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New insights into malignant cell survival mechanisms in medulloblastoma

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Abstract

mRNA translation and protein synthesis is an important determinant for cell metabolism and cell homeostasis. Perturbations in cellular homeostasis often result in activation of negative feedback loops as compensatory mechanisms. Although, these mechanisms are important for mammalian cells to adjust to environmental changes, they also pose a major challenge for targeted cancer therapy as they provide escape mechanisms for cancer cells. The mammalian target of rapamycin (mTOR) is a crucial regulator of mRNA translation, protein synthesis and metabolism and represents an attractive target for anticancer therapy. We have recently reported that selective inhibition of mTORC1 by rapamycin or its analogs in medulloblastoma cells results in phosphorylation of eukaryotic translation initiation factor 4E (eIF4E) on serine-209, an event known to be associated with induction of protein translation and cell transformation. We have also previously established that this event is mediated by mitogen-activated protein (MAP) kinase-interacting kinase 2 (Mnk2) independently of MAPKs, the conventional activators of Mnk. Here we discuss the implications for our current understanding of negative feedback regulation by mTOR and Mnk in cancer.

Keywords

mTOR; Mnk; rapamycin; medulloblastoma

The development of small-molecule inhibitors that target kinase signaling initially led to promising results for targeted cancer therapy. However, over the last decade there has been emerging evidence that tumor cells can develop resistance to these inhibitors. Resistance can be either intrinsic to the tumor or acquired during treatment, and this poses a major problem for targeted therapeutic approaches^[1]. One mechanism for such resistance is the rewiring of signaling networks via feedback regulation, resulting in activation of alternative pathways.

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For instance, selective inhibition of mTORC1 is known to initiate such negative feedback responses. S6 kinase (p70-S6K) is a direct substrate of mTORC1 and it inhibits the activity of mTORC2 by negative regulation of the crucial mTORC2 components Rictor and Sin1 [2–4]. Consequently, inhibition of mTORC1 results in concomitant inhibition of p70-S6K, which allows for activation of mTORC2 as a compensatory survival mechanism [5]. Thus, targeted therapeutic approaches require concomitant inhibition of the survival pathways anticipated to be activated after treatment.

We and others have reported that selective inhibition of mTORC1 by rapalogs induces phosphorylation of eIF4E in a variety of malignant cell types, thereby inducing the translation of eIF4E target proteins such as c-myc and cyclin D [6–10]. Importantly, phosphorylation of Ser-209 in eIF4E has been shown to be elevated in tumors and is associated with cell transformation, indicative of activation of a survival mechanism in cancer cells [11–14]. As OSI-027, an ATP competitive inhibitor of mTOR, prevented rapamycin from inducing eIF4E phosphorylation in medulloblastoma cells, mTOR kinase activity seems to be indirectly required for rapamycin-induced phosphorylation of eIF4E in medulloblastoma cells. The requirement for mTOR kinase activity was corroborated by a previous study, demonstrating that knockdown of mTOR by siRNA inhibits phosphorylation of eIF4E in the presence of rapamycin [10]. This raised the possibility that selective inhibition of mTORC1 results in activation of another mTOR complex, such as mTORC2, which may indirectly regulate via engagement of various effectors eIF4E phosphorylation. However, mTORC2 activity does not appear to be required for phosphorylation of eIF4E because both p70-S6K and Sin1 were dispensable for rapamycin-induced p-eIF4E in our study [6]. Similarly, others have found that knockdown of Rictor does not decrease rapamycin-induced p-eIF4E levels [10]. Thus, it appears that rapamycin-induced phosphorylation of eIF4E does not rely on the conventional mTORC1-p70-S6K-mTORC2 negative feedback loop. As there has been extensive prior evidence for rapamycin-insensitive (RI) mTORC1 signals (reviewed in [5]), it is tempting to speculate that there exists another rapamycin-insensitive (RI) mTOR kinase complex that is activated by rapamycin in a negative feedback regulatory manner (Fig. 1).

It is well established that phosphorylation of Ser-209 in eIF4E is mediated by Mnk1 and Mnk2 [6]. Mnks are activated by T-loop phosphorylation mediated by the MAPKs p38 and/or Erk1/2 in response to stress or mitogenic stimuli [15]. Our recent findings established that rapamycin increases p-eIF4E levels independently of MAPK signaling in medulloblastoma cells [6]. Moreover, we demonstrated that rapamycin engages Mnk2, but not Mnk1, for eIF4E phosphorylation [6]. This challenges a prior dogma, as so far it was generally accepted that induction of eIF4E phosphorylation is mainly triggered by MAPK-activated Mnk1, while Mnk2 is responsible for basal eIF4E phosphorylation [16]. This assumption needs to be revisited because based on our recent report [6] it is now clear that Mnk2 phosphorylates eIF4E in a way independent of MAPKs and dependent on mTOR kinase activity in medulloblastoma cells [6]. Interestingly, there is also some evidence that this phosphorylation of eIF4E is phosphatidylinositol 3-kinase (PI3K) dependent. First, rapamycin is unable to induce phosphorylation of eIF4E in the presence of the PI3K inhibitor LY294002 [9]. Second, mouse embryonic fibroblasts (MEFs) deficient of the PI3K-regulatory subunits p85 α and p85 β (p85-DKO MEF) fail to increase p-eIF4E levels in

response to rapamycin ^[10]. Thus, rapamycin treatment results in increased Mnk2 activity for eIF4E phosphorylation and this event may require both mTOR and PI3K activity. Taken together, it is likely that eIF4E phosphophorylation is regulated in two different ways; either through MAPK-activated Mnk1 or through mTOR/PI3K-activated Mnk2 (see Fig. 1). Therefore, it is possible that different signaling pathways may converge to Mnk/eIF4E phosphorylation to ensure survival in response to environmental challenges. This suggests that inhibition of eIF4E phosphorylation might be a promising target for combination cancer therapy and in particular in combination with mTOR and PI3K inhibitors.

While Mnks are required for eIF4E-mediated oncogenic transformation, they are dispensable for normal development ^[16]. This suggests Mnk inhibitors could be an attractive tool for cancer therapy and in particular for targeted combination therapies with inhibitors that induce eIF4E phosphorylation as a survival mechanism. Consistent with this, our recent findings have suggested that inhibition of Mnks, and in particular Mnk2, may represent a promising combination strategy for medulloblastoma treatment in targeted therapies with rapalogs ^[6]. The enhanced antineoplastic effects mediated by Mnk2 inhibition are most likely due to preventing activation of the Mnk2-eIF4E axis that triggers survival in rapamycin treated cells. The rapamycin-induced survival pathway engaging Mnk2-eIF4E may reflect intrinsic resistance to rapamycin, and simultaneous treatment with Mnk inhibitors may be a promising approach for this type of resistance. It will be also interesting to see whether the Mnk2-eIF4E axis is important for acquired resistance to rapalogs and whether Mnk inhibition might be beneficial in the treatment of these tumors.

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Abbreviations

eIF4E	eukaryotic translation initiation factor 4E
mTOR	mammalian target of rapamycin
MAP	mitogen-activated protein
MEF	mouse embryonic fibroblast
PI3K	phosphatidylinositol 3-kinase
RI	rapamycin insensitive
p70-S6K	S6 kinase

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Figure 1.
Proposed model for rapalog-induced phosphorylation of eIF4E in malignant medulloblastoma cells.