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Mini-review

AKT and ERK dual inhibitors: The way forward?

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ABSTRACT

Phosphatidylinositol 3-kinase (PI3K)/AKT pathway regulates cell growth, proliferation, survival, mobility and invasion. Mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway is also an important mitogenic signaling pathway involved in various cellular progresses. AKT, also named protein kinase B (PKB), is a primary mediator of the PI3K signaling pathway; and ERK at the end of MAPK signaling is the unique substrate and downstream effector of mitogen-activated protein/extracellular signal-regulated kinase (MEK). The AKT and ERK signaling are both aberrantly activated in a wide range of human cancers and have long been targeted for cancer therapy, but the clinical benefits of these targeted therapies have been limited due to complex cross-talk. Novel strategies, such as AKT/ERK dual inhibitors, may be needed.

1. Introduction

The PI3K/AKT pathway is an intracellular signaling cascade consisting of phosphatidylinositol 3-kinase (PI3K), AKT and downstream effectors. PI3K is a family of lipid enzymes that phosphorylate 3′-OH group of the inositol ring in phosphatidylinositols (PI) on plasma membrane [1]. Based on the primary structure and lipid substrate specificity, PI3Ks are divided into four classes, i.e., class I-IV. Class II PI3Ks produce phosphatidylinositol 3-phosphate [PI(3)P] from PI and phosphatidylinositol 3,4-bisphosphate [PI(3,4)P_2] from phosphatidylinositol 4-bisphosphate [PI(4)P]; Class III PI3Ks catalyze production of PI(3)P from PI [2,3]; and Class IV PI3Ks are a group of distantly related Ser/Thr protein kinases, including ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3 related (ATR), DNA-dependent protein kinase (DNA-PK), and mammalian target of rapamycin (mTOR) [4]. Only Class I PI3Ks, heterodimers consisting of a catalytic subunit and a regulatory subunit, are involved in production of phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) and activation of AKT [5,6]. PI3Ks discussed in this article indicates the Class I PI3Ks. The Class I PI3Ks are further divided into Class IA and Class IB; Class IA PI3Ks are composed of a p110 catalytic subunit and a p85 regulatory subunit, and Class IB PI3Ks consist of a p110γ catalytic subunit and a p101 regulatory subunit [4]. AKT, also named protein kinase B (PKB), is a serine/threonine kinase and primary mediator of PI3K signaling cascade [1,7]. To date, three highly conserved AKT isoforms are identified: AKT1 (i.e., AKT), AKT2 and AKT3 [8]. AKT is a cardinal node in diverse signaling pathways and has a wide range of downstream substrates, such as forkhead family of transcription factors (FOXO), IκKα, GSK3β, MDM2, procaspase-9, Bim, Bad, p21 < SUP > CIP1 < /SUP > and p27 < SUP > KIP1 < /SUP > [9].

The MAPK/ERK pathway is composed of a group of protein kinases including RAS, RAF, MEK and ERK [10]. Mitogen-activated protein/extracellular signal-regulated kinase (MEK) has two similar isoforms, MEK1 and MEK2 [11]; extracellular signal-regulated kinase (ERK) also includes ERK1 and ERK2 [12]. In this MAPK/ERK signaling cascade, ERK1/2 is the unique substrate of MEK1/2 [10]. Activated ERK1/2 phosphorylates numerous targets in the cytoplasm, such as p90Rsk and IKK, or migrates into the nucleus to phosphorylate a range of transcription factors, such as Ets-1, Elk-1, c-Fos, c-Jun and c-Myc, thus driving cell survival, growth and proliferation [9].

The PI3K/AKT and MAPK/ERK pathways both are important intracellular signal transduction cascades, regulating cell growth and proliferation, survival and apoptosis, and mobility and invasion [8,9,13]. In tumorigenesis, oncogenic amplifications and/or mutations of the effectors in PI3K/AKT and MAPK/ERK pathways occur frequently, leading to aberrant activation of signaling [14–21]. PI3K/AKT

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and MAPK/ERK pathways are dysregulated in almost one-third of human cancers, and in many cancers, the PI3K/AKT and MAPK/ERK pathways are concurrently activated and thus are hot targets for cancer therapy [10,22].

In the past decades, substantial efforts have been invested in the development of targeted therapy with small molecule inhibitors of the PI3K/AKT or MAPK/ERK pathway; however, clinical benefits are limited. This is largely ascribed to broad crosstalk between these two pathways and subsequent drug resistance [23]. Combination of agents that target PI3K/AKT and MAPK/ERK pathways improves objective response, but serious adverse effects occur [24]. It is a challenge for basic and clinical scientists to inhibit the activity of both PI3K/AKT and MAPK/ERK pathways with favorable host adverse effects. The development of an AKT/ERK dual inhibitor ONC201 shed light on this exploration. This mini review summarizes hurdles in PI3K/AKT and MAPK/ERK targeted therapy and updates the basic and clinical investigation of the AKT/ERK dual inhibitors.

2. Pi3k/Akt and Mapk/Erk pathways in cancers

During the process of tumorigenesis, a cell escapes from normal control of growth and acquires capability of invasion. Aberrant activation of the PI3K/AKT and/or MAPK/ERK pathways promotes cell survival and unlimited growth and proliferation, driving carcinogenesis.

2.1. PI3K/AKT signaling in tumorigenesis

The PI3K/AKT pathway is activated by receptor tyrosine kinases (RTKs) or G-proteins coupled receptors (GPCRs) [8]. RTKs are cell surface receptors composed of an extracellular domain for ligand binding, a transmembrane domain and an intracellular tyrosine kinase domain [25]. RTKs have high affinity to ligands: growth factors, cytokines and hormones. Binding with ligands, two monomer RTKs form a functional dimer, activating the tyrosine kinase domain by auto-phosphorylation. Phosphorylated tyrosine residues serve as a dock for the regulatory subunit p85 of PI3K and then recruit the catalytic subunit p110 for an active PI3K complex [26]. Ligand-activated receptors could also trigger formation of a Shc/GRB2/SOS protein complex, activating Ras [27]. Activated Ras could induce membrane translocation and activation of the PI3K subunit p110, further activating PI3K [28]. The activated PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP_2) to generate phosphatidylinositol-3,4,5-trisphosphate (PIP_3) [6]. PIP_3 functions as a scaffold to recruit phosphoinositol-dependent kinase-1 (PDK1) and AKT through the pleckstrin homology (PH) domain to the plasma membrane, where PDK1 phosphorylates AKT at Thr308 in kinase domain (T-loop) [29]. mTORC2 (also named PDK2) then phosphorylates the residue of Ser473 within the hydrophobic motif (HM) in the C-terminal tail for full activation of AKT, triggering downstream signaling [30].

AKT is the node of the PI3K/AKT signaling cascade [14]. Activated AKT drives carcinogenesis through phosphorylation to activate or inhibit numerous downstream effectors. Mammalian target of rapamycin (mTOR) is a key downstream effector protein of AKT. Activated AKT may directly phosphorylate Ser2448 of mTOR, activating mTOR signaling [31]; AKT may also catalyze Thr1462 phosphorylation of TSC2 and inhibit TSC1/TSC2 complexes, a master negative regulator of mTOR [32]. Therefore, AKT activates mTOR signaling through both direct phosphorylation of mTOR protein and inhibition of the TSC1/TSC2 complex. Activated mTOR then phosphorylates 4E-BP1 and enhances formation of translation initiation complexes, driving protein synthesis [33]. In addition, mTOR phosphorylates and activates p70S6K, triggering ribosomal 40S subunit binding to translation complexes and protein synthesis [34]. Therefore, activation of the AKT/mTOR signaling pathway stimulates cell growth and proliferation, driving cancer progression [35]. AKT also activates IκB kinase (IKK) to trigger NF-κB signaling cascade [36], and mouse double minute 2 homolog (MDM2) to inhibit p53 [37]. In addition, AKT also phosphorylates and inhibits Bad, p27, glycogen synthase kinase-3 (GSK-3) and FOXO transcription factors 1 and 4 (FOXO1/4). Inhibition of Bad and p27 suppresses apoptosis and promotes cell cycle [38,39]; and phosphorylation inactivation of GSK-3 by AKT triggers WNT signaling [40]. FOXO1/4 belong to the forkhead family of transcription factors with a conserved DNA-binding domain (Forkhead box) and participate in regulation of apoptosis and cell cycle progression [41,42]. Therefore, AKT activates multiples oncogenic signaling to promote cancer; and AKT hyperactivation is associated with poor differentiation and worse prognosis of cancer, being a negative prognostic marker of cancer [43,44].

Theoretically, any activating events that occur in effectors of the whole signaling cascade could trigger this PI3K/AKT pathway. In fact, aberrant hyperactivation of the PI3K/AKT signaling in human cancers has been widely documented and is considered as a hallmark of cancer [14,15,45]. Oncogenic amplification or mutations of *Akt* gene and upstream regulatory effector genes, such as *RTKs*, *PI3K*, phosphatase tensin homolog (*PTEN*) and *Ras* have been well recognized in human tumors [15–17]. For instance, the E17K mutant of *Akt* gene leads to constitutive localization at the cell membrane and growth factor independent hyper-phosphorylation of AKT protein [46].

2.2. MAPK/ERK signaling in tumorigenesis

The MAPK/ERK pathway is also activated by RTKs and GPCRs, with a conformational change of Ras [9]. Ras is a family of small GTP-binding proteins, including three most notable members, H-Ras, K-Ras and N-Ras [47,48]. Activation of receptors by interaction with ligands switches GDP-bound Ras (inactive) to a GTP-bound Ras (active). The active Ras recruits and activates serine/threonine protein kinase Raf. There are three isoforms of Raf kinases, i.e., A-Raf, B-Raf and C-Raf (Raf-1). Raf is activated through a series of complicated events, including the recruitment of Raf to the plasma membrane by Ras, dimerization, phosphorylation/dephosphorylation on different domains, disassociation from the Raf kinase inhibitory protein (RKIP) and association with scaffolding complexes (e.g., kinase suppressor of Ras, KSR) [9]. Activated Raf phosphorylates tyrosine/threonine kinases MEK1/2 that in turn phosphorylates and activates serine/threonine protein kinase ERK1/2 [49]. The MAPK/ERK signaling pathway regulates cell growth, proliferation and differentiation and profoundly affects cell survival by post-translational phosphorylation modifications of apoptotic proteins, such as Bad, Bim, Mcl-1, Bcl-2 and caspases [9].

ERK1/2 is at the end of the MAPK pathway and mediates various cellular processes regulated by MAPK pathway. ERK1/2 kinase has a broad range of substrates that are differentiated by spatial localization [50]. For instance, cytosolic ERK1/2 may phosphorylate TSC2, ribosomal S6 kinases (RSK), cytoskeletal proteins and L1 adhesion molecule to regulate cell metabolism, adhesion, movement and trafficking [51,52]. Minutes after activation, ERK1/2 translocates into the nucleus, where ERK1/2 phosphorylates and activates various transcription factors, such as carbamoyl phosphate synthetase II (CPS II) and p90RSK for DNA synthesis and cell cycle progression [53]. Timing, duration and signal intensity also determine the cellular effects of the MAPK/ERK pathway activation. An early gene product, c-FOS, may work as a sensor of the duration of MAPK/ERK activation, and pro-carcinogenic or pro-apoptotic role of ERK signaling depends on the timing and duration of activation [54].

In human cancers, the MAPK/ERK signaling pathway is frequently activated, driving malignant transformation of cells and tumor growth through promotion of cell growth and proliferation and prevention of apoptosis [18]. Like the AKT signaling, activating events of the MAPK/ERK signaling pathway in cancers are well documented, including chromosomal translocation (e.g., BCR-ABL), gene amplification (e.g., Ras), mutations (e.g., RTKs, Ras and B-Raf) and aberrant expression of

effectors in this pathway [19–21]. The activating mutations in Ras codons 12, 13, 59 and 61 occur in approximately 30% of human cancer, leading to constitutive activation of Ras and carcinogenesis [19]. B-Raf gene is mutated in 22% colorectal cancer, 30% ovarian cancer, 53% papillary thyroid cancer and 70% melanoma [9], and upstream receptors (e.g., EGFR) are frequently mutated in lung cancer [55]. The constitutive activation of the MAPK/ERK signaling is associated with poor prognosis and is thus a negative prognostic marker and therapeutic target of cancer [56].

2.3. Cross-talk between PI3K/AKT and MAPK/ERK signaling cascades

The PI3K/AKT and MAPK/ERK pathways are both important cellular signaling cascades with large functional overlaps in cell growth, proliferation and survival/apoptosis, and are both activated by RTKs and GPCRs. Targeted inhibition of one pathway at downstream of receptors may not affect the activity of the other. More importantly, broad crosstalk exists between the PI3K/AKT and MAPK/ERK pathways. The early hint of cross-talk between the PI3K/AKT and MAPK/ERK pathways arose in early 1990s from finding of PI3K-dependent and independent activation of p70S6K [57]. To date, multiple mechanisms of crosstalk between the PI3K/AKT and MAPK/ERK pathways are described.

Ras as a node of AKT and ERK signaling: Ras protein is associated with plasma membrane by farnesylation or geranylgeranylation on the cysteine residue [58] and functions as a binary switch, cycling between an active GTP-bound and an inactive GDP-bound status [47]. Binding of ligands, such as cytokines and growth factors, to RTK or GPCR receptors activates the coupling complex Shc/GRB2/SOS, which in turn triggers exchanges of GDP for GTP in Ras, activating Ras [27,59]. Activated Ras recruits and activates Raf, triggering the MAPK/ERK pathway [60]. The activated Ras also recruits and activates the PI3K/AKT pathway. The p110 catalytic subunit of Class I PI3K contains a Ras binding site, and thus the active Ras can recruit the p110 catalytic subunit of PI3K to plasma membrane and triggers the AKT signaling pathway (Fig. 1) [61].

PIP_2 as a lipid second messenger mediating AKT and ERK signaling: Phosphatidylinositol 4,5-bisphosphate (PIP_2) is an cellular lipid second messenger that mediates both PI3K/AKT and MAPK/ERK signaling cascades (Fig. 2). Phosphatidylinositol (PI), a class of phosphatidylglycerides, is a precursor of PIP_2. PI 4-kinase or PI 5-kinase converts PI into phosphatidylinositol 4-phosphate [PI(4)P] or phosphatidylinositol 5-phosphate [PI(5)P], both of which are then converted into PI (4,5)P_2 (i.e., PIP_2) by type I or type II PIP kinases [62]. PIP_2 is then phosphorylated into phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) by PI3K, activating the AKT signaling [63].

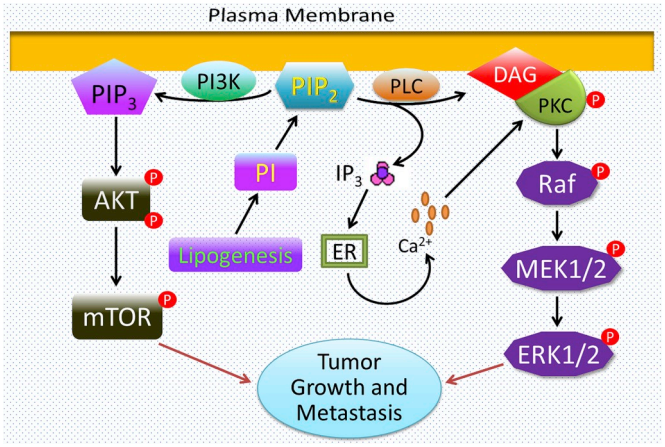

Fig. 2. PIP_2 lipid second messenger mediates activation of PI3K/AKT and MAPK/ERK pathways. PIP_2 may be phosphorylated into PIP_3 by PI3K, activating AKT signaling; PIP_2 may also be hydrolyzed by phospholipase C into DAG and IP_3. DAG remains on membrane to recruit and activate protein kinase C (PKC). IP_3 diffuses into cytosol triggering calcium release from endoplasmic reticulum, activating calcium-dependent PKC isoforms. Activated PKC phosphorylates and activates Raf, triggering ERK signaling. In cancer cells, increased glycolysis and conversion of glucose to lipids leads to elevation of membrane lipid PI which is then converted to PIP_2, activating the PIP_2 lipid messenger and AKT and ERK signaling cascades.

The MAPK/ERK pathway is also mediated by the PIP_2 messenger system. PIP_2 is hydrolyzed by phospholipase C (PLC) to produce diacylglycerol (DAG) and inositol trisphosphate (IP_3). Hydrophobic DAG remains in the plasma membrane, activating protein kinase C (PKC); IP_3 diffuses into cytosol and stimulates calcium release from endoplasmic reticulum (ER), activating calcium-dependent PKC [64]. Active PKC activates Raf by phosphorylation of the Ser497 and Ser499 and thus trigger the MAPK/ERK pathway [65]. PKC isoforms also phosphorylate RKIP on Ser153 and thus disassociate Raf from RKIP, activating Raf [66].

Through the PIP_2 second messenger, the PI3K/AKT and PKC/ERK signaling cascades are both activated. Targeted inhibition of one pathway may lead to the flow of PIP_2 signals to the other. More importantly, with glycolysis of glucose (aerobic glycolysis: Warburg Effect [67]) as a carbon source, *de novo* lipogenesis is increased in cancers [68–70]. This glucose-lipogenic conversion occurs at early stage of cancer development and expands as malignant progression. The activation of *de novo* lipogenesis is required for tumor cell survival and

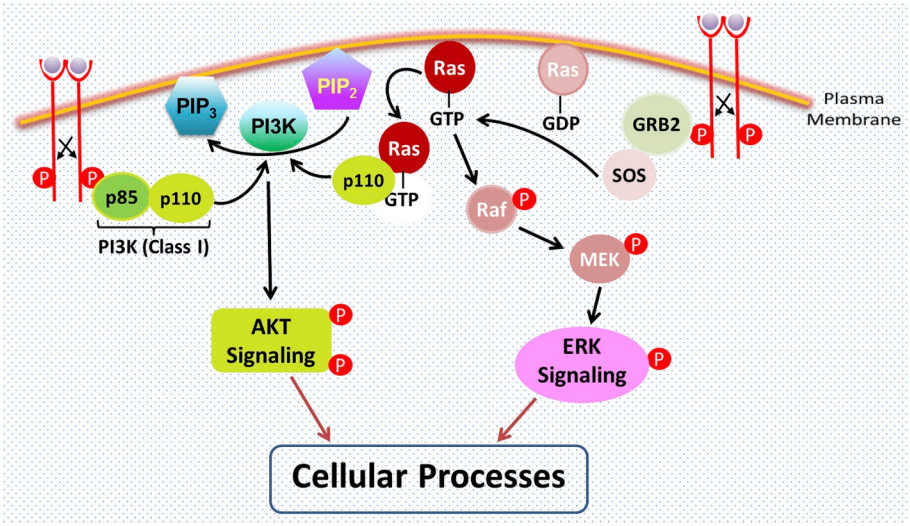

Fig. 1. Ras as a node for activation of MAPK/ERK and PI3K/AKT pathways. Receptor tyrosine kinases (RTKs) is activated through autophosphorylation of tyrosine residues after binding of ligands, which recruits GRB2 and SOS complexes to convert Ras-GDP (inactive) to Ras-GTP (active). Activated Ras recruits and activates Raf, activating ERK signaling. Activated Ras could also recruits p110 catalytic subunit of PI3K, which phosphorylates membrane lipid PIP_2 to PIP_3. PIP_3 then functions as a docking site for AKT recruitment and phosphorylation by PDK1 and PDK2, triggering AKT signaling. Activated RTKs could also serve as a dock for p85 regulatory subunit of PI3K, which then recruits p110 catalytic subunit of PI3K, forming an active complex.

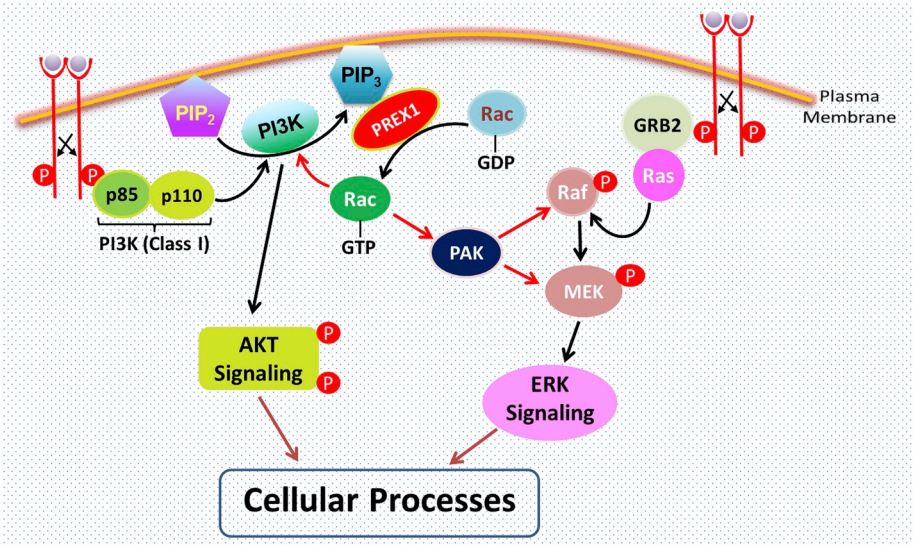

Fig. 3. PREX1 as an activator of both PI3K/AKT and MAPK/ERK pathways. Receptor tyrosine kinases (RTKs) and G-protein coupled receptors (GPCRs) activate PI3K that phosphorylates PIP_2 to PIP_3. PIP_3 recruits PREX1 to the membrane via PH domain to convert Rac-GDP (inactive) to Rac-GTP (active). Activated Rac activates PI3K/p110β, triggering AKT signaling; active Rac also stimulates PAK-mediated Raf/MEK/ERK signaling cascade.

serves as an indicator of poor prognosis [69,71]. Yet tumor cells synthesize *de novo* up to 95% of saturated and mono-unsaturated fatty acids even in sufficiency of dietary lipid availability [72]. The newly synthesized lipids serve as components of plasma membrane and form lipid rafts, facilitating signaling transduction. In cancer cells, the elevated *de novo* lipogenesis leads to increase of membrane PI, activating the PIP_2 second messenger system [73]. We found that aldo-keto reductase 1B10 (AKR1B10) promotes *de novo* lipogenesis by preventing ubiquitination and proteasomal degradation of acetyl-CoA carboxylase-α (ACCA), a rate-limiting enzyme in *de novo* synthesis of long chain fatty acids [74], and thus increases cellular PIP_2 levels and PKC/ERK signaling activity [75]. Targeted expression or siRNA-mediated silencing of AKR1B10 affects the *de novo* lipid synthesis, cellular PIP_2/DAG levels, and PKC/ERK signaling activity [75].

PREX1 as an activator of both AKT and ERK signaling: Phosphatidylinositol-3,4,5-trisphosphate (PIP_3)-dependent Rac exchange factor 1 (PREX1) is a Rac guanine exchange factor (GEF) [76]. PREX1 activates both PI3K/AKT and MAPK/ERK signaling pathways promoting cancer cell proliferation (Fig. 3). Binding to PIP_3 through the PH domain, PREX1 is recruited onto plasma membrane where it activate Rho family GTPases Rac1/2/3 by exchanging associated GDP to GTP [77]. Active Rac can activate the p110β isoform of Class IA PI3K [78]; Rac also activates Raf/MEK/ERK signaling cascade through a PAK-mediated mechanism [79,80]. Particularly in PI3K/AKT signaling, PREX1 lies downstream and upstream of PI3K; PI3K- catalyzed production of PIP_3 activates PREX1, and activated PREX1 triggers the p110β of PI3K [78]. Hence, PREX1 is an important guanine nucleotide exchange factor in PI3K signaling and a bridge for crosstalk between the PI3K/AKT and MAPK/ERK pathways.

Other cross-talk between PI3K/AKT and MAPK/ERK pathways: AKT could directly inhibit the MAPK/ERK pathway. Activity of Raf is negatively regulated by phosphorylation on the conserved region (CR) 2 regulatory domain; AKT can phosphorylate Ser259 on Raf-1 or Ser364 and Ser428 on B-Raf in CR2 domain and inhibits Raf activity [81,82].

In summary, the PI3K/AKT and MAPK/ERK pathways are two important signaling cascades in various cellular activities, but their crosstalk makes them as two ends of a balance in targeted cancer therapies. Inhibition of one pathway may compensatively provoke the other, attenuating the targeted therapeutic efficacy (Fig. 4).

3. PI3K/AKT and MAPK/ERK targeted cancer therapy

The oncogenic role and aberrant activation of PI3K/AKT and MAPK/ERK signaling cascades in cancer have driven tremendous efforts in exploration of specific inhibitors for targeted cancer therapy; numerous small molecule inhibitors have been developed and tested in preclinical models. However, only a few inhibitors have been approved by FDA for clinical use thus far and the benefit of cancer treatment is limited due to rapid drug resistance.

3.1. PI3K/AKT targeted cancer therapy

To date, numerous small molecule inhibitors have been developed, which target PI3K, AKT or mTOR; over 40 compounds have been tested in clinical use in a range of cancers, but clinical development of many chemicals was terminated before late-phase randomized trials due to limited antitumor activity and/or prohibitive host toxicities. Only two PI3K inhibitors (idelalisib and copanlisib) and two mTOR inhibitors (temsirolimus and everolimus) have been approved by FDA for clinical treatment of cancers [83]. AKT inhibitors are still in early phase of clinical tests, and agents that target AKT E17K mutant appear encouraging [84]. Table 1 summarizes the selected PI3K/AKT inhibitors approved by FDA or under clinical investigation.

Idelalisib is a selective inhibitor of the PI3K catalytic subunit δ and was the first PI3K inhibitor approved for treatment of chronic lymphocytic leukemia (CLL) in combination with rituximab [85]. This regimen improves overall response rate (ORR), progression-free survival (PFS) and overall survival (OS) of CLL patients when compared to rituximab alone. Idelalisib plus rituximab improved PFS at 24 weeks to 93% vs. 46% in rituximab plus placebo and 1 year overall survival to 92% vs. 80% [85]. However, grade 3 or 4 adverse events occurred in 56% of patients in the idelalisib arm, including severe diarrhea, rash, and a high risk of serious immune-mediated hepatotoxicity, pneumonitis, and infection [85]. Idelalisib is also approved by FDA as a monotherapy for relapsed follicular B cell non-Hodgkin's lymphoma and relapsed small lymphocytic leukemia (SLL), but over 50% of patients had serious side effects [86]. In fact, six clinical trials of idelalisib in combination with other agents were halted due to safety concerns (FDA alerts, 2016) [87]. Copanlisib is a pan PI3K inhibitor that received accelerated approval from FDA for treatment of refractory follicular lymphoma in September 2017. Hyperglycaemia and nausea are the major side effects [88].

Temsirolimus is a prodrug of the allosteric mTORC1 inhibitor rapamycin and is approved for the treatment of advanced renal cell carcinoma. Temsirolimus as a monotherapy improved overall survival to 10.9 months vs. 7.3 months in controls, but the objective response was observed only in 8.6% of patients [89]. Everolimus is also a derivative of rapamycin administered orally and approved by FDA for treatment of

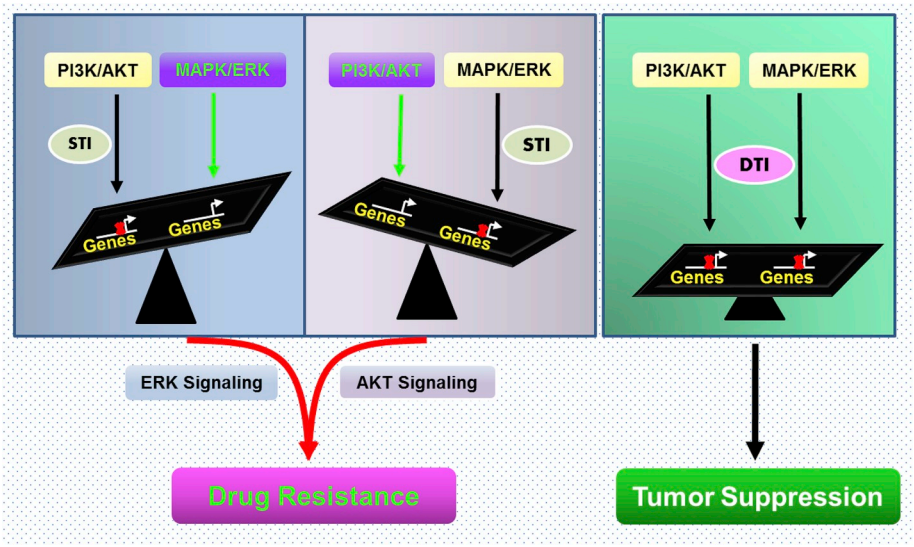

Fig. 4. Compensation between PI3K/AKT and MAPK/ERK pathways. Due to the broad cross-talk between the PI3K/AKT and MAPK/ERK pathways, targeted inhibition by small molecules of either pathway may lead to compensative activation of the other. As the PI3K/AKT and MAPK/ERK pathways are both important signaling with largely functional overlaps in regulation of cell growth, proliferation, survival and apoptosis, a compensative activation of one pathway would largely attenuate the effect of targeted inhibition on the other, causing drug resistance. This drawback may be solved potentially by AKT/ERK dual inhibitors. STI, single target inhibitors; DTI, dual target inhibitors.

advanced renal cell carcinoma, hormone receptor-positive HER2-negative breast cancer, pancreatic neuroendrocrine tumors [83]. Everolimus as a monotherapy for advanced renal cell carcinoma slightly improved PFS (median 4.0 months vs. 1.9 months in control), but improved PFS was not translated to overall survival [90]. The overall response rate of everolimus was low at 1%–5% [90,91]. A higher response rate of 9.5% was gotten only in advanced hormone receptor-positive HER2-negative breast cancer when combined with aromatase inhibitors letrozole or anastrozole [92].

In general, considerable efforts have been invested in the preclinical and clinical development of agents targeting the PI3K/AKT pathway, but clinical achievements are incomparable to the investment. A few agents are approved by FDA for clinical use, but overall responsive rate is low, resistance emerges quickly, and safety concerns arise due to on-target and off-target toxicity that often causes limitation of exposure doses. A few potential strategies may be applied to improve the PI3K/AKT targeted cancer therapy. First, combination with other therapeutic agents, such as chemotherapy or other target therapy, may improve the objective response rate. For instance, the combination of mTOR inhibitor everolimus with aromatase inhibitor exemestane is approved by FDA for treatment of hormone receptor-positive breast cancer [92]. However, safety profile of the combined therapy is often a concern. Some combination trials were halted due to prohibitive adverse effects, such as the combination of pan-PI3K inhibitor buparlisib with fulvestrant, a selective estrogen receptor degrader [93]. Second, optimization of dosing schedules, such as intermittent vs. continuous dosing strategies may reduce dose limiting toxicities and improve the depth and duration of target inhibition [94]. Finally, development of predictive markers and personalization of treatment may improve objective response rate of PI3K/AKT inhibitors and patient survival. For instance, PI3Kα-specific inhibitors demonstrated promising response in patients with *PIK3CA* mutants when compared to patients with wild type *PIK3CA* [95].

3.2. MAPK/ERK targeted cancer therapy

The MAPK/ERK signaling pathway has also been targeted for cancer therapy (Table 2). In four important effectors of the MAPK/ERK pathway, efforts targeting Ras protein is challenging although aberrant activation of Ras occurs frequently. This is ascribed to the smooth and floppy tertiary structure of Ras protein and lack of binding pocket for small molecules [96]. In addition, affinity of Ras to GTP is extremely high so that it is nearly impossible to develop competitive small molecules [97]. Agents that target the K-Ras mutant G12C appear promising, but are at the stage of laboratory modeling [98].

A few clinical successes have been achieved in Raf and MEK inhibitors. Sorafenib, an orally available Raf inhibitor is approved for treatment of renal and hepatocellular carcinomas [99,100]. Sorafenib at 400 mg twice daily improved PFS with a median 167 days for sorafenib vs. 84 days for placebo [99]. Selective B-Raf small inhibitor vemurafenib is a B-Raf V600E mutant selective inhibitor approved for metastatic and unresectable B-Raf mutated melanoma [101]; Vemurafenib had a median OS of 13.6 months compared to 9.7 months for chemotherapy agent dacarbazine [102]. Selective B-Raf inhibitor dabrafenib is approved for B-Raf V600K mutated metastatic melanoma [103]. Trametinib is only MEK inhibitor approved by FDA for B-Raf V600E mutant metastatic melanoma [104]. Interestingly, combination of B-Raf inhibitors with MEK inhibitors demonstrated better tolerance than respective inhibitor used alone via inhibition of paradoxical activation of MAPK signaling in B-Raf wild type tissues [105]; and FDA has approved dabrafenib in combination with trametinib for treatment of B-Raf V600 E/K mutant metastatic melanoma [106]. A phase III clinical trial of 423 patients with metastatic B-Raf V600 E/K mutant melanomas demonstrated that the 3-year progression-free survival was 22% for dabrafenib plus trametinib vs. 12% with dabrafenib monotherapy [107].

ERK1/2 at the end of the MAPK signaling pathway carries out cellular processes and is targeted to overcome acquired resistance of upstream kinase inhibitors [108,109]. To date, two ERK1/2 inhibitors have reached clinical trials (Phase I or I/IIa). BVD-523 is an ATP competitive selective inhibitor with an IC_{50} at 300 and 40 pM for ERK1 and ERK2. BVD-523 demonstrated antiproliferative activity in cells resistant to MAPK inhibitors and is in two phase I/II clinical trials for solid tumors and hematologic malignancy [110]. GDC-0994, with an IC_{50} of 6.1 and 3.1 nM for ERK1 and ERK2 [111], is in a dose escalation trial in patients with locally advanced or metastatic solid tumors.

In general, a few MAPK/ERK pathway inhibitors have achieved benefits in melanoma patients, but drug resistance occurs frequently; a single drug resistance may lead to cancer resistance to multiple drugs [112], and selective B-Raf mutant inhibitors may work only in metastatic melanoma, but not in other cancers with same mutants, such as colorectal cancer, glioblastoma, and non-small cell lung cancer. In addition, B-Raf mutant inhibitors may cause the paradoxical activation of the MAPK/ERK signaling in B-Raf wild type cells, leading to host toxicities [113,114]. Unique to inhibitors targeting the MAPK/ERK pathway, combination of different agents for the same target or for different targets in the same pathway may markedly reduce resistance and improve efficacy compared to a single agent [112]. Combination of

Table 1
Selected PI3K/AKT inhibitors approved by FDA or under clinical trials.

Inhibitors [References]	Targets	Disease Settings	Clinical Stage	Dose & Delivery	Efficacy	Toxicities
Idelalisib [85]	PI3Kδ	Relapsed follicular B cell non-Hodgkin's lymphoma, relapsed small lymphocytic leukemia, and chronic lymphocytic leukemia	FDA approved	150 mg twice a day, oral	ORR 81%, PFS (24 weeks) 93%, OS (1 year) 92%	Grade 3 or 4 diarrhea and rash in > 50% cases; high risk of serious hepatotoxicity, pneumonitis, and infection
Copanlisib [133]	Pan-PI3K	Refractory follicular lymphoma	FDA approved	60 mg on days 1, 8 and 15 of a 28-day cycle, i.v.	ORR 59%, CR 12%, PFS 11.2 mon.	Grade 3 or 4 hyperglycemia (41%) and hypertension (24%)
Temsirolimus [89]	mTORC1	Advanced renal cell carcinoma	FDA approved	25 mg weekly, i.v.	ORR 8.6%, PFS (6 mon) 32.1%, OS (10.9 mon) and PFS (3.8 mon)	Grade 3 or 4 AEs in 67% cases, e.g., rash, peripheral edema, hyperglycemia & hyperlipidemia
Everolimus [90]	mTORC1	Advanced renal cell carcinoma, HR-positive HER2-negative breast cancer, neuroendrocrine tumors	FDA approved	10 mg once a day, oral	ORR 1–5%, PFS (4.0 mon); OS not improved	Grade 3 pneumonitis (2.9%), mild or moderate stomatitis (40%), rash (25%), and fatigue (20%)
Alpelisib [95]	PI3Kα	Solid tumors	Phase I/II	30–450 mg once daily or 120–200 mg twice daily for 28 days, oral	At ≥ 270 mg once daily, ORR 6.0%, disease control rate 58.2%.	Hyperglycemia (51.5%), nausea (50.0%), decreased appetite (41.8%), diarrhea (40.3%), and vomiting (31.3%)
GSK2636771 [134]	PI3Kβ	Solid tumors	Phase I/II	25–500 mg once daily, Oral.	Response: > 1 year in 1 case and ≥24 weeks in 10 patients	Diarrhea (48%), nausea (40%), and vomiting (31%)
Ridaforolimus [135]	mTORC1	Advanced soft tissue sarcoma	Phase III	40 mg once a day for 5 days/week oral	Modest, OS 90.6 weeks vs. 85.3weeks in placebo	Grade 3 or 4 AEs in 67% cases, e.g., stomatitis, infections, fatigue, pneumonitis
Ipatasertib [136]	AKT	Breast or prostate cancer	Phase II/III (ongoing)	25–800 mg once daily for 21 days, followed by 7 days off, oral	Stable disease: 30% (16 of 52 patients)	Grade 3 or 4 AEs: asthenia, skin eruption, diarrhea, and hyperglycemia

AEs, adverse events; CR, complete response; ORR, overall response rate; PFS, progression-free survival in median, PR, partial response; and OS, overall survival.

Table 2
Selected MAPK/ERK inhibitors approved by FDA or under clinical trials.

Inhibitors [References]	Targets	Disease Settings	Clinical Stage	Dose & Delivery	Efficacy	Toxicities
Sorafenib [99]	Raf	Renal and hepatocellular carcinomas	FDA approved	400 mg, twice daily, oral	PFS 167 days vs. 84 days for placebo; Overall preliminary	Grade 3 AEs: hand-foot skin reaction (6%), fatigue (5%), and hypertension (3%)
Vemurafenib [137]	B-Raf V600E mutant	B-Raf V600E mutated metastatic and unresectable melanoma	FDA approved	960 mg twice daily, oral	ORR 48%; OS at 6 mon 84%, PFS 5.3 mon	Grade 2 or higher AEs > 5%, e.g., arthralgia, headache, fatigue, rash, nausea, vomiting and diarrhea.
Dabrafenib [103]	B-Raf V600K mutant	B-Raf V600K mutated metastatic melanoma	FDA approved	150 mg, twice daily, oral	PFS 5.1 mon,	Grade 2 or higher AEs 53%, e.g., skin toxic effects, fever, fatigue, arthralgia & headache
Trametinib [104]	MEK	B-Raf V600E mutated metastatic melanoma	FDA approved	0·125–4·0 mg once daily, oral	CR 6.7%, PR 33.3%, PFS 5.7 mon	Grade 2 or lower AEs: rash/dermatitis acneiform 82% and diarrhea 45%
Binimetinib [138]	MEK	Ras mutated relapsed or refractory or naïve acute myeloid leukemia	Phase II	30 or 45 mg twice daily, oral	CR 8.0% (1 0f 13 patients), not responded 92% (12 of 13 patients)	Grade 3 or 4 AEs: hypokalemia (6%), hypotension (6%), lung infection (6%), and febrile neutropenia (6%)
Selumetinib [139]	MEK	B-Raf V600 E/K mutated melanoma	Phase II	75 mg twice daily, oral	No response in 10 cases with high pAKT; PR or nearly PR in 3 of 5 cases with low pAKT	Grade 3 or 4 AEs: rash, elevated liver function tests, lymphopenia, hypoalbuminemia, and dyspnea
BVD-523 [140]	ERK1/2	Solid tumors and hematologic malignancy	Phase I	10–900 mg twice daily, oral	PR 17% at 600 mg twice daily or higher	Common AEs: diarrhea (48%), fatigue (42%), nausea (41%) and dermatitis acneiform (31%)
GDC-0994 [NCT01875705]	ERK1/2	Locally Advanced or Metastatic solid tumors	Phase I	Oral, Escalating doses	Data not posted	Data not posted
SML-8-73-1 [98]	K-Ras mutant G12C	Not available	Laboratory	Not available	Not available	Not available
SML-10-70-1 [98]	K-Ras mutant G12C	Not available	Laboratory	Not available	Not available	Not available

AEs, adverse events; CR, complete response; ORR, overall response rate; PFS, progression-free survival in median, PR, partial response; and OS, overall survival.

MAPK/ERK inhibitors with other therapies, such as immunotherapy and PI3K/Akt target therapy (see below) may also improve the treatment efficacy. Also, optimization of dosing schedules and development of predictive markers and personalization of MAPK/ERK inhibitors are also practical approaches to improve the effectiveness.

3.3. Combination of PI3K/AKT inhibitors and MAPK/ERK inhibitors for cancer therapy

In view of close crosstalk between PI3K/AKT and MAPK/ERK pathways and limited clinical efficacy in cancer therapy targeting either pathway alone [23], combination therapy with PI3K/AKT inhibitors and MAPK/ERK inhibitors has emerged as a promising strategy. Yet numerous preclinical studies have demonstrated synergistic antiproliferative activity in various cancer cell lines and tumor xenografts in animals, including basal-like breast cancer models [22–24,115,116]. These promising preclinical achievements have encouraged active clinical studies in the combination of PI3K/AKT inhibitors with MAPK/ERK inhibitors.

A phase I clinical trial with a PI3K inhibitor pictilisib and an MEK inhibitor cobimetinib in 78 patients yielded therapeutic activity, but also severer side effects [24]. A clinical trial with a PI3K inhibitor buparlisib plus a B-Raf inhibitor vemurafenib was terminated due to dose limiting toxicities HYPERLINK "http://[ClinicalTrials.gov" \o "http://[ClinicalTrials.gov"**Error! Hyperlink reference not valid.** Identifier: NCT01512251]. The mTOR inhibitor everolimus in combination with a MEK inhibitor trametinib was evaluated in 67 patients with advanced solid tumors, but failed to obtain a recommended dose and schedule for a phase II study due to tolerability issues [117]. A combination of mTOR inhibitor temsirolimus with MEK inhibitor pimasertib showed significantly greater incidence and severity of mucositis when compared to monotherapy of these agents [118].

Combination of AKT inhibitors with MEK inhibitors are being tested in clinics (Table 3). This combination also showed a tolerability issue. In a clinical trial with trametinib and uprosertib (ClinicalTrials.gov Identifier: NCT01907815), prohibitive adverse events occurred in 11/16 (68.75%) participants at 2.0 mg trametinib plus 25 mg uprosertib. The side effects occurred even in 6/7 (85.71%) participants when 1.5 mg trametinib and 50 mg uprosertib were used. Clinical trials of AKT inhibitor afuresertib or uprosertib with MEK inhibitor trametinib in patients with advanced solid tumors showed similar tolerability issues [24,119].

Certainly, the rationale for simultaneous targeting of cross-talked PI3K/AKT and MAPK/ERK pathways is concrete, but clinical tests of combinatory regimens are not encouraging due to tolerability and resultant preclusion of optimal therapeutic doses. Other challenges include drug resistance and high variability of patient responses.

4. Akt/erk dual inhibitors: a bend in the road to targeted therapy?

ONC201, also known as NSC350625 or TRAIL-inducing compound 10 (TIC10), is identified by a screening of small molecules that induce tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) [120]. ONC201 dually inhibits AKT and ERK and upregulated TRAIL that triggers apoptosis by activation of death receptors 4 and 5 (DR4 and DR5) [120–122]. Recent studies showed that ONC201 also triggers cell death through TRAIL-independent mechanisms, such as activation of the integrated stress response (ATF4/CHOP) pathway and down-regulation of cyclin D1, inhibitor of apoptosis (IAP) and Bcl-2 family members [123,124]. ONC201 induces cell death in a wide range of solid and hematopoietic malignancies independent of tumor types or mutations, including colon cancer, breast cancer, brain cancer, acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia, chronic lymphocytic leukemia, diffuse large B-cell lymphoma, Burkitt's lymphoma, anaplastic large cell lymphoma, cutaneous T-cell lymphoma, mantle cell lymphoma, Hodgkin's lymphoma, and multiple myeloma [122,125]. Furthermore, ONC201 is featured with stability, oral availability, excellent tolerability, and penetration of the blood brain barrier [121].

ONC201 has soon entered clinical tests in view of the encouraging efficacy, well tolerated safety and most importantly the novel mechanisms of dual inhibition of AKT and ERK. A first-in-human phase I study of ONC201 that recruited 28 patients with advanced solid tumors showed that ONC201 at 625 mg, a dose of 5 fold higher than that in preclinical models, achieved 3.9–19.4 μmol/L plasma concentrations with a mean half-life of 11.3 h, and at this dose, no grade > 1 drug-related adverse events were recorded [126]. A Phase II study of 17 patients with recurrent or refractory glioblastoma at 625 mg ONC201 every 3 weeks achieved promising response, and one patient remained disease-free for > 12 months; no drug-related serious adverse events occurred and no treatment was discontinued due to toxicities [127]. Currently, 15 active phase I/II clinical trials are going on for various advanced cancers, including neuroendocrine tumors, endometrial cancer, glioma, breast cancer, myeloma, leukemia and lymphoma (Table 4). One trial was suspended due to a funding issue, and one was withdrawn for a protocol issue. The ONC201 is a highly promising dual inhibitor of AKT and ERK and cancer therapy agent.

5. Conclusion

The PI3K/AKT and MAPK/ERK pathways are important oncogenic signaling cascades. These two cascades are aberrantly activated in many human cancers due to amplification, mutations and/or overexpression of effector genes, which makes a solid rationale for targeted therapy of cancers. In the past decades, tremendous efforts have been

Table 3
Clinical trials of AKT inhibitors in combination with MEK inhibitors.

	Agent Combination			
	AKT inhibitors	MEK/ERK inhibitors	Diseases/Conditions	ClinicalTrials.gov Identifier Number
1	Afuresertib (**GSK2110183**)	Trametinib (GSK1120212)	Multiple myeloma or solid tumors	NCT01476137
2	Uprosertib (GSK2141795)	Trametinib	Recurrent or persistent endometrial cancer	NCT01935973
3	Uprosertib	Trametinib	Melanoma	NCT01979523
4	Uprosertib	Trametinib	Relapsed or refractory myeloma	NCT01989598
5	MK-2206	Selumetinib (AZD6244)	Advanced colorectal carcinoma	NCT01333475
6	MK-2206	Selumetinib	Multiple pancreatic cancer	NCT01658943
7	MK-2206	Selumetinib	Lung cancer and thymic Malignancies	NCT01306045
8	Uprosertib	Trametinib	ER − /HER2- invasive breast cancer	NCT01964924
9	Ipatasertib (RG7440)	**Cobimetinib**	Locally advanced or metastatic cancer	NCT01562275
10	Ipatasertib	**Cobimetinib**	Breast cancer and ER^+ breast cancer	NCT03395899
11	MK-2206	Selumetinib	Advanced solid tumors	NCT01021748
12	Uprosertib	Trametinib	Metastatic triple negative breast cancer	NCT01964924
13	MK2206	Selumetinib	Advanced melanoma	NCT01519427
14	MK2206	Selumetinib	Advanced colorectal cancer	NCT01333475

Table 4
Clinical trials of AKT and ERK dual inhibitor ONC201.

	Agents	Phases	Diseases/Conditions	Identifier Number
1	ONC201	II	Recurrent and metastatic Neuroendocrine Tumor	NCT03034200
2	ONC201	I	Malignant glioma and diffuse intrinsic pontine glioma	NCT03416530
3	ONC201	I/II	Multiple Myeloma	NCT02863991
4	ONC201 + Cytarabine	I	Leukemia	NCT02392572
5	ONC201 + Methionine-restricted diet	II	Triple negative breast cancer	NCT03733119
6	ONC201	II	Glioma	NCT03295396
7	ONC201 + Behavioral (Phone Call)	I/II	Relapsed/refractory non-Hodgkin's lymphoma	NCT02420795
8	ONC201	I	Glioblastoma, H3 K27 M glioma, and midline glioma	NCT02525692
9	ONC201	**II**	Recurrent endometrial cancer	NCT03485729
10	ONC201	**II**	Triple negative breast cancer, hormone receptor positive, her2 negative breast cancer and endometrial cancer	NCT03394027
11	ONC201	II	Endometrial cancer	NCT03099499
12	ONC201 + Ixazomib + Dexamethasone	I/II	Multiple myeloma	NCT03492138
13	ONC201 + Biomarkers	I	Unspecified adult solid tumor	NCT02324621
14	ONC201, multiple doses	I/II	Metastatic colorectal cancer	NCT03791398
15	ONC201 +	I	Advanced solid tumors and multiple myeloma	NCT02609230

put in the development and clinical investigation of small molecule inhibitors for targeted inhibition of the PI3K/AKT or MAPK/ERK pathway, but just a few inhibitors are eventually approved by FDA for clinical use. While these approved inhibitors achieve clinical benefits in cancer patients, a fact well recognized is the limited response rate and duration because of rapidly developed drug resistance. Evidenced cross-talk between the PI3K/AKT and MAPK/ERK pathways endorses a rational combination of PI3K/AKT inhibitors and MAPK/ERK inhibitors, but clinical practice fails to achieve encouraging results because of prohibitive adverse effects and limitation of exposure doses. ONC201 as an AKT and ERK dual inhibitor has achieved promising success in preclinical models and early clinical tests. It may be expected that this chemical would soon be approved by FDA for clinical use in one or multiple cancers. Exploration of small molecules that dually inhibit the PI3K/AKT and MAPK/ERK pathways may represent a novel practical strategy in the development of new anticancer agents.

It is noteworthy to keep in mind the heterogeneity and complexity of signaling network in cancer cells. PI3K/AKT and MAPK/ERK pathways are important oncogenic cascades and play a critical role in cancer development and progression, but other oncogenic pathways, such as WNT signaling in colorectal cancer, breast cancer and hepatocellular cancer [128], may be intrinsically activated or induced in response to inhibition of the PI3K/AKT and MAPK/ERK pathways. Therefore, even promising AKT and ERK dual inhibitors may face unexpected limits in clinical practice. Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (PIN1) isomerizes the phospho-Serine/Threonine-Proline motifs and regulates protein function in a post phosphorylation manner [129,130]. PIN1 thus function as a central signaling regulator in many pathways that are regulated by proline-directed phosphorylation and has emerged as a promising target for cancer therapy [131]. PIN1 inhibitors have been developed and demonstrated encouraging anti-proliferative activity by blocking multiple cancer-driving signaling pathways in cancer cells, thus being a new class of small molecules for targeted cancer therapy [131,132].

List of abbreviations

ACCA, acetyl-CoA carboxylase-α; AKR1B10, aldo-keto reductase 1B10; CLL, chronic lymphocytic leukemia; CR, conserved region; DAG, diacylglycerol; DR, death receptor; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; FOXO3a, Forkhead box O3; GPCR, G proteins coupled receptors; GSK3β, glycogen synthase kinase 3 beta; IκKα, IκB kinase alpha; IP_3, inositol trisphosphate; KSR, kinase suppressor of Ras; MAPK, mitogen-activated protein kinase; MDM2, mouse double minute 2 homolog; MEK, mitogen-activated protein/extracellular signal-regulated kinase; NF-κB, nuclear factor kappa B; ORR, overall response rate; OS, overall survival; PDK1, phosphoinositol-dependent kinase-1; PFS, progression-free survival; PH, pleckstrin homology; PI, phosphatidylinositol; PI3K, phosphatidylinositol 3-kinase; PIP_2, phosphatidylinositol (4,5)-trisphosphate; PIP_3, phosphatidylinositol (3,4,5)-trisphosphate; PKB, protein kinase B; PKC, protein kinase C; PLC, phospholipase C; PTEN, phosphatase tensin homolog; RKIP, Raf kinase inhibitory protein; RTK, receptor tyrosine kinase; SLL, small lymphocytic leukemia; TIC10, TRAIL-inducing compound 10.

Author contributions

ZC did literature search and wrote the draft, MS and KH aided in literature, QL contributed to proof-reading, JJ worked on revisions and DC revised and finalized the manuscript.

Competing interests

Authors declare that they have no competing interests.

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