Role of mTOR signaling pathway in acute myeloid leukemia

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Abstract: The mammals target protein of rapamycin (mTOR) signaling pathway is crucial to cell survival and proliferation. Alteration of the important components of the mTOR pathway has significant effects on leukemogenesis and resistance to conventional chemotherapy. Thus, we reviewed recent researches of mTOR signaling pathway in acute myeloid leukemia (AML). The inhibitors targeting phosphatidylinositol 3-kinease (PI3K)/protein kinase B (AKT)/mTOR pathway are applied to many experiments, alone or in combination with cytotoxic drugs. However, some inhibitors alone did not block off the pathway successfully, even lead to reactivation of the pathway because of the existence of feedback mechanism, while the combination of inhibitors or cytotoxic drugs acquire stronger inhibitive effect. Liver kinase B1 (LKB1)/adenosine monophosphate-activated protein kinase (AMPK)/mTOR pathway is known as a tumor suppression axis while some experiments demonstrate it prolongs the proliferation of AML cells. In a word, the role of mTOR pathway in AML is complicated and needs further investigation.

Keywords: mTOR, AML, PI3K/AKT, LKB1/AMPK

Introduction

Acute myeloid leukemia (AML), the most common malignancy of hematological system, is characterized by a deregulated proliferation of immature myeloid progenitors, leading to the accumulation of leukemic cells in the bone marrow and inhibition of normal hematopoiesis. It has a low complete recession and short disease free survival [1]. Mammals target protein of rapamycin (mTOR) is a vital downstream effector of phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) and liver kinase B1 (LKB1)/adenosine monophosphate-activated protein kinase (AMPK) pathway, and it controls cell growth, proliferation and survival through essential signaling pathway [2]. In AML, deregulation of mTOR signaling pathway may enhance the survival and proliferation of abnormal hematopoietic cells, and contribute to the development and occurrence of AML [3]. We reviewed recent researches on PI3K/AKT/mTOR, LKB1/AMPK/mTOR pathway, and their roles in AML, by which we hope to find new inspirations for AML treatment.

Structure of mTOR

mTOR, an extremely conservative protein, is a 289 kDa serine/threonine kinase belonging to the PI3K-related protein kinase (PIKKs) family [2]. It plays a central role in the regulation of cell growth and metabolism [4, 5] and encompasses two functionally distinct protein complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [6].

mTORC1, consisting of mTOR, raptor, mLST8, PRAS40 and DEPTOR, is sensitive to rapamycin and its analogue [7]. It is the main downstream effector of PI3K/AKT pathway, through activating p70S6K and 4E-BP1 associated with mRNA translation [8]. mTORC2, which is insensitive to rapamycin differently, contains Mtor, Rictor, mLST8, mSin1 and Protor [9]. It regulates cellular survival and proliferation indirectly by phosphorylating AKT at Ser473 [10].

Physiological functions of mTOR related pathways

As a conservative serine/threonine kinase, mTOR plays a considerable role in cellular sur-
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**Figure 1.** Diagram of the mTOR signaling pathway. RTKs (for example, IGF-1R) stimulate class IA PI3K activity. PI3K generates PIP3 from PIP2. PIP3 attracts PDK1 which phosphorylates Akt at Thr 308, to the plasma membrane. Full Akt activation requires Ser 473 phosphorylation by mTORC2. Active Akt phosphorylates TSC2 to inhibit TSC1/TSC2 complex activity. TSC1/TSC2 complex inactivation allows Rheb to accumulate in a GTP-bound state. Rheb-GTP then stimulates mTORC1. mTORC1 targets p70S6K, 4E-BP1 and eIF4E which are critical for mRNA translation. p70S6K controls activation of PI3K through an inhibitory loop which involves IRS-1/2. Active Akt inhibit FOXO family activation of stimulating the expression of RTKs. Another pathway involves LKB1 and AMPK. LKB1 phosphorylates AMPK at Thr 172. Active AMPK stimulates TSC1/TSC2 complex which can inhibits the activation of Rheb, negatively regulating mTORC1 and inhibiting the translation of proteins. (→"represents “promote”; “┻” represents “inhibit”).

vival, proliferation and metabolism. mTORC1, one of the mTOR complex, is an important downstream effector of PI3K/AKT and LKB1/AMPK pathway which play critical roles in hematopoiesis [11].

**PI3K/AKT/mTOR pathway**

PI3K can be classified into three classes (PI3K-I-III) according the differences of substrate, and all of them belong to serine/threonine kinase [9]. Class IA PI3K is a heterodimer composed of a p110 catalytic subunit and a p85 (p50/p55) regulatory subunit and is activated by receptors tyrosine kinase (RTKs), such as insulin growth factor-1 receptor (IGF-1R) and insulin receptor (IR) [1, 8]. PI3K controls and regulates survival, proliferation, differentiation and apoptosis of various cells by phosphorylating AKT [10, 12-14]. AKT, also known as Protein Kinase B (PKB), is a 57 kDa serine/threonine kinase [15] who contributes to proliferation and apoptosis of cells [16]. The activation of AKT, which influences the conformation and development of cells, can be found in many types of tumors [17].

PI3K is activated by the combination of growth factors and RTKs. Activated PI3K phosphorylates the phosphatidyl-inositol bisphosphate (PIP2) to generate phosphatidyl-inositol triphosphate (PIP3) [8]. PIP3 recruits PDK1 and
AKT to the plasma membrane where PDK1 phosphorylates AKT on Thr308. The proteins recruited by PI3K, including PDK1 and AKT, have the pleckstrin-homology domain (PHD). At the same time, mTORC2 phosphorylates AKT on Ser473. So far, AKT is fully activated [1, 18]. PTEN, located in chromosome 10, can negatively regulate PI3K/AKT pathway by dephosphorylating PIP3 to PIP2, leading to a reduced recruitment of AKT to the cell membrane [3] (Figure 1).

Activated AKT stimulates its substrate mTORC1 through deactivating tuberous sclerosis complex (TSC). TSC2 is a downstream effector of phosphorylated AKT. Phosphorylation of TSC2 prevents TSC1/TSC2 complex formation, which inhibits the small GTPase Rheb into the GTP-bound active state, leading to the activation of mTORC1 at Ser2448 [2]. Therefore, lost of TSC1 or overexpression of TSC2 can lead to over activation of PI3K/AKT/mTOR pathway [19, 20]. Activated mTORC1 phosphorylates downstream effectors, including p70S6 kinase 1 (p70S6K1) and 4E-binding protein 1 (4EBP1). Phosphorylated p70S6K1 regulates survival and proliferation of cells through enhancing translation of mRNA. 4EBP1 inhibits initiation of translation via combining eukaryotic translation initiation factor 4E (eIF4E). 4EBP1 can be phosphorylated by mTORC1 on multi-sites, which promotes the dissociation of eIF4E and 4EBP1. As a consequence, the inhibition of translation is removed and the proliferation started [18, 21].

**LKB1/AMPK/mTOR pathway**

LKB1, encoded by STKII gene in humans, is a serine/threonine kinase [7] and was recognized as a tumor inhibitor primarily [22]. LKB1 mutation was first found to be associated with Peutz-Jeghers syndrome [22, 23] that is characterized by gastrointestinal hamartomatous polyps, elevated risk of various neoplasms [24], and an extremely poor prognosis.

AMPK, a heterotrimer serine/threonine kinase, consists of one α-catalytic subunit, one β-regulatory subunit and one γ-regulatory subunit. AMPK is known as “energy receptor” for the cells, and it is a crucial element for energy metabolism. Energy stress reduces cellular adenosine triphosphate (ATP) concentrations and increases adenosine monophosphate (AMP) concentrations, increasing the AMP-to-ATP ratio. Under this condition, LKB1/AMPK pathway is activated, leading to ATP production and blocking anabolic processes that consume ATP. Thus, the AMP-to-ATP ratio recovers [25, 28].

When cells are in the shortage of energy, LKB1 activates AMPK via phosphorylating AMPK on Thr172 [29]. Activated AMPK recruits TSC1/TSC2 complex which inhibits the activation of Rheb, thereby negatively regulating mTORC1 and inhibiting the translation of proteins [23, 30, 31]. Hence, LKB1/AMPK pathway maintains the metabolic balance by inhibiting anabolic metabolism, and exerts anti-tumor effects by negatively regulating proliferation of cells [28]. In addition, p53 is also a downstream effector of AMPK, and it inhibits protein translation through dephosphorylating 4E-BP1 and p70S6K [32] (Figure 1). Interestingly, LKB1/AMPK is also involved in the regulation of protein degradation or autophagy through two main proteolytic systems, ubiquitin-proteasome system (UPS) and macroautophagy [33, 34].

**mTOR signaling pathways in AML**

Deregulation of mTOR signaling pathways is associated with poor prognosis, resistance to conventional chemotherapy and relapse in AML [35]. Therefore, a better comprehension of the pathway deregulation mechanism is extremely significant for the treatment of AML patients.

**PI3K/AKT/mTOR pathway in AML**

The deregulation of PI3K/AKT/mTOR pathway can be detected in 50%-80% AML patients [10, 36, 37]. It influences survival, proliferation and differentiation of leukemic cells [13] and contributes to the resistance to chemotherapeutic agents and recurrence of AML [38]. But, the distinct mechanism of abnormal activation of the pathway is still unclear. Thus, it is very significant to identify deregulation mechanism of PI3K/AKT/mTOR pathway in AML.

PI3K/AKT/mTOR pathway is upregulated by the overexpression or mutation of RTKs in cytomembrane. In bone marrow samples of 40 primary AML patients, down regulation of PI3K/AKT/mTOR pathway is found after using insulin growth factor-1 (IGF-1) inhibitor and specific
siRNA, or IGF-1R antigen, which indicates that IGF-1/IGF-1R signaling is responsible for constitutive PI3K/AKT activation in AML [39]. Insulin receptor substrate 1 (IRS1) that contains multiple tyrosine phosphorylation sites is the main substrate of IGF-1R. Its stimulation results in the activation of PI3K/AKT/mTOR and MAPK pathway (Figure 1). Inhibition of IRS1 reduces proliferation and downregulates PI3K/AKT/mTOR pathway in K562 cells [40]. FMS-like tyrosine kinase-3 (FLT3) stimulates PI3K/AKT/mTOR pathway that promotes survival of FLT3-mutated AML cells, which means that FLT-3 may be a upstream regulator of this pathway and can be taken as a target for the treatment of FLT3 mutation AML patients [18]. Spleen Tyrosine Kinase (SYK), a non-receptor tyrosine kinase that is broadly expressed in hematopoietic cells, regulates PI3K/AKT/mTOR pathway that is certificated via inhibiting or overexpressing SYK in various AML cell lines [41]. T-cell immunoglobulin and mucin domain 3 (Tim-3), located in plasma membrane, is highly expressed in human AML cells. It triggers growth factor type responses through integrating its specific ligand galectin-9 in AML cells, resulting in activating PI3K/AKT/mTOR pathway [42]. In addition, c-KIT, platelet derived growth factor receptor alpha (PDGFRa), epidermal growth factor receptor (EGFR), fibroblast growth factor receptor (FGFR) or colony-stimulating growth factor I (CSF-I) are also the activator of PI3K/AKT/mTOR pathway [43].

It is worth mentioning that the acute monocytic leukemia (M5)-associated antigen-34 (MLAA-34), a new M5 associated antigen, is identified by the method of serologic analysis of recombinant cDNA expression library (SEREX) in U937 cell. MLAA-34 may be a novel anti-apoptotic factor closely related to carcinogenesis or progression of M5 through downregulating MLAA-34 expression using its shRNA. The results indicate that inhibiting MLAA-34 can significantly suppress the proliferation of U937 cells in vitro, and increase the spontaneous apoptosis of these leukemia cells [44, 45]. Co-immunoprecipitation (Co-IP), shotgun and bioinformatics analysis has identified 71 proteins interacting with MLAA-34 in U937, including mTOR signaling pathway elements [46]. In most situations, the subcellular localization of MLAA-34 in U937 cells is determined on the cytomembrane [46] where the chain reaction of PI3K/AKT/mTOR from RTKs to AKT takes place as well. We hypothesize that MLAA-34 might work as an anti-apoptosis protein through interacting with RTKs, IRS1/2, PI3K, PDK1, PTEN or AKT protein. But this needs further verification.

Besides those upstream regulators of PI3K/AKT/mTOR pathway mentioned above, changes of other elements of pathway can also induce the deregulation of the pathway. For instance, PHD mutation of AKT is detected to activate the PI3K/AKT/mTOR pathway in some AML patients [47]. On the contrary, Raoul Tibes proposes there is no mutation in PHD of AKT in the bone marrow or peripheral blood samples of 49 primary AML patients [48]. Due to the limited sample size, this conclusion needs further research. Down-regulation or mutation of PTEN is related to leukemic stem cells (LSCs) survival, while normal PTEN maintains hematopoietic stem cells (HSCs) in their quiescent state [49]. Deletion [50] or aberrant transcripts [43] of PTEN are contributed to the deregulation of PI3K/AKT/mTOR pathway. PTEN deletions or mutations can be detected in AML, and it is worthwhile to investigate whether the presence of this alteration is associated with chemotherapy resistance and relapse or not [51]. PI3K mutation can be found in solid tumor, but not in AML so far [47]. DEPTOR, a naturally inhibitory subunits of both mTORC1 and mTORC2, is degraded by a kind of ubiquitin ligase, leading to mTOR activation and cell survival or proliferation in cancer [52].

Deregulation of PI3K/AKT/mTOR pathway in AML might be induced by the mutation of its own components or malformed upstream regulators. Therefore, targeting PI3K/AKT/mTOR pathway may be a new effective therapy for AML patients. There have been lots of researches on inhibitors targeting mTOR pathway, such as RTK inhibitors, dual PI3K/mTOR inhibitor, AKT inhibitors [53], especially MTIs [54, 55].

Deficiency of Raptor, a considerable subunit of mTORC1, can interrupt mTOR pathway, reduce the number of leukemia cells in the blood and bone marrow of AML mice model, and prolong the survival of the mice markedly [56, 57]. This outcome indicates that mTORC1 is closely related with carcinogenesis and progression of AML. But a subgroup of AML cells with undifferentiated phenotypes survive long time in the absence of mTORC1 activity in vitro [58], which
shows us that lacking of mTORC1 may contribute to proliferation of undifferentiated AML cells. So, what is the real role of mTORC1 in AML cells? It is complicated and needs further investigation. MTIs, the inhibitor of mTORC1, have already been studied for years in many fields [59]. Rapamycin (also named sirolimus), the first generation of MTIs, used as immunodepressant for post-kidney transplant treatment, can integrate mTORC1 and restrain proliferation or induce apoptosis of cells [60]. Rapamycin inhibits pre-B acute lymphoblastic leukemia cells via downregulating DNA and RNA polymerases and activating autophagy [61], as well as AML cells [62]. The second generation of MTIs, including temsirolimus, everolimus, deferolimus, is better than the first generation in water solubility and bioavailability. Moreover, dual mTORC1/2 targeting results in potent suppressive effects on AML progenitors than rapamycin alone in vitro [55, 63]. The mTOR inhibitor PP242, targeting of mTORC1/2, induces apoptosis in AML cells within the analogous bone marrow microenvironment [64]. Besides, polystyrene nanoparticles with amino groups (PS-NH2), functioning as a type of mTOR inhibitor, can induce cell cycle arrest and inhibit proliferation through inhibiting the activation of mTOR in leukemia cells [65]. Above all, targeting mTOR may be an efficient way for treatment of AML patients.

Activation of mTOR pathway relies on the combination of growth factors and RTKs, so RTKs inhibitors (RTKIs) may interrupt this pathway successfully. RTKIs exert antitumor effects and enhance growth inhibition via inhibiting mTOR pathway in multiple cell lines of hematological malignancies [66]. In addition, inhibition of PI3K activity impairs proliferation and triggers apoptosis of NB-4 cells in vitro [67].

However, reactivation of PI3K/AKT/mTOR pathway can be caused by some inhibitors alone, because of sundry feedback loops [68]. A negative regulation of PI3K/AKT/mTOR activity is dependent on p7OS6K-mediated phosphorylation of IRS-1/2 adapter proteins, downstream of the insulin receptor (IR) and/or IGF-1R. IRS-1/2 is required to activate PI3K after stimulation of IR/IGF-1R tyrosine kinase activity. When mTORC1 is activated, p7OS6K promotes the phosphorylation of IRS-1/2 on Ser residues, targeting them for proteasomal degradation [9] (Figure 1). When mTORC1 is inhibited, p7OS6K-mediated negative feedback is blocked and the degradation of IRS-1/2 is decreased. As a result, PI3K/AKT/mTOR and MAPK pathway is re/overactivated [69]. Another negative feedback is related to AKT inhibition inducing the expression and phosphorylation of multiple RTKs in cell membrane, and this may be mediated by a direct AKT kinase substrate, the Forkhead box O (FOXO) family of transcription factors. Unphosphorylated FOXOs reside in the nucleus where they regulate transcription of several target genes, including the activators of the PI3K/AKT/mTOR pathway IR and IGF-1R. Conversely, phosphorylated AKT determines nuclear exclusion and degradation of FOXOs [37, 70, 71]. That is, there is a converse relationship between the level of phosphorylated AKT and FOXOs activity. As a consequence, inhibition of AKT reduces degradation of its substrate FOXOs. FOXOs enhance the expression and phosphorylation of RTKs that could in turn activate and sustain PI3K/AKT/mTOR signaling pathway (Figure 1). In addition, interaction of pathways contributes to the reactivation of pathway or resistance to inhibitors as well.

In vitro, STAT5 activation by FLT3 can protect cells treated with the PI3K/AKT pathway inhibitors from apoptosis by interacting with mTORC1/4EBP1 pathway [72]. These results demonstrate the necessity of combining inhibitors with other targeted agents or chemotherapeutic drugs for the treatment of AML patients.

Combination of PI3K and mTOR inhibitors exerts synergistic antiproliferative effect in diverse cell lines of neoplastic hematologic disorders [73] and AML bone marrow samples [74]. This outcome is also detected in primary AML bone marrow samples by dual inhibitor of PI3K and mTOR NVP-BEZ235 [8] and S9 [12]. In some solid tumors, a new PI3K/mTOR dual inhibitor VS-5584 preferentially targets cancer stem cells (CSCs) in vivo and in vitro [75], but its function in AML has not been tested yet. Combining rapamycin with RTKs inhibitors shows more powerful depressant effect to leukemic cells in vitro [76]. Moreover, using PI3K/AKT/mTOR and other pathway inhibitors together displays extraordinarily synergistic antiproliferative and pro-apoptotic effect. For example, combination of PI3K inhibitor and Bcl-2/xL blockade [77, 78] or mitogen-extracellular activated protein kinase (MEK) inhibitor [79] can
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elevate the effect of inducing apoptosis of AML cells.

Besides, combination of inhibitors and cytotoxic drugs improves lethal effect of leukemic cells as well. PI3K inhibitor synergizes with the arsenic trioxide to eradicate AML stem cells through inducing differentiation [80]. Rapamycin cuts down the apoptotic threshold of the cells in the presence of anti-tumor drug etoposide [81]. A combination of temsirolimus, an allosteric MTIs, with clofarabine has synergistic pro-apoptosis effect in vitro [82]. Furthermore, blockade of mTOR signaling potentiates the ability of histone deacetylase inhibitor to induce growth arrest, differentiation and apoptosis of AML cells [83]. All of these results suggest that inhibitors of pathway may improve the effect of cytotoxic drugs, and may be a powerful therapy for AML patients.

Despite extensive studies of PI3K/AKT/mTOR pathway in AML have been made, it remains unsettled about the role of upstream regulators and whether these molecules can be targeted clinically. Leukemic cells' survival and proliferation can be weakened by targeting RTKs. But normal cells can also be influenced unavoidably because the activation of PI3K/AKT/mTOR pathway in both normal and leukemic cells is dependent on the activation of RTKs. Therefore, searching for specific upstream regulators of the deregulated pathway is vital and necessary for targeting therapy in AML patients.

Most of studies about PI3K/AKT/mTOR inhibitors show its strong effect of anti-proliferation and pro-apoptosis. Unfortunately, it doesn’t always work effectively in every AML patient. The failure of antileukemic effect of PI3K/AKT/mTOR pathway inhibitors may be related to the overexpression of cell division cycle 25B (CDC25B) which mediate resistance of rapamycin in AML mice models [84]. Therefore, further study is supposed to determine different effects of PI3K/AKT/mTOR inhibitors in primary AML patients and, in particular, search for differences in gene expression that could explain the differences in antileukemic effects between individuals.

LKB1/AMPK/mTOR pathway in AML

LKB1/AMPK/mTOR signaling pathway has tumor suppressor activity in AML through the repression of mTOR-dependent oncogenic mRNA translation [23]. Inhibiting expression of LKB1 gene induces tumorigenesis according to animal experiment in mice [22, 29]. Co-activation of AMPK and mTORC1 can induce cytotoxicity in cell lines of AML, so activating AMPK may represent a hopeful therapeutic opportunity in mTORC1-overactivated AML [85]. For these reasons, stimulation of LKB1/AMPK may be a useful therapy for AML or other malignancy [86]. Metformin belongs to the biguanide class of oral hypoglycemic agents and is a widely used antidiabetic drug. Interestingly, metformin inhibits cancer cell growth through activating the AMPK/mTOR axis in solid tumor and acute leukemia (AL) [7, 87-89]. There is an inverse correlation between the degree of ERK and AMPK activation after stimulating with either glucose deprivation or metformin. Due to negative regulation of LKB1/AMPK pathway by ERK, inhibition of ERK pathway combining with metformin significantly strengthens apoptosis of AML cells in vitro [90].

On the contrary, inhibition of AMPK enhances apoptosis of MLL-rearranged pediatric B-acute lymphoblastic leukemia (ALL) cells [91], induces apoptosis in multiple myeloma cells [92] and sensitizes human leukemia K562 cells to nontoxic concentration of doxorubicin [93]. All of these results show AMPK protein may contributes to survival and proliferation of tumor cells. Hence, the role of AMPK in AML needs further investigation.

Conclusion

In AML, deregulation of mTOR pathways enhances survival/proliferation of myeloid cells and contributes to leukemogenesis. PI3K/AKT/mTOR pathway can be activated by overexpression of RTKs, deletion of PTEN protein or mutation of AKT. MLAA-34, a new related antigen for M5, is located in plasma membrane of U937 cells. We hypothesize MLAA-34 exerts anti-apoptosis function via interacting with the PI3K/AKT/mTOR pathway elements, such as RTKs, IRS1/2, PI3K, PTEN, PDK1 and AKT. But it needs to be further investigated.

Nowadays, the compound directly against PI3K/AKT and mTOR is developing, and those compounds may be treated as novel and potent agents for treating AML. Furthermore, combination of inhibitors and cytotoxic drugs is proved...
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to be more efficient than alone in many trials. As we known, mTOR signaling pathway is essential for survival and proliferation of leukemic cells, as well as normal cells. Accordingly, an effort should be made in looking for specific upstream regulators leading to activation of mTOR pathway in AML, and searching for the compounds specifically targeting the deregulated pathway in leukemic cells, in order to avoid damaging normal cells. Since different patients respond to inhibitors of pathway differently, future clinical studies should probably focus on the patients showing biological characteristics related with susceptibility or resistance to these drugs, and this would be the foundation of individualized treatment of AML in the future.

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Disclosure of conflict of interest

None.

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