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Dual targeting of eIF4E by blocking MNK and mTOR pathways in leukemia

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Abstract

Dysregulation of mRNA translation leads to aberrant activation of cellular pathways that promote expansion and survival of leukemic clones. A key element of the initiation translation complex is eIF4E (eukaryotic translation initiation factor 4E). The mitogen-activated protein kinase (MAPK) and mammalian target of rapamycin (mTOR) pathways play important roles in the regulation of eIF4E expression and downstream functional outcomes. Mitogen-activated protein kinase interacting protein kinases (Mnks) control translation by phosphorylation of eIF4E, whereas the mTOR kinase phosphorylates/de-activates the eIF4E inhibitor, 4E-BP1, to release translational repression. Both pathways are often abnormally activated in leukemia cells and promote cell survival events by controlling expression of oncogenic proteins. Targeting these pathways may provide approaches to avoid aberrant proliferation and neoplastic transformation.

Keywords

translation initiation; eIF4E; MNK; mTOR; acute myeloid leukemia

1. Introduction

Acute myeloid leukemia (AML) is a genetically heterogeneous leukemia characterized by subtypes with different molecular abnormalities and by the activation of multiple signalling pathways that promote cell survival and proliferation. Despite the currently available therapies, most subtypes of AML remain difficult to treat [1]. The control of mRNA translation plays a pivotal role in the regulation of expression of genes that are responsible for many cellular processes such as cell proliferation, differentiation and apoptosis. Translation processes are tightly regulated. The critical step for initiating translation of mRNAs is the availability of eIF4E (eukaryotic translation initiation factor 4E) to participate in the eukaryotic initiation complex 4F, along with RNA helicase eIF4A and the scaffolding

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protein eIF4G [2, 3] (Figure 1). eIF4E is a key component of this complex because it recognizes and directly binds the 5′-cap of the mRNA structure, which includes a 7-methylguanosine (m^7G) moiety [4, 5]. The eIF4G scaffolding protein also binds to mRNA by interaction with eIF4E and the m^7G cap structure. This complex also includes the eIF4B protein that helps in the RNA-helicase function of eIF4A, thus regulating the translation of mRNAs that contain 5′-UTRs (untranslated regions) [6, 7]. The study of eIF4E has become a major focus in cancer research due to its key role in controlling translation of mRNAs that lead to the expression of tumor-associated proteins, such as c-Myc, cyclins D1 and D3, and Mcl-1 (Myeloid cell leukemia 1). The activity of these proteins has been linked to proliferation of leukemic cells and other type of malignant cells [8, 9, 10]. In contrast, the activation of eIF4E plays a minor role in the expression of mRNAs for housekeeping genes, such as GAPDH (glyceraldehyde-3-phosphate dehydrogenase) and β-actin [11]. In addition to its role in translation, eIF4E also seems to facilitate the nucleocytoplasmic transport of certain mRNAs, which is enhanced by eIF4E phosphorylation. This process enables the production of proteins that are involved in cell cycle progression and cell survival [12]. This could represent an independent mechanism required for expression of oncogenic proteins and potentially provide a unique cellular target for therapeutic approaches.

The function of eIF4E is strictly regulated in cells under normal physiological conditions and is controlled by its repressor proteins, 4E-BPs (eIF4E-binding proteins), whose function does not allow formation of eIF4F complex [8]. eIF4E activity can be regulated by two major signalling pathways which play critical roles in leukemogenesis, the MAPK (Mitogen-activated protein kinases) and mTOR (mammalian target of rapamycin) pathways [13, 14]. The selective targeting of these pathways, alone or in combination with other therapies, could conceptually increase the anti-leukemic activity of the currently available and generally insufficient treatments for patients with AML and has been the major focus of study by different groups in the field.

2. The oncogenic activity of eIF4E and its phosphorylation by MNKs

The oncogenic activity of eIF4E can be modified by its phosphorylation at Serine209 (Ser209) by the MAPK-interacting kinases MNK1 and MNK2 (Figure 1). MNK1 and MNK2 belong to a family of serine/threonine protein kinases that are activated downstream of either ERK or p38 MAPK in response to extracellular factors (growth factors and stress) [15, 9, 16]. In human cells, two Mnk genes have been identified as *MNK1* and *MNK2*. Each one of these genes, after alternative splicing events, translate two protein isoforms: MNK1a, MNK1b, MNK2a, and MNK2b, respectively, which share a similar N-terminal region (involved in binding to eIF4G), but vary in their C-terminal domains [17, 18]. The C-terminal regions of the longer MNK1a and MNK2a isoforms have a MAPK-biding site that allows their interaction/phosphorylation by ERK and p38 MAPK [19]. However, unlike MNK1a, which has a high affinity for both kinases, MNK2a has greater affinity for ERK. Moreover, the activation of ERK or p38 MAPK increases the low basal activity of MNK1a, but does not have a significant impact on the constitutive high basal activity of MNK2a [20, 17]. On the other hand, the shorter b-isoforms of Mnks lack the MAP-kinase biding site in their C-terminal region. Initial studies have shown that basal activity of MNK1b is higher than that of MNK2b [17, 21].

Recent studies have shown that phosphorylation and activation of eIF4E at Ser209 by MNK1/MNK2 is critical for eIF4E to promote oncogenic activity [22], but not essential for normal development [15, 16]. As a proof of concept of this perspective, a study using Mnk1/2 double knockout PTEN$^{-/-}$ mice (T-cell-specific PTEN conditional knockout mice) showed resistance to lymphogenesis in these mice, when compared to the parental PTEN$^{-/-}$ mice [23]. Phosphorylation of eIF4E is also involved in development and progression of other type of cancer [11]. Notably, phosphorylation of eIF4E was shown to promote invasion, metastasis and epithelial to mesenchymal transition [24]. eIF4E is overexpressed in many types of cancer and in most cases is connected with poor prognosis (increased cancer recurrence and decreased patient survival) [11]. There has been accumulating evidence implicating Mnks in the pathophysiology of leukemogenesis. AML is often characterized by a collection of several mutations that support proliferation and survival of leukemic clones [25, 26]. It has been shown that MNK1 activity is induced by several AML fusion genes and has an important role in hematopoietic proliferation [27]. A recent study on chronic myeloid leukemia (CML) provides evidence that targeting the MNK-eiF4E axis can inhibit the function of blast crisis leukemia stem cells (BC LSCs) by affecting production of β-catenin, without affecting normal hematopoietic stem cell functions [28]. Thus, Mnks can play an important role in leukemia progression and, therefore, future development of new small molecule inhibitors could be important for the treatment of leukemia.

3. Regulation of eIF4E by the mTOR pathway

The mTOR signaling cascade controls initiation of mRNA translation and plays significant roles in cellular processes such as protein synthesis, lipid production, cell growth and proliferation, and ribosome biogenesis [29, 30]. This kinase is present in two unique, separate complexes: mTORC1 and mTORC2 [31]. In those complexes there are common and distinct subunits and effectors [32, 33]. mTORC1 contains the common subunits mLST8 (mammalian lethal with sec13 protein 8) and Deptor (DEP domain-containing mTOR-interacting protein), and the unique components PRAS 40 (proline rich Akt substrate of 40 kDA) and Raptor (regulatory-associated protein of mTOR) [34, 29, 32]. The mTORC2 includes the unique subunits Rictor (rapamycin-insensitive companion of mTOR), mSin1 (mammalian stress activated protein-kinase interacting protein), and Protor, in addition to the common proteins mLST8 and Deptor [30]. A defining function of mTORC2 is the control of phosphorylation of AKT at Ser473, a site which is essential for activation of AKT and anti-apoptotic downstream effectors [31, 32, 35]. Additionally, mTORC2 regulates cytoskeletal organization and glucose and lipid metabolism [36]. It has also been shown that in addition to AKT, mTORC2 also phosphorylates SGK (glucocorticoid-regulated kinase) and PKCα (protein kinase C-α) [37, 38, 39].

mTORC1 is activated upstream by engagement of the PI3K/AKT cascade. PI3K (phosphoinositide 3-kinase) is a membrane-associated lipid kinase that when activated promotes conversion of phosphatidyl inositol 3,4 (PIP_2) to phosphatidyl inositol 3,4,5 (PIP_3) [29, 40]. Once active, PI3K and PIP_3 bind PDK1 which phosphorylates AKT at Threonine 308 [41, 42]. In its active form, AKT plays an important role in regulation of mTORC1 activity by phosphorylation of PRAS40 and tuberous sclerosis complex 2 (TSC2) [43, 44, 45].

mTORC1 signaling is induced by multiple growth factors, hormones, cytokines, as well as, stress [46, 40] and regulates pathways involved in initiation of mRNA translation of oncogenic proteins, cellular growth, cell cycle progression and autophagy [30, 47]. Two key substrates of mTORC1 are S6K and translation repressor 4E-BP1 [48, 49, 50]. After its phosphorylation by mTOR, 4E-BP1 is inactivated, leading to its dissociation from eIF4E. Consequently, eIF4E can become phosphorylated on Ser209 by MNK1 and MNK2, triggering initiation of mRNA translation of-mitogenic proteins [51] (Figure 1). S6K regulates two downstream substrates, the S6 ribosomal protein (rpS6) and eukaryotic initiation factor 4B (eIF4B). eIF4B is recruited, along with eIF3, to contribute in the formation of the translation initiation complex [52].

Dysregulation of mTOR pathways plays an important role in tumorigenesis as shown by promotion of proliferation and survival of various types of malignant cells [31, 53]. In fact, PI3K/AKT/mTOR pathways were shown to promote leukemic cell proliferation and survival and have been implicated in resistance to antileukemic drugs [54, 55]. It has been reported that in 50-80% of AML cases there is constitutive activation of the PI3K and mTOR kinases [56]. Therefore, targeting the PI3K/AKT/mTOR pathway could improve pro-apoptotic an antiproliferative effects on hematological malignancies.

4. Strategies for targeting eIF4E in AML

4.1 Direct eIF4E inhibitors

The use of eIF4E anti-sense oligonucleotides was one of the first approaches used to specifically and directly target eIF4E, which inhibited the expression of eIF4E–regulated proteins, such as cyclin D1, c-Myc and Bcl-2, and induced apoptosis [57]. However, this approach does not appear to be clinically meaningful [58]. A more effective approach seems to be the use of the antiviral drug ribavirin. There is evidence that ribavirin mimics the m^7G cap, binding eIF4E, and thus blocking its activity. A clinical trial with ribavirin has shown to reduce eIF4E activity and to induce partial remission in some patients with M4/M5 subtypes of AML [59, 60]. This approach appears to be promising, but future studies are warranted to confirm its value in a larger cohort of patients.

4.2 MNK inhibitors

Another strategy to reduce the activity of eIF4E is by developing compounds that target MNK and inhibit eIF4E phosphorylation on Ser209. Until now, only a few small-molecule inhibitors have been described. CGP57380 is a Mnk inhibitor which exhibits cytotoxic effects against cancer cells at low micromolar concentration, yet was also shown to inhibit other kinases [10, 61]. CGP57380 blocks eIF4E phosphorylation on Ser209, but also was shown to decrease rpS6 phosphorylation in CML cell lines [62]. Another Mnk inhibitor, cercosporamide, is a natural product isolated from *Cercosporidium henningsii* and was initially identified as a broad spectrum antifungal agent [63]. However, cercosporamide also targets other kinases, including Jak3 [64]. We have previously reported that cercosporamide suppresses tumor growth in an AML xenograft model [65]. Additionally, cercosporamide blocks MNK1/2-induced phosphorylation of eIF4E in human AML cells and such inhibitory effects correlate with decreased cell viability [65]. Most recently, Diab *et al.* [66] identified a

series of 5-(2-(phenyloamino)pyrymidin-4-yl)thiazole-2(3H)-one derivatives as Mnk inhibitors, most of which showed potent MNK2 inhibition and resulted in reduced levels of the anti-apoptotic protein Mcl-1 and promoted apoptosis in MV4-11 AML cells.

4.3 Allosteric mTOR inhibitors

It has been known that deregulation of the mTOR pathway plays an important role in AML pathogenesis. Notably, constitutive activation of PI3K is essential for survival of AML blasts [67, 68]. This has provided the rationale for investigating the potential use of mTOR inhibitors in the treatment of AML. Initial studies investigated the antileukemic effects of the first generation of mTOR inhibitors, rapamycin and several analogs (everolimus, temsirolimus, ridaforolimus), which target mTORC1 [69]. It has been shown that RAD001 (everolimus) induced apoptosis and enhanced tumor necrosis factor-related apoptosis-inducing ligand (TRIAL) in Jurkat T leukemia cells [70]. In addition, CCI-779 (temsirolimus) has been shown to co-operate with ABT-737 (Bcl-2 family proteins inhibitor) to induce apoptotic cell death in acute lymphoblastic leukemia (ALL), which was accompanied by downregulation of Mcl-1 expression [71]. Unfortunately, the efficacy of rapamycin analogs in leukemia treatment has been clinically lower than expected [72, 73, 11]. Directly blocking mTORC1 activity results in only some cytostatic effects on AML cells *in vitro* [67]. In addition, rapamycin incompletely blocks 4E-BP1 phosphorylation, while inhibiting the negative feedback loops between mTORC1-S6K and AKT, thus resulting in activation of AKT that can promote cell survival [74, 75]. For all these reasons, the future clinical use of first generation mTOR inhibitors is unclear in leukemia.

4.4 Catalytic mTOR inhibitors

Because of the limited success of rapalogs, most recent therapeutic studies have been focused on developing mTOR catalytic inhibitors which can target both mTORC1 and mTORC2. PP242 and OSI-027 have both showed potent suppressive effects on cell viability in BCR-ABL transformed cell lines when compared with rapamycin treatment [39, 70]. This dual inhibition of the mTOR complexes has also demonstrated tumor growth suppressive effects in a human AML xenograft mouse model [69]. Moreover, PP242 treatment reduced colony formation of leukemic progenitor cells derived from CML patients [76] Additionally, in AML models, OSI-027 has shown anti-leukemic effects in cell lines, as well as, in primary leukemic precursors from patients with AML. Importantly, when compared to rapamycin treatment, OSI-027 was able to completely block phosphorylation of 4E-BP1 in AML cells [40]. Other recent studies have shown that Torin-2, a second-generation ATP-competitive inhibitor, induces apoptosis and autophagy in B-precursor acute lymphoblastic leukemia (B-pre ALL) cell lines [77].

4.5 Dual PI3K/mTOR inhibitors

Another potential approach to avoid negative feedback mechanisms is the development of inhibitors that can target both mTOR and PI3K, which is the main AKT-activating kinase [78]. PI-103, a dual PI3K/mTOR inhibitor, was found to strongly induce antiproliferative effects compared to rapamycin in various leukemia cell lines [79, 80, 81]. NVP-BEZ-235 is a new orally available inhibitor, which is currently under clinical investigation, including in acute leukemia (European Clinical Trials Database number EUDRACT2011-005050-61)

[82]. These dual inhibitors were shown to fully inhibit the rapamycin-resistant phosphorylation of 4E-BP1, resulting in suppression of cell proliferation and induction of apoptosis in AML cells [83] and chronic lymphocytic leukemia (CLL) cells [84]. NVP-BEZ-235 induces cytostatic effects in resistant lymphoid malignant models, by reducing Mcl-1 expression and activating BAX (BCL2-Associated × Protein), which results in cellular death [85]. Further studies have also demonstrated that NVP-BEZ-235 induces apoptosis in primary AML blast cells, and inhibits growth/viability of AML cell lines [54]. Additionally, this PI3K/mTOR inhibitor delayed tumor growth and prolonged survival in an AML xenograft mouse model [56]. NVP-BGT226, a novel dual PI3K/mTOR inhibitor, has shown greater anti-leukemic and pro-apoptotic effects against a broad range of leukemic cells [86].

4.6 Combinatorial targeting of MNK-eIF4E-mTOR and other pathways in leukemia treatment

Based on most recent data there is strong evidence indicating that combinatorial inhibition of mTOR-eIF4E-MNK pathways may provide an important strategic advantage in cancer treatment [65, 87, 88]. Inhibition of mTORC1 pathways leads to activation of pro-survival pathways/negative feedback loops, which results in upregulation of eIF4E activity. Thus, combination of MNK inhibitors with rapalogs may provide anti-leukemic synergistic effects, by blocking eIF4E phosphorylation, which may result in greater anti-tumorigenic effects. In fact, cercosporamide and rapamycin treatment resulted in greater inhibition of colony formation of leukemic progenitor cells derived from AML cell lines and patients with AML, when compared to each drug used alone [65]. Additionally, Teo *et al.* [87] established that sensitivity of different leukemia cell lines to Mnk inhibitors correlates with the levels of phosphorylated 4E-BP1 at Thr70. Co-treatment of leukemic cells with Mnk inhibitors and rapamycin showed superior anti-leukemic effects compared to each drug alone [87], further suggesting that combined MNK and mTORC1 inhibition can provide an advantage in inducing antileukemic responses.

Bruton's tyrosine kinase (BTK) plays a key role in malignant cell proliferation and survival in several B-cell malignancies [89]. Recently, Wu *et al.* [90] suggested that dual inhibition of BTK and Mnks may provide an effective approach for the treatment of B-cell malignancies. The authors successfully developed a first selective dual BTK/MNK inhibitor, QL-X-138, which induced antiproliferative effects in leukemia cell lines *in vitro*, as well as, in CLL/AML-patient derived primary leukemia cells [90]. Another recent study showed that treatment of *MLL*-rearranged AML cells, which can lead to activation of both Ras/Raf/MEK/ERK and PI3K/AKT/mTOR pathways, with the dual PI3K/mTOR inhibitor NVP-BEZ-235 and selumetinib MEK-inhibitor strongly induces apoptosis in patient-derived cells [56]. Additionally, co-treatment with selumetinib and the catalytic mTOR inhibitor AZD8055 resulted in further induction of apoptosis in AML cell lines and primary AML samples, when compared to each drug alone [91].

In another study, co-treatment of BCR-ABL-positive leukemia cells with the dual PI3K/mTOR inhibitor NVP-BEZ-235 and nilotinib, a BCR-ABL kinase inhibitor, effectively blocked phosphorylation of 4E-BP1, AKT and S6K, induced cellular apoptosis, and

suppressed colony formation and *in vivo* tumor growth [92]. Additionally, in primary blast cells from AML patients, targeting of AKT and mTOR by allosteric inhibitors was shown to induce AKT phosphorylation and activation of compensatory mechanisms, such as activation of receptor tyrosine kinase (RTK) pathways [93, 94]. Therefore, treatment of AML with RTK inhibitors (linstinib, sunitinib, guizartinib) in combination with dual PI3K/mTOR inhibitors could provide a better therapeutic strategy in the treatment of this disease [94].

5. Conclusions

Significant progress has been made to understand the role of MAPK and mTOR in oncogenic transformation and development. Recent evidence suggests a dysregulation of both of these pathways, in addition to either upstream or downstream effectors, in hematologic malignancies, which has led to development of new targeted therapeutics drugs. Nevertheless, there are still some limitations due to activation of multiple feedback loops and due to crosslinking between different pathways that hinder the effective targeted treatment of cancer. One approach that could improve potency of future anti-leukemic therapy is the use of multimodal treatment strategies. The combination of mTORC1/mTORC2 pathways inhibitors with MAPK signalling inhibitors warrant further development and clinical evaluation.

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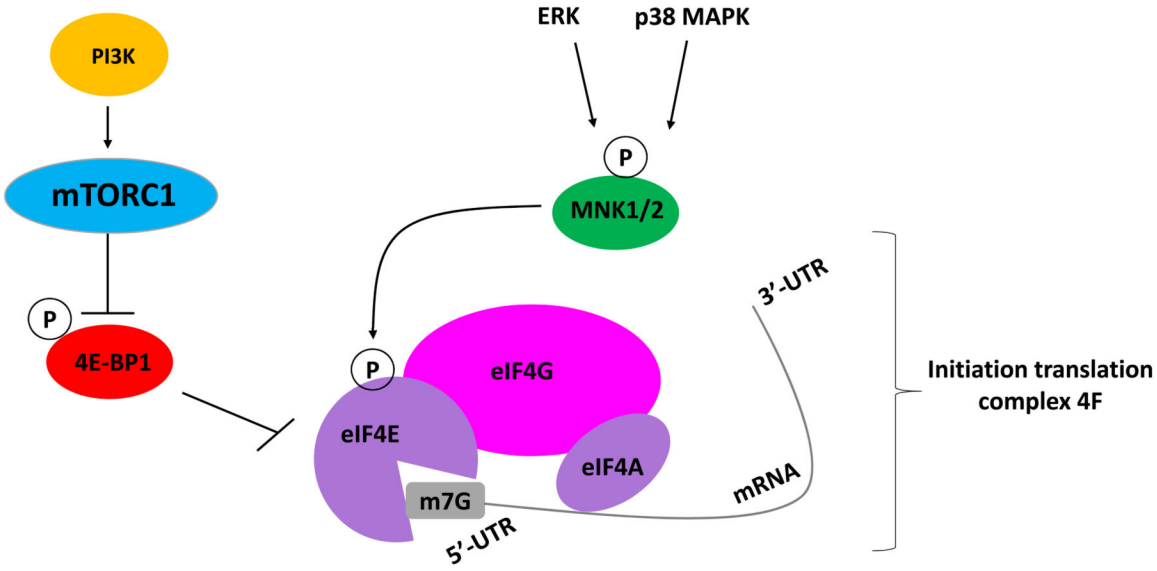

Figure 1.
Schematic illustration depicting the cellular pathways that lead to eIF4E activation.