and halting tumor cell proliferation (Yap et al., 2008; Vakana et al., 2010).

Natural Phytochemicals as mTOR Inhibitors

Numerous important anticancer drugs in the market are either obtained from natural sources, by structural modification of natural compounds, or by synthesis of new compounds using natural compound as lead (Cragg et al., 1997; da Rocha et al., 2001). Therefore, sourcing out new drugs and the continuous interest in using natural compounds for cancer therapy is a global effort. Numerous preclinical investigations have shown that some herbs and natural phytochemicals, such as curcumin, resveratrol, timosaponin III, gallic acid, diosgenin, pomegranate, epigallocatechin gallate (EGCC), genistein, and 3,3'-diindolylmethane inhibit mTOR pathway either directly or indirectly (Table 1). Some of them are undergoing clinical trials as chemotherapeutic agents, chemopreventive compounds and/or combination therapy to improve the efficacy of the standard chemotherapy. These natural phytochemicals with mTOR inhibitory activities have great potential in cancer prevention. This is in view that higher consumption of fruits and vegetables was associated with lower risk of cancer (Gullett et al., 2010).

Curcumin, a polyphenol natural compound extracted from the plant *Curcuma longa* L., is commonly used

as spice in India and Southeast Asia. It is used as food additive and traditional Indian medicine for the treatment of various diseases such as biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis (Shishodia et al., 2007). Curcumin has shown exceptional chemopreventive and anti-tumor activities in some pre-clinical studies. In HCT116 colorectal cancer cells, curcumin downregulates protein and mRNA expression of mTOR, Raptor and Rictor, suggesting that curcumin exerts its anti-proliferative effects by inhibiting the mTOR signalling pathway and thus may represent a novel class of mTOR inhibitor (Johnson et al., 2009). In human Rh1 and Rh30 rhabdomyosarcoma cells, DU145 prostate cancer cells, MCF-7 breast cancer cells and Hela cervical cancer cells, curcumin rapidly inhibits the phosphorylation of mTOR and its downstream effector molecules such as p70S6K and 4E-BP1, indicating that curcumin may execute its anticancer activity primarily by blocking mTOR-mediated signalling pathways in these tumor cells (Beevers et al., 2006). Furthermore, curcumin induces apoptosis, inhibits cell growth and inhibits the basal or type I insulin-like growth factor-induced motility of the Rh1 and Rh30 cells (Beevers et al., 2006). Curcumin is found to dissociate Raptor, at low concentration, and Rictor, at high concentration, from mTOR complex. However, it is unclear if curcumin disrupts the mTOR complex by direct binding to mTOR or to a component of the mTOR complexes (Beevers et al., 2009). In human PC3 prostate cancer cells, curcumin suppresses murine double minute 2 (MDM2) oncogene expression through the erythroblastosis virus transcription factor 2 (EST2) by modulating PI3K/mTOR/ETS2 signalling pathway (Li et al., 2007a). In both human U87-MG and U373-MG malignant glioma cells, curcumin inhibits the Akt/mTOR/p70S6K pathway and activates the extracellular signal-regulated kinase (ERK) pathway, resulting in the induction of autophagy. On the other hand, activation of Akt pathway by recombinant full-length human active Akt1 protein (rAkt1) inhibited curcumin-induced autophagy and decreased curcumin-inhibited phosphorylation of Akt and p70S6K, suggesting that curcumin-induced inactivation of Akt/mTOR/p70S6K pathway plays a role in induction of autophagy (Aoki et al., 2007). As combined treatment, curcumin and dual PI3K/Akt and mTOR inhibitor induce apoptosis through p53-dependent Bcl-2 mRNA down-regulation at the transcriptional level and Mcl-1 protein down-regulation at the post-transcriptional level in human renal carcinoma Caki cells (Seo et al., 2014).

The promising effect of curcumin at the preclinical phases has led to the initiation of several clinical trials. In Phase I clinical studies, it has been shown that curcumin is not toxic to human; and in Phase II clinical trial, curcumin is well tolerated and produces some biological activity in patients with advanced pancreatic cancer (Cheng et al., 2001; Sharma et al., 2001; Lao et al., 2006; Dhillon et al., 2008). Curcumin taken orally for 3 months produces histologic improvement of precancerous lesions in 1 out of 2 patients with recently resected bladder cancer, 2 out of 7 patients of oral leucoplakia, 1 out of 6 patients of intestinal metaplasia of the stomach, 1 out of 4 patients with uterine cervical intraepithelial neoplasm (CIN) and 2 out of 6 patients with Bowen's disease (Cheng et al., 2001). Radiologically stable colorectal cancer was demonstrated in 5 out of 15 patients after 2-4 months of treatment with curcuma extract at doses between 440 and 2200 mg/day, containing 36-180 mg of curcumin (Sharma et al., 2001). In a Phase II, nonrandomized, open-label clinical trial in 44 eligible smokers with eight or more aberrant crypt foci (ACF) on screening colonoscopy, a significant 40% reduction in ACF number occurred with the 4-g dose of curcumin for 30 days. The ACF reduction in the 4-g group was associated with a significant, five-fold increase in post-treatment plasma curcumin/conjugate levels (Carroll et al., 2011). A Phase I/II study of gemcitabine-based chemotherapy plus curcumin for patients with gemcitabine-resistant pancreatic cancer reported that 8 g oral curcumin daily with gemcitabine-based chemotherapy was safe and feasible in patients with pancreatic cancer (Kanai et al., 2011). However, all these are short term studies and the unremarkable response rates were not surprising and it certainly warrants longer trials.

Interestingly, a randomized, double-blind, placebo-controlled clinical trial of 30 breast cancer patients revealed that oral curcumin, 6.0 g daily during radiotherapy, reduced the severity of radiation dermatitis in breast cancer patients (Ryan et al., 2013). Curcumin in improved formulations have also proven to be safe and acceptable among patients in pilot studies (Irving et al., 2013; Kanai et al., 2013). Other ongoing clinical trials include Phase II combination therapy with standard radiation therapy and chemotherapy (capecitabine) in rectal cancer, Phase II trial to prevent colon cancer in smokers with aberrant crypt foci, Phase II trial in patients with pancreatic cancer, Phase II trial in patients with colorectal cancer, Phase I trial in patients with advanced cancer as well as Phase I trial to prevent colorectal cancer in patients undergoing colorectal endoscopy or colorectal surgery (Table 1).

Resveratrol is a polyphenolic compound present in grapes and red wine with potential anti-inflammatory and anticancer properties (Pervaiz, 2003; Marques et al., 2009). It is used in traditional Chinese and Japanese medicine to treat dermatitis, gonorrhea, athlete's foot and hyperlipemia (Aggarwal et al., 2004). In human LNCaP prostate carcinoma cells, resveratrol inhibits PI3K/Akt signalling pathway and induces apoptosis (Aziz et al., 2006). Resveratol is also shown to down-regulate the PI3K/Akt/mTOR signalling pathway, and combination with rapamycin further enhances the resveratrol-induced cell death in human U251 glioma cells (Jiang et al., 2009). In smooth muscle cells (SMC), resveratrol blocks the oxidized LDL (oxLDL)-induced activation of the mTOR pathway via PI3K/PDK1/Akt, thereby inhibiting oxLDL-induced SMC proliferation (Brito et al., 2009). In MDA-MB-231 and MCF-7 human breast cancer cells, resveratrol decreases mTOR and p70S6K phosphorylation, and in combination with rapamycin, suppresses the phosphorylation of Akt. An additive effect of resveratrol and rapamycin combination suggests some therapeutic value in breast cancer (He et al., 2010). In both estrogen receptor (ER)-positive and ER-negative breast cancer cells, resveratrol activates AMP-activated kinase (AMPK) and subsequently downregulates mTOR, 4E-BP1

Table 1. The List Of Natural Compounds And Clinical Trial Phases

No	Natural compounds	Target	Natural Source	Clinical trial phase	Reference	Status
1	Curcumin	Akt and mTOR	*Curcuma longa* L.	In Phase I-II for pancreatic cancer[1], colorectal cancer[2], colon cancer[3], rectal cancer[4], advanced cancer[5]. In clinical trial for familial adenomatous polyposis[6]	1. http://clinicaltrials.gov/show/NCT00094445 2. http://clinicaltrials.gov/show/NCT00118989 3. http://clinicaltrials.gov/show/NCT00365209 4. http://clinicaltrials.gov/show/NCT00745134 5. http://clinicaltrials.gov/show/NCT01201694 6. http://clinicaltrials.gov/show/NCT00641147	1-5: ongoing, but not recruiting participants 6: currently recruiting participants
2	Resveratrol	PI3K, Akt and mTOR	Grapes and red wine	In clinical trial for neuroendocrine tumor[7]	7. http://clinicaltrials.gov/show/NCT01476592	7: ongoing, but not recruiting participants
3	Pomegranate	PI3K, Akt and mTOR	*Punica granatum* L.	In Phase II for prostate cancer[8,9,10]	8. http://clinicaltrials.gov/show/NCT00060086 9. http://clinicaltrials.gov/show/NCT00731848 10. http://clinicaltrials.gov/show/NCT00732043	8-10: ongoing, but not recruiting participants
4	Genistein	Akt and mTOR	*Glycine max* (L.) Merr. and several plants	In Phase I-II for prostate cancer[11,12], breast cancer[13, 16], pancreatic cancer[14] bladder cancer[15], endometrial cancer[16]	11. http://clinicaltrials.gov/show/NCT00499408 12. http://clinicaltrials.gov/show/NCT01126879 13. http://clinicaltrials.gov/show/NCT00244933 14. http://clinicaltrials.gov/show/NCT00376948 15. http://clinicaltrials.gov/show/NCT00118040 16. http://clinicaltrials.gov/show/NCT00099008	11, 13-16: completed 12: currently recruiting participants
5	3, 3-diindolylmethane	PI3K, Akt and mTOR	Cruciferous vegetables	In Phase II-III for breast cancer[17] and prostate cancer[18]	17. http://clinicaltrials.gov/show/NCT01391689 18. http://clinicaltrials.gov/show/NCT00888654	17-18: currently recruiting participants
6	Green tea extract or polyphenon E	Akt and mTOR	Green tea	In Phase I-II for breast cancer[19,20], leukemia[21], monoclonal gammopathy of undetermined significance and/or smoldering multiple myeloma[22], prostatic hyperplasia[23], premalignant lesions of the head and neck[24]	19. http://clinicaltrials.gov/show/NCT00516243 20. http://clinicaltrials.gov/show/NCT00676793 21. http://clinicaltrials.gov/show/NCT00262743 22. http://clinicaltrials.gov/show/NCT00942422 23. http://clinicaltrials.gov/show/NCT00596011 24. http://clinicaltrials.gov/show/NCT01116336	19,20,22,23: ongoing, but not recruiting participants 21: completed 24: currently recruiting participants
7	Epigallocatechin gallate (EGCG)	Akt and mTOR	Green tea	In preclinical study for human hepatoma cells and keloid fibroblast	Huang et al., 2009; Zhang et al., 2006	
8	Timosaponin AIII	Akt and mTOR	Anemarrhena asphodeloides Bunge	In pre-clinical study for BT-549 and MDAM231 breast cancer cells	King et al., 2009	
9	Gallic acid	Akt and mTOR	*Phaleria macrocarpa* (Scheff.) Boerl.	In pre-clinical study for TE-2 esophageal cancer cells	Faried et al., 2007	
10	Diosgenin	Akt and mTOR	*Dioscorea* spp.	In pre-clinical study for AU565 breast adenocarcinoma cells	Chiang et al., 2007	

and mRNA translation (Lin et al., 2010).

Resveratrol has undergone numerous clinical investigations for its putative cancer chemopreventive properties. A pilot study of SRT501, a micronized resveratrol preparation, given as 5.0 g daily for 14 days, to patients with colorectal cancer and hepatic metastases scheduled to undergo hepatectomy, revealed a marked increase of cleaved caspase-3, a marker of apoptosis, in malignant hepatic tissue compared with tissue from the placebo-treated patients (Howells et al., 2011). In healthy volunteers, the ingestion of resveratrol caused a significant decrease in circulating IGF-1 and IGFBP-3 in all volunteers, suggesting chemopreventive activities (Brown et al., 2010). In another study with healthy volunteers, daily intake of 1 g of resveratrol for 4 weeks revealed an induction of GST-pi level and UGT1A1 activity in individuals with low baseline enzyme level/activity, indicating that resveratrol can modulate enzyme systems involved in carcinogen activation and detoxification, suggesting a possible mechanism by which resveratrol inhibits carcinogenesis (Chow et al., 2010).

Unfortunately, a Phase II study of SRT501 (resveratrol) with bortezomib in patients with relapsed and/or refractory multiple myeloma has to be terminated recently (Popat et al., 2013). Out of 24 patients, 9 patients receiving SRT501 and bortezomib were withdrawn from the study, mainly due to serious adverse reactions. The predominant study finding was an unexpected renal toxicity and low efficacy of SRT501 with nausea and vomiting which could have

resulted in disease progression and dehydration. This study has demonstrated an unacceptable safety profile and minimal efficacy in patients with relapsed/refractory multiple myeloma (Popat et al., 2013). At least two more clinical trials on colorectal cancer were completed but no published data was noted on the outcome. Currently an intervention study to examine the effects of resveratrol on neuroendocrine tumor is ongoing (Table 1).

Pomegranate, an ancient and mystical fruit of the tree *Punica granatum* L., has been used for centuries for the treatment of inflammatory diseases and disorders of the digestive tract (Faria and Calhau, 2010). In A/J mice, pomegranate fruit extract decreases carcinogen-induced lung tumorigenesis. Analysis of the murine lung tissue sample showed that pomegranate fruit extract down-regulates mTOR signalling by inhibiting the phosphorylation of PI3K, Akt and mTOR, and downstream molecules such as p70S6K and 4E-BP1 (Khan et al., 2007a). Other anti-carcinogenic effects of pomegranate fruit in numerous animal and cell culture models are well demonstrated in various studies (Kim et al., 2002b; Malik et al., 2005; Khan et al., 2007b).

In a Phase II clinical trial for men with rising PSA (prostate serum antigen) after surgery or radiotherapy for localized prostate cancer, patients were treated with 8 ounces of pomegranate juice daily (Pantuck et al., 2006). This study shows statistically significant prolongation on PSA doubling time over a period of 13 months. However, it was uncertain if improvements in biomarker like PSA doubling time are likely to serve as surrogate for clinical benefit. In a randomized Phase II study of pomegranate extract for men with rising PSA following initial therapy for localized prostate cancer, pomegranate extract treatment was associated with more than 6 months increase in PSA doubling time without adverse effects. Unfortunately, the significance of slowing of PSA doubling time remains unclear (Paller et al., 2013). Currently, clinical trials using either pomegranate juice or extract on prostate cancer patients are still ongoing (Table 1).

Genistein, the predominant isoflavone found in soybean (*Glycine max* (L.) Merr.), was found to have potent anti-tumor effects on prostate, brain, breast and colon cancers (Ravindranath et al., 2004; Hwang et al., 2009; Nakamura et al., 2009; Das et al., 2010; Sakamoto et al., 2010). In Hela and CaSki cervical cancer cells, genistein inhibits cell growth by modulating various mitogen-activated protein kinases (MAPK) and inhibiting Akt phosphorylation (Kim et al., 2009). In MCF-7 breast cancer cells, genistein decreases protein expression of total Akt and phosphorylated Akt, suggesting that genistein could offer protection against breast cancer through down-regulation of the PI3K/Akt signalling pathway (Anastasius et al., 2009). Combination of genistein and indol-3-carbinol induces apoptosis and autophagy in HT-29 colon cancer cells by inhibiting Akt and mTOR phosphorylation (Nakamura et al., 2009). In addition, it inhibits Akt kinase activity and abrogates the EGF-induced activation of Akt in PC3 prostate cancer cells (Li and Sarkar, 2002). Genistein is also found to augment the efficacy of cisplatin in pancreatic cancer by down-regulating Akt expression (Banerjee et al., 2007).

The promising anti-cancer effects of genistein has led to Phase II clinical trials involving combination therapy of genistein with gemcitabine hydrochloride in stage IV breast cancer, genistein with gemcitabine and erlotinib in locally advanced or metastatic pancreatic cancer as well as genistein with vitamin D in men with early stage prostate cancer (Table 1). Other clinical trials of genistein include Phase II study in patients who are undergoing surgery for bladder cancer, Phase II study in patients with prostate cancer as well as Phase I study of genistein in preventing breast or endometrial cancer in healthy postmenopausal women (Table 1). A Phase II randomized, placebo-controlled trial was carried out to investigate whether daily, oral genistein (300 or 600 mg/d) as purified soy extract for 14 to 21 days before surgery alters molecular pathways in bladder epithelial tissue in 59 subjects diagnosed with urothelial bladder cancer (Messing et al., 2012). Overall, genistein treatment was well tolerated and the observed toxicities were primarily mild to moderate. A significant reduction in bladder cancer tissue p-EGFR staining was observed in low dose treatment group as compared with placebo. However, there were no significant differences in tumor tissue staining between treatment groups for COX-2, Ki-67, activated caspase-3, Akt, p-Akt and MAPK (Messing et al., 2012).

3,3'-diindolylmethane is a potential anticancer component found in cruciferous vegetables with anti-proliferative and antiandrogenic properties in human prostate cancer cells (Le et al., 2003; Garikapaty et al., 2006). In DU145 human prostate cancer cells, the anti-proliferative effect of 3,3'-diindolylmethane was mediated by downregulation of PI3K, total Akt and phosphorylated Akt (Garikapaty et al., 2006). BR-DIM, a formulated 3,3'-diindolylmethane with higher bioavailability, inhibits phosphorylation of Akt in C4-2B prostate cancer cells (Li et al., 2007b) and inhibits phosphorylation of Akt, mTOR, 4E-BP1 and p70S6K in platelet-derived growth factor-D-overexpressing PC3 prostate cancer cells (Kong et al., 2008). A Phase I dose-escalation study of oral BR-DIM in castrate-resistant, non-metastatic prostate cancer patients revealed that BR-DIM was well tolerated and modest efficacy was demonstrated (Heath et al., 2010). In a pilot study to demonstrate the protective effect of BR-DIM supplements in postmenopausal women with a history of early-stage breast cancer, daily DIM (108 mg DIM/day) supplements for 30 days increased the 2-hydroxylation of estrogen urinary metabolites (Dalessandri et al., 2004). Currently, Phase II/III studies in patients with breast cancer and Phase II study in patients with stage I or stage II prostate cancer undergoing radical prostatectomy are ongoing (Table 1).

EGCG, a polyphenolic compound, is the major catechin found in green tea (Nagle et al., 2006). High consumption of green tea is associated with decreased risk of carcinogenesis and EGCG is a potent antioxidant that may have anticancer properties (Nagle et al., 2006; Katiyar et al., 2007; Pyrko et al., 2007). EGCG induces AMPK in both p53 positive and negative human hepatoma cells, resulting in the suppression of mTOR and 4E-BP1, and a general decrease in mRNA translation (Huang et al., 2009).

In keloid fibroblast, EGCG inhibits the phosphorylation of Akt, p70S6K and 4E-BP1 (Zhang et al., 2006). Further studies are needed to establish the relationship between EGCG and PI3K/Akt/mTOR pathway and to determine whether mTOR mediates the effects of EGCG in treating brain, prostate, cervical and bladder cancers (Hsieh and Wu, 2009; Philips et al., 2009; Qiao et al., 2009; Das et al., 2010). However, many current clinical studies focus on using green tea extract or polyphenon E in a wide range of cancers such as breast cancer, leukemia, multiple myeloma and head and neck lesions (Table 1).

Timosaponin AIII is a steroidal saponin isolated from *Anemarrhena asphodeloides* Bunge (Liliaceae), a traditional Chinese medicine with anti-diabetic, anti-platelet aggregation and diuretic activities (Zhang et al., 1999). Timosaponin AIII has been reported to exhibit cytotoxicity towards HeLa cervical cancer cells and HCT-15 human colorectal cancer cells (Sy et al., 2008; Kang et al., 2011). Timosaponin AIII selectively induces cell death in BT474 and MDAM231 breast carcinoma cells, but not in normal MCF10A immortalized mammary epithelial cells. It exerts its anti-proliferative activity by inhibiting phosphorylation of Akt and mTOR, as well as p70S6K and 4E-BP1 (King et al., 2009). This compound is still in pre-clinical stages and has not progressed into clinical trials.

Gallic acid is a natural antioxidant polyhydroxyphenolic compound found in various plants and fruits (Chu et al., 2002; Sun et al., 2002). Gallic acid is also isolated from *Phaleria macrocarpa* (Scheff.) Boerl, an Indonesian medicinal plant which is used in traditional medicine to control cancer, impotency, hemorrhoids, diabetes mellitus, allergies, liver and heart disease. In preclinical studies, gallic acid induces apoptosis and inhibits cell growth of various cancer cell lines, including human TE-2 esophageal cancer, MKN-28 gastric cancer, HT-29 and Colo201 colon cancer, MCF-7 breast cancer, CaSki cervix cancer and mouse colon-26 colon cancer cells (Faried et al., 2007). It up-regulates the pro-apoptotic Bax protein, induces the caspase-cascade and down-regulates anti-apoptotic protein such as Bcl-2 (Faried et al., 2007). In human TE-2 esophageal cancer cells, gallic acid reduces the phosphorylation of Akt, mTOR and p70S6K, suggesting that the inhibitory effect of gallic acid was mediated by down-regulation of Akt/mTOR pathway (Faried et al., 2007).

Diosgenin is a naturally occurring plant steroid with potential antineoplastic activities as it induces apoptosis in various human cancer cell lines (Moalic et al., 2001; Liu et al., 2005). In human AU565 HER2-overexpressing breast adenocarcinoma cells, diosgenin down-regulates protein levels of fatty acid synthase (FAS), phosphorylated Akt and phosphorylated mTOR, suggesting that diosgenin may suppress FAS expression in AU565 cells through PI3K/Akt/mTOR signal transduction pathway (Chiang et al., 2007). High levels of FAS are associated with poor prognosis in human cancers, and it is highly elevated in HER2-overexpressing breast cancer cells (Kuhajda, 2000; Kumar-Sinha et al., 2003). In another study to determine effect of diosgenin on breast cancer cells, diosgenin is found to inhibit p-Akt expression and Akt kinase activity without affecting PI3 kinase levels. It causes G1 cell cycle arrest by down-regulating cyclin D1, cdk-2 and cdk-4 expression in breast tumor cells, resulting in inhibition of cell proliferation and induction of apoptosis. Interestingly, no significant toxicity was seen in the normal breast epithelial cells (MCF-10A). *In vivo* tumor studies indicate that diosgenin significantly inhibits tumor growth in both MCF-7 and MDA-231 xenografts in nude mice, indicating that it is a potential chemotherapeutic agent (Srinivasan et al., 2009). Diosgenin, timosaponin AIII and gallic acid are still in pre-clinical stages and have not progressed to clinical trials.

Conclusion

Hyperactivation of the PI3K/Akt/mTOR signalling pathway is a prominent hallmark of cancer and is frequently implicated in resistance to anticancer therapies such as biologics, tyrosine kinase inhibitors, radiation, and cytotoxics (Ballou and Lin, 2008). In therapeutic sensitivity restoration, inhibitors of the PI3K/Akt/mTOR pathway are often evaluated in combination with the other anticancer therapies in preclinical models and in clinical studies. Current preclinical and clinical evidences suggest that inhibitors of the PI3K/Akt/mTOR pathway in combination with other anticancer therapies are able to circumvent resistance by cancer cells. One of the important considerations of mTOR inhibitors would be the general tolerability and safety profile of the drugs. Although most of the reported toxicities are mild to moderate in severity and can be managed clinically by dose modification and supportive measures, efforts should continue to optimize leads with greater safety and better pharmacological profile. It is quite interesting that mTOR signalling pathway is not only implicated in various cancers but appears to be involved in multiple disease conditions. For example, rapamycin was also investigated for its longevity activity and lifespan extension possibilities. The relationship between age-associated diseases with mTOR and its signalling systems are intriguing. The mTOR signalling pathway clearly offers tremendous opportunities for discovery of new drugs that target both aging and its associated diseases (Sharp and Richardson, 2011).

Rapamycin and its analogues are versatile drugs with proven efficacy in cancer and new drugs produced promising results in various cancer-related clinical trials. Potential chemopreventive activities of some natural phytochemicals such as curcumin, green tea extract and pomegranate are convincing as more and more trials were carried out to provide evidence-based data to advocate chemoprevention of cancer. The challenge for the future will be to further dissect the molecular signalling pathway to fully understand the mechanisms underpinning sensitivity or resistance to mTOR inhibition. The uncover of these pathways and identification of novel drug targets will provide insight into rational combinations of mTOR inhibitors with classic cytotoxic agents, radiation, and other molecular targeted therapies in the treatment and prevention of cancer as well as to discover novel uses of this class of drugs.

References

Abdulkareem IH, Blair M (2013). Phosphatase and tensin homologue deleted on chromosome 10. *Niger Med J,* **54**, 79-86.

Abraham RT (1998). Mammalian target of rapamycin: immunosuppressive drugs uncover a novel pathway of cytokine receptor signalling. *Curr Opin Immunol,* **10**, 330-6.

Aggarwal BB, Bhardwaj A, Aggarwal RS, et al (2004). Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. *Anticancer Res,* **24**, 2783-840.

Anastasius N, Boston S, Lacey M, Storing N, Whitehead SA (2009). Evidence that low-dose, long-term genistein treatment inhibits oestradiol-stimulated growth in MCF-7 cells by down-regulation of the PI3-kinase/Akt signalling pathway. *J Steroid Biochem Mol Biol,* **116**, 50-5.

Aoki H, Takada Y, Kondo S, et al (2007). Evidence that curcumin suppresses the growth of malignant gliomas *In vitro* and *In vivo* through induction of autophagy: role of Akt and extracellular signal-regulated kinase signalling pathways. *Mol Pharmacol*, **72**, 29-39.

Asnaghi L, Bruno P, Priulla M, et al (2004). mTOR: a protein kinase switching between life and death. *Pharmacol Res* **50**, 545-9.

Aziz MH, Nihal M, Fu VX, et al (2006). Resveratrol-caused apoptosis of human prostate carcinoma LNCaP cells is mediated via modulation of phosphatidylinositol 3'-kinase/Akt pathway and Bcl-2 family proteins. *Mol Cancer Ther* **5**, 1335-41.

Ballou LM, Lin RZ (2008). Rapamycin and mTOR kinase inhibitors. *J Chem Biol*, **1**, 27-36.

Banerjee S, Zhang Y, Wang Z, et al (2007). *In vitro* and *In vivo* molecular evidence of genistein action in augmenting the efficacy of cisplatin in pancreatic cancer. *Int J Cancer*, **120**, 906-17.

Baselga J (2011). Targeting the phosphoinositide-3 (PI3) kinase pathway in breast cancer. *Oncologist*, **16**, 12-9.

Beevers CS, Chen L, Liu L, et al (2009). Curcumin disrupts the mammalian target of rapamycin-raptor complex. *Cancer Res,* **69**, 1000-8.

Beevers CS, Li F, Liu L, et al (2006). Curcumin inhibits the mammalian target of rapamycin-mediated signalling pathways in cancer cells. *Int J Cancer*, **119**, 757-64.

Bharti AC, Aggarwal BB (2002). Nuclear factor-kappa B and cancer: its role in prevention and therapy. *Biochem Pharmacol*, **64**, 883-8.

Brito PM, Devillard R, Negre-Salvayre A, et al (2009). Resveratrol inhibits the mTOR mitogenic signalling evoked by oxidized LDL in smooth muscle cells. *Atherosclerosis* **205**, 126-34.

Brown VA, Patel KR, Viskaduraki M, et al (2010). Repeat dose study of the cancer chemopreventive agent resveratrol in healthy volunteers: safety, pharmacokinetics, and effect on the insulin-like growth factor axis. *Cancer Res,* **70**, 9003-11.

Caron E, Ghosh S, Matsuoka Y, et al (2010). A comprehensive map of the mTOR signalling network. *Mol Syst Biol,* **6**, 453.

Carroll RE, Benya RV, Turgeon DK, et al (2011). Phase IIa clinical trial of curcumin for the prevention of colorectal neoplasia. *Cancer Prev Res*, **4**, 354-64.

Chan S (2004). Targeting the mammalian target of rapamycin (mTOR): a new approach to treating cancer. *Br J Cancer* **91**, 1420-4.

Chen HX, Sharon E (2013). IGF-1R as an anti-cancer target--trials and tribulations. *Chin J Cancer,* **32**, 242-52.

Cheng AL, Hsu CH, Lin JK, et al (2001). Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Res*, **21**, 2895-900.

Chiang CT, Way TD, Tsai SJ, et al (2007). Diosgenin, a naturally occurring steroid, suppresses fatty acid synthase expression in HER2-overexpressing breast cancer cells through modulating Akt, mTOR and JNK phosphorylation. *FEBS Lett*, **581**, 5735-42.

Chow HH, Garland LL, Hsu CH, et al (2010). Resveratrol modulates drug- and carcinogen-metabolizing enzymes in a healthy volunteer study. *Cancer Prev Res,* **3**, 1168-75.

Chu Y-F, Sun J, Wu X, et al (2002). Antioxidant and antiproliferative activities of common vegetables. *J Agr Food Chem*, **50**, 6910-6.

Cragg GM, Newman DJ, Snader KM (1997). Natural products in drug discovery and development. *J Nat Prod*, **60**, 52-60.

da Rocha AB, Lopes RM, Schwartsmann G (2001). Natural products in anticancer therapy. *Curr Opin Pharmacol*, **1**, 364-9.

Dalessandri KM, Firestone GL, Fitch MD, et al (2004). Pilot study: effect of 3,3'-Diindolylmethane supplements on urinary hormone metabolites in postmenopausal women with a history of early-stage breast cancer. *Nutr Cancer*, **50**, 161-7.

Das A, Banik NL, Ray SK (2010). Flavonoids activated caspases for apoptosis in human glioblastoma T98G and U87MG cells but not in human normal astrocytes. *Cancer*, **116**, 164-76.

Dhillon N, Aggarwal BB, Newman RA, et al (2008). Phase II trial of curcumin in patients with advanced pancreatic cancer. *Clin Cancer Res*, **14**, 4491-9.

Dowling RJ, Topisirovic I, Fonseca BD, et al (2010). Dissecting the role of mTOR: lessons from mTOR inhibitors. *Biochim Biophys Acta,* **1804**, 433-9.

Faria A, Calhau C (2010). Chapter 36 - Pomegranate in human health: an overview (W. Ronald Ross and R. P. Victor, Ed.^ Eds). Academic Press, San Diego, **pp**. 551-63.

Faried A, Kurnia D, Faried LS, et al (2007). Anticancer effects of gallic acid isolated from Indonesian herbal medicine, Phaleria macrocarpa (Scheff.) Boerl, on human cancer cell lines. *Int J Oncol*, **30**, 605-13.

Fingar DC, Richardson CJ, Tee AR, et al (2004). mTOR controls cell cycle progression through its cell growth effectors S6K1 and 4E-BP1/eukaryotic translation initiation factor 4E. *Mol Cell Biol,* **24**, 200-16.

Fruman DA, Rommel C (2014). PI3K and cancer: lessons, challenges and opportunities. *Nat Rev Drug Discov*, **13**, 140-56.

Galluzzi L, Vicencio JM, Kepp O, et al (2008). To die or not to die: that is the autophagic question. *Curr Mol Med*, **8**, 78-91.

Ganley IG, Lam du H, Wang J, et al (2009). ULK1.ATG13. FIP200 complex mediates mTOR signalling and is essential for autophagy. *J Biol Chem*, **284**, 12297-305.

Garcia-Echeverria C (2010). Allosteric and ATP-competitive kinase inhibitors of mTOR for cancer treatment. *Bioorg Med Chem Lett*, **20**, 4308-12.

Garikapaty VP, Ashok BT, Tadi K, et al (2006). 3,3'-Diindolylmethane downregulates pro-survival pathway in hormone independent prostate cancer. *Biochem Biophys Res Commun*, **340**, 718-25.

Gera JF, Mellinghoff IK, Shi Y, et al (2004). AKT activity determines sensitivity to mammalian target of rapamycin (mTOR) inhibitors by regulating cyclin D1 and c-myc expression. *J Biol Chem*, **279**, 2737-46.

Ghayad SE, Cohen PA (2010). Inhibitors of the PI3K/Akt/mTOR pathway: new hope for breast cancer patients. *Recent Pat Anticancer Drug Discov*, **5**, 29-57.

Gomez-Pinillos A, Ferrari AC (2012). mTOR signalling pathway and mTOR inhibitors in cancer therapy. *Hematol Oncol Clin North Am*, **26**, 483-505.

Guertin DA, Kim D-H, Sabatini DM (2004). Growth control through the mTOR network. In 'Cell Growth: Control of Cell

Size' (Hall MN et al., eds), Cold Spring Harbor Laboratory Press, pp. 193-234.
Guertin DA, Sabatini DM (2009). The pharmacology of mTOR inhibition. *Sci Signal*, **2**, 24.
Gullett NP, Ruhul Amin AR, Bayraktar S, et al (2010). Cancer prevention with natural compounds. *Semin Oncol*, **37**, 258-81.
Han D, Li SJ, Zhu YT, et al (2013). LKB1/AMPK/mTOR signalling pathway in non-small-cell lung cancer. *Asian Pac J Cancer Prev*, **14**, 4033-9.
Hardwick JS, Kuruvilla FG, Tong JK, et al (1999). Rapamycin-modulated transcription defines the subset of nutrient-sensitive signalling pathways directly controlled by the Tor proteins. *Proc Natl Acad Sci USA*, **96**, 14866-70.
Hasskarl J (2014). Everolimus. *Recent Results Cancer Res*, **201**, 373-92.
Hay N, Sonenberg N (2004). Upstream and downstream of mTOR. *Genes Dev*, **18**, 1926-45.
He X, Wang Y, Zhu J, et al (2010). Resveratrol enhances the anti-tumor activity of the mTOR inhibitor rapamycin in multiple breast cancer cell lines mainly by suppressing rapamycin-induced AKT signalling. *Cancer Lett*, **28**, 168-76
He Y, Li D, Cook SL, et al (2013). Mammalian target of rapamycin and Rictor control neutrophil chemotaxis by regulating Rac/Cdc42 activity and the actin cytoskeleton. *Mol Biol Cell*, **24**, 3369-80.
Heath EI, Heilbrun LK, Li J, et al (2010). A Phase I dose-escalation study of oral BR-DIM (BioResponse 3,3'-Diindolylmethane) in castrate-resistant, non-metastatic prostate cancer. *Am J Transl Res*, **2**, 402-11.
Hidalgo M, Rowinsky EK (2000). The rapamycin-sensitive signal transduction pathway as a target for cancer therapy. *Oncogene*, **19**, 6680-6.
Hosokawa N, Sasaki T, Iemura S, et al (2009). Atg101, a novel mammalian autophagy protein interacting with Atg13. *Autophagy*, **5**, 973-9.
Houghton PJ (2010). mTOR and Cancer Therapy: General Principles (V. A. Polunovsky and P. J. Houghton, Ed.^ Eds.), pp. 113-31. Humana Press, Springer New York Dordrecht Heidelberg London.
Howells LM, Berry DP, Elliott PJ, et al (2011). Phase I randomized, double-blind pilot study of micronized resveratrol (SRT501) in patients with hepatic metastases--safety, pharmacokinetics, and pharmacodynamics. *Cancer Prev Res*, **4**, 1419-25.
Hsieh TC, Wu JM (2009). Targeting CWR22Rv1 prostate cancer cell proliferation and gene expression by combinations of the phytochemicals EGCG, genistein and quercetin. *Anticancer Res*, **29**, 4025-32.
Huang CH, Tsai SJ, Wang YJ, et al (2009). EGCG inhibits protein synthesis, lipogenesis, and cell cycle progression through activation of AMPK in p53 positive and negative human hepatoma cells. *Mol Nutr Food Res*, **53**, 1156-65.
Huang S, Houghton PJ (2003). Targeting mTOR signalling for cancer therapy. *Curr Opin Pharmacol*, **3**, 371-7.
Hwang YW, Kim SY, Jee SH, et al (2009). Soy food consumption and risk of prostate cancer: a meta-analysis of observational studies. *Nutr Cance*r, **61**, 598-606.
Inoki K, Li Y, Zhu T, et al (2002). TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Biol*, **4**, 648-57.
Irving GR, Howells LM, Sale S, et al (2013). Prolonged biologically active colonic tissue levels of curcumin achieved after oral administration--a clinical pilot study including assessment of patient acceptability. *Cancer Prev Res (Phila)*, **6**, 119-28.
Jacinto E, Loewith R, Schmidt A, et al (2004). Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol*, **6**, 1122-8.
Jiang H, Shang X, Wu H, et al (2009). Resveratrol downregulates PI3K/Akt/mTOR signalling pathways in human U251 glioma cells. *J Exp Ther Oncol*, **8**, 25-33.
Johnson SM, Gulhati P, Arrieta I, et al (2009). Curcumin inhibits proliferation of colorectal carcinoma by modulating Akt/mTOR signalling. *Anticancer Res*, **29**, 3185-90.
Jung CH, Jun CB, Ro SH, et al (2009). ULK-Atg13-FIP200 complexes mediate mTOR signalling to the autophagy machinery. *Mol Biol Cell*, **20**, 1992-2003.
Kadowaki M, Kanazawa T (2003). Amino acids as regulators of proteolysis. *J Nutr*, **133**, 2052-6.
Kanai M, Otsuka Y, Otsuka K, et al (2013). A Phase I study investigating the safety and pharmacokinetics of highly bioavailable curcumin (Theracurmin) in cancer patients. *Cancer Chemother Pharmacol*, **71**, 1521-30.
Kanai M, Yoshimura K, Asada M, et al (2011). A phase I/II study of gemcitabine-based chemotherapy plus curcumin for patients with gemcitabine-resistant pancreatic cancer. *Cancer Chemother Pharmacol,* **68**, 157-64.
Kang Y-J, Chung H-J, Nam J-W, et al (2011). Cytotoxic and antineoplastic activity of timosaponin a-iii for human colon cancer cells. *J Nat Prod*, **74**, 701-6.
Katiyar S, Elmets CA, Katiyar SK (2007). Green tea and skin cancer: photoimmunology, angiogenesis and DNA repair. *J Nutr Biochem*, **18**, 287-96.
Khan N, Afaq F, Kweon MH, et al (2007a). Oral consumption of pomegranate fruit extract inhibits growth and progression of primary lung tumors in mice. *Cancer Res*, **67**, 3475-82.
Khan N, Hadi N, Afaq F, et al (2007b). Pomegranate fruit extract inhibits prosurvival pathways in human A549 lung carcinoma cells and tumor growth in athymic nude mice. *Carcinogenesis*, **28**, 163-73.
Kim DH, Sarbassov DD, Ali SM, et al (2002a). mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell*, **110**, 163-75.
Kim ND, Mehta R, Yu W, et al (2002b). Chemopreventive and adjuvant therapeutic potential of pomegranate (Punica granatum) for human breast cancer. *Breast Cancer Res Treat*, **71**, 203-17.
Kim SH, Kim YB, Jeon YT, et al (2009). Genistein inhibits cell growth by modulating various mitogen-activated protein kinases and AKT in cervical cancer cells. *Ann NY Acad Sci*, **1171**, 495-500.
King FW, Fong S, Griffin C, et al (2009). Timosaponin AIII is preferentially cytotoxic to tumor cells through inhibition of mTOR and induction of ER stress. *PloS one*, **4**, 7283.
Kirken RA, Wang YL (2003). Molecular actions of sirolimus: sirolimus and mTor. *Transplant Proc*, **35**, 227-30.
Kong D, Banerjee S, Huang W, et al (2008). Mammalian target of rapamycin repression by 3,3'-Diindolylmethane inhibits invasion and angiogenesis in platelet-derived growth factor-D-overexpressing PC3 cells. *Cancer Res*, **68**, 1927-34.
Lao CD, Ruffin MTt, Normolle D, et al (2006). Dose escalation of a curcuminoid formulation. *BMC Complement Altern Med*, **6**, 10.
Laplante M, Sabatini DM (2012). mTOR signalling in growth control and disease. *Cell*, **149**, 274-93.
Laplante M, Sabatini DM (2013). Regulation of mTORC1 and its impact on gene expression at a glance. *J Cell Sci*, **126**, 1713-9.
Law BK (2005). Rapamycin: an anti-cancer immunosuppressant? *Crit Rev Oncol Hematol*, **56**, 47-60.
Le HT, Schaldach CM, Firestone GL, et al (2003). Plant-derived 3,3'-Diindolylmethane is a strong androgen antagonist in human prostate cancer cells. *J Biol Chem*, **278**, 21136-45.

Li JC, Zhu HY, Chen TX, et al (2013). Roles of mTOR and p-mTOR in gastrointestinal stromal tumors. *Asian Pac J Cancer Prev*, **14**, 5925-8.

Li M, Zhang Z, Hill DL, et al (2007). Curcumin, a dietary component, has anticancer, chemosensitization, and radiosensitization effects by down-regulating the MDM2 oncogene through the PI3K/mTOR/ETS2 pathway. *Cancer Res*, **67**, 1988-96.

Li Y, Sarkar FH (2002). Inhibition of nuclear factor kappaB activation in PC3 cells by genistein is mediated via Akt signalling pathway. *Clin Cancer Res*, **8**, 2369-77.

Li Y, Wang Z, Kong D, et al (2007). Regulation of FOXO3a/beta-catenin/GSK-3beta signalling by 3,3'-Diindolylmethane contributes to inhibition of cell proliferation and induction of apoptosis in prostate cancer cells. *J Biol Chem*, **282**, 21542-50.

Lin JN, Lin VC, Rau KM, et al (2010). Resveratrol modulates tumor cell proliferation and protein translation via SIRT1-dependent AMPK activation. *J Agric Food Chem*, **58**, 1584-92.

Liu MJ, Wang Z, Ju Y, et al (2005). Diosgenin induces cell cycle arrest and apoptosis in human leukemia K562 cells with the disruption of Ca_2+ homeostasis. *Cancer Chemother Pharmacol,* **55**, 79-90.

Liu P, Cheng H, Roberts TM, et al (2009a). Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discov*, **8**, 627-44.

Liu Q, Thoreen C, Wang J, Sabatini D, Gray NS (2009b). mTOR mediated anti-cancer drug discovery. *Drug Discov Today Ther Strateg*, **6**, 47-55.

Loewith R, Jacinto E, Wullschleger S, et al (2002). Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. *Mol Cell*, **10**, 457-68.

LoPiccolo J, Blumenthal GM, Bernstein WB, et al (2008). Targeting the PI3K/Akt/mTOR pathway: effective combinations and clinical considerations. *Drug Resist Updat*, **11**, 32-50.

Malik A, Afaq F, Sarfaraz S, et al (2005). Pomegranate fruit juice for chemoprevention and chemotherapy of prostate cancer. *Proc Natl Acad Sci USA*, **102**, 14813-8.

Manning BD, Cantley LC (2007). AKT/PKB signalling: navigating downstream. *Cell*, **129**, 1261-74.

Marques FZ, Markus MA, Morris BJ (2009). Resveratrol: cellular actions of a potent natural chemical that confers a diversity of health benefits. *Int J Biochem Cell Biol*, **41**, 2125-8.

Messing E, Gee JR, Saltzstein DR, et al (2012). A phase 2 cancer chemoprevention biomarker trial of isoflavone G-2535 (genistein) in presurgical bladder cancer patients. *Cancer Prev Res*, **5**, 621-30.

Mita MM, Mita A, Rowinsky EK (2003). The molecular target of rapamycin (mTOR) as a therapeutic target against cancer. *Cancer Biol Ther*, **2**, 169-77.

Moalic S, Liagre B, Corbiere C, et al (2001). A plant steroid, diosgenin, induces apoptosis, cell cycle arrest and COX activity in osteosarcoma cells. *FEBS Lett*, **506**, 225-30.

Moschetta M, Reale A, Marasco C, et al (2014). Therapeutic targeting of the mTOR-signalling pathway in cancer: benefits and limitations. *Br J Pharmacol*, [Epub ahead of print]

Nagle DG, Ferreira D, Zhou YD (2006). Epigallocatechin-3-gallate (EGCG): chemical and biomedical perspectives. *Phytochemistry*, **67**, 1849-55.

Nakamura Y, Yogosawa S, Izutani Y, et al (2009). A combination of indol-3-carbinol and genistein synergistically induces apoptosis in human colon cancer HT-29 cells by inhibiting Akt phosphorylation and progression of autophagy. *Mol Cancer*, **8**, 100.

Nave BT, Ouwens M, Withers DJ, et al (1999). Mammalian target of rapamycin is a direct target for protein kinase B: identification of a convergence point for opposing effects of insulin and amino-acid deficiency on protein translation. *Biochem J*, **344**, 427-31.

Nicholson KM, Anderson NG (2002). The protein kinase B/Akt signalling pathway in human malignancy. *Cell Signal* **14**, 381-95.

Noh WC, Mondesire WH, Peng J, et al (2004). Determinants of rapamycin sensitivity in breast cancer cells. *Clin Cancer Res*, **10**, 1013-23.

O'Reilly KE, Rojo F, She QB, et al (2006). mTOR inhibition induces upstream receptor tyrosine kinase signalling and activates Akt. *Cancer Res*, **66**, 1500-8.

Pal I, Mandal M (2012). PI3K and Akt as molecular targets for cancer therapy: current clinical outcomes. *Acta Pharmacol Sin*, **33**, 1441-58.

Paller CJ, Ye X, Wozniak PJ, et al (2013). A randomized Phase II study of pomegranate extract for men with rising PSA following initial therapy for localized prostate cancer. *Prostate Cancer Prostatic Dis*, **16**, 50-5.

Pandurangan AK (2013). Potential targets for prevention of colorectal cancer: a focus on PI3K/Akt/mTOR and Wnt pathways. *Asian Pac J Cancer Prev,* **14**, 2201-5.

Pantuck AJ, Leppert JT, Zomorodian N, et al (2006). Phase II study of pomegranate juice for men with rising prostate-specific antigen following surgery or radiation for prostate cancer. *Clin Cancer Res*, **12**, 4018-26.

Pervaiz S (2003). Resveratrol: from grapevines to mammalian biology. *FASEB J*, **17**, 1975-85.

Peterson TR, Laplante M, Thoreen CC, et al (2009). DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. *Cell*, **137**, 873-86.

Philips BJ, Coyle CH, Morrisroe SN, et al (2009). Induction of apoptosis in human bladder cancer cells by green tea catechins. *Biomed Res*, **30**, 207-15.

Plas DR, Thompson CB (2005). Akt-dependent transformation: there is more to growth than just surviving. *Oncogene*, **24**, 7435-42.

Popat R, Plesner T, Davies F, et al (2013). A phase 2 study of SRT501 (resveratrol) with bortezomib for patients with relapsed and or refractory multiple myeloma. *Br J Haematol* **160**, 714-7.

Porta C, Paglino C, Mosca A (2014). Targeting PI3K/Akt/mTOR signalling in cancer. *Fron Oncol*, **4**, 64.

Powers T, Walter P (1999). Regulation of ribosome biogenesis by the rapamycin-sensitive TOR-signalling pathway in Saccharomyces cerevisiae. *Mol Biol Cell*, **10**, 987-1000.

Proud CG (2002). Regulation of mammalian translation factors by nutrients. *Eur J Biochem*, **269**, 5338-49.

Pyrko P, Schonthal AH, Hofman FM, et al (2007). The unfolded protein response regulator GRP78/BiP as a novel target for increasing chemosensitivity in malignant gliomas. *Cancer Res*, **67**, 9809-16.

Qiao Y, Cao J, Xie L, et al (2009). Cell growth inhibition and gene expression regulation by (-)-epigallocatechin-3-gallate in human cervical cancer cells. *Arch Pharm Res*, **32**, 1309-15.

Ravindranath MH, Muthugounder S, Presser N, et al (2004). Anticancer therapeutic potential of soy isoflavone, genistein. *Adv Exp Med Biol*, **546**, 121-65.

Rodon J, Dienstmann R, Serra V, et al (2013). Development of PI3K inhibitors: lessons learned from early clinical trials. *Nat Rev Clin Oncol*, **10**, 143-53.

Ryan JL, Heckler CE, Ling M, et al (2013). Curcumin for radiation dermatitis: a randomized, double-blind, placebo-

controlled clinical trial of thirty breast cancer patients. *Radiat Res*, **180**, 34-43.

Sabatini DM, Erdjument-Bromage H, Lui M, et al (1994). RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. *Cell*, **78**, 35-43.

Sabers CJ, Martin MM, Brunn GJ, et al (1995). Isolation of a protein target of the FKBP12-rapamycin complex in mammalian cells. *J Biol Chem*, **270**, 815-22.

Sakamoto T, Horiguchi H, Oguma E, et al (2010). Effects of diverse dietary phytoestrogens on cell growth, cell cycle and apoptosis in estrogen-receptor-positive breast cancer cells. *J Nutr Biochem*, **21**, 856-64.

Sancak Y, Thoreen CC, Peterson TR, et al (2007). PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. Mol. *Cell*, **25**, 903-15.

Sansal I, Sellers WR (2004). The biology and clinical relevance of the PTEN tumor suppressor pathway. *J Clin Oncol*, **22**, 2954-63.

Sarbassov DD, Ali SM, Kim DH, et al (2004). Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol*, **14**, 1296-302.

Sarbassov DD, Ali SM, Sengupta S, et al (2006). Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol Cell*, **22**, 159-68.

Sehgal SN, Baker H, Vezina C (1975). Rapamycin (AY-22,989), a new antifungal antibiotic. II. Fermentation, isolation and characterization. *J Antibiot (Tokyo)*, **28**, 727-32.

Seo BR, Min KJ, Cho IJ, et al (2014). Curcumin significantly enhances dual PI3K/Akt and mTOR inhibitor NVP-BEZ235-induced apoptosis in human renal carcinoma Caki cells through down-regulation of p53-dependent Bcl-2 expression and inhibition of Mcl-1 protein stability. *PloS one*, **9**, 95588.

Sharma RA, McLelland HR, Hill KA, et al (2001). Pharmacodynamic and pharmacokinetic study of oral curcuma extract in patients with colorectal cancer. *Clin Cancer Res*, **7**, 1894-900.

Sharp Z, Richardson A (2011). Aging and cancer: can mTOR inhibitors kill two birds with one drug? *Target Oncol*, **6**, 41-51.

Shimobayashi M, Hall MN (2014). Making new contacts: the mTOR network in metabolism and signalling crosstalk. *Nat Rev Mol Cell Biol*, **15**, 155-62.

Shishodia S, Chaturvedi MM, Aggarwal BB (2007). Role of curcumin in cancer therapy. *Curr Probl Cance*, **31**, 243-305.

Shor B, Gibbons JJ, Abraham RT, et al (2009). Targeting mTOR globally in cancer: thinking beyond rapamycin. *Cell Cycle* **8**, 3831-7.

Soliman GA, Acosta-Jaquez HA, Dunlop EA, et al (2010). mTOR Ser-2481 autophosphorylation monitors mTORC-specific catalytic activity and clarifies rapamycin mechanism of action. *J Biol Chem*, **285**, 7866-79.

Srinivasan S, Koduru S, Kumar R, et al (2009). Diosgenin targets Akt-mediated prosurvival signalling in human breast cancer cells. *Int J Cancer*, **125**, 961-7.

Sun J, Chu Y-F, Wu X, et al (2002). Antioxidant and antiproliferative activities of common fruits. *J Agric Food Chem*, **50**, 7449-54.

Sun SY, Rosenberg LM, Wang X, et al (2005). Activation of Akt and eIF4E survival pathways by rapamycin-mediated mammalian target of rapamycin inhibition. *Cancer Res*, **65**, 7052-8.

Sy LK, Yan SC, Lok CN, et al (2008). Timosaponin A-III induces autophagy preceding mitochondria-mediated apoptosis in HeLa cancer cells. *Cancer Res*, **68**, 10229-37.

Tabernero J, Rojo F, Calvo E, et al (2008). Dose- and schedule-dependent inhibition of the mammalian target of rapamycin pathway with everolimus: a Phase I tumor pharmacodynamic study in patients with advanced solid tumors. *J Clin Oncol* **26**, 1603-10.

Takahashi Y, Kohashi K, Yamada Y, et al (2014). Activation of the Akt/mammalian target of rapamycin pathway in myxofibrosarcomas. *Human pathol*, **45**, 984-93.

Tang KD, Ling MT (2014). Targeting Drug-Resistant Prostate Cancer with Dual PI3K/MTOR Inhibition. *Curr Med Chem*, [Epub ahead of print].

Tokunaga E, Oki E, Egashira A, et al (2008). Deregulation of the Akt pathway in human cancer. *Curr Cancer Drug Targets*, **8**, 27-36.

Tsang CK, Qi H, Liu LF, et al (2007). Targeting mammalian target of rapamycin (mTOR) for health and diseases. *Drug Discov Today*, **12**, 112-24.

Vakana E, Sassano A, Platanias LC (2010). Induction of autophagy by dual mTORC1-mTORC2 inhibition in BCR-ABL-expressing leukemic cells. *Autophagy*, **6**, 966-7.

Vezina C, Kudelski A, Sehgal SN (1975). Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. *J Antibiot*, **28**, 721-6.

Vivanco I, Sawyers CL (2002). The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nat Rev Cancer*, **2**, 489-501.

Vogt PK, Hart JR, Gymnopoulos M, et al (2010). Phosphatidylinositol 3-kinase: the oncoprotein. *Curr Top Microbiol Immunol*, **347**, 79-104.

Wan X, Harkavy B, Shen N, et al (2007). Rapamycin induces feedback activation of Akt signalling through an IGF-1R-dependent mechanism. *Oncogene*, **26**, 1932-40.

Wang XW, Zhang YJ (2014). Targeting mTOR network in colorectal cancer therapy. *World J Gastroenterol*, **20**, 4178-88.

Wu Y, Kim J, Elshimali Y, et al (2014). Activation of Akt1 accelerates carcinogen-induced tumorigenesis in mammary gland of virgin and post-lactating transgenic mice. *BMC Cancer*, **14**, 266.

Yap TA, Garrett MD, Walton MI, et al (2008). Targeting the PI3K-AKT-mTOR pathway: progress, pitfalls, and promises. *Curr Opin Pharmacol*, **8**, 393-412.

Yu K, Toral-Barza L, Discafani C, et al (2001). mTOR, a novel target in breast cancer: the effect of CCI-779, an mTOR inhibitor, in preclinical models of breast cancer. *Endocr Relat Cancer*, **8**, 249-58.

Zhang J, Meng Z, Zhang M, et al (1999). Effect of six steroidal saponins isolated from anemarrhenae rhizoma on platelet aggregation and hemolysis in human blood. *Clin Chim Acta*, **289**, 79-88.

Zhang Q, Kelly AP, Wang L, et al (2006). Green tea extract and (-)-epigallocatechin-3-gallate inhibit mast cell-stimulated type I collagen expression in keloid fibroblasts via blocking PI-3K/AkT signalling pathways. *J Invest Dermatol*, **126**, 2607-13.

Zhao JJ, Cheng H, Jia S, et al (2006). The p110α isoform of PI3K is essential for proper growth factor signalling and oncogenic transformation. *Proc Natl Acad Sci USA*, **103**, 16296-300.