Signalling to the Ribosome in Cancer – It's More than Just mTORC1

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It is becoming increasingly clear that dysregulation of protein synthesis contributes to a range of diseases characterised by tissue overgrowth. These include arterial stenosis, cardiac hypertrophy, hamartomas and cancer. The central hub for the regulation of protein synthesis is the ribosome, where the key signalling pathways downstream of RAS, MYC and PI3K converge to confer exquisite, coordinated control of ribosome synthesis and function. Such cooperation ensures strict regulation of protein synthesis rates and cell growth. This review will focus on the role the PI3K/AKT/mTORC1 pathway plays in regulating ribosome function during both health and disease, its interaction with the other key growth regulatory pathways activated by RAS and MYC, and the therapeutic potential for targeting this network.

The PI3K/AKT/mTORC1 Pathway

The Class IA phosphatidylinositol-3-kinases (PI3K) are lipid kinases predominantly activated by growth factor receptor tyrosine kinases to convert phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-trisphosphate (PIP_3). This reaction is directly antagonised by the lipid phosphatase PTEN or Src-homology 2 containing phosphatases SHIP1 and SHIP2. PI3K initiates a complex signalling cascade (**Fig. 1**). The core module of this network is activated when PIP3 binds to AKT, localising it to the cell membrane to be phosphorylated by PDK1 and the mTORC2 complex. mTORC2 is a complex of the mTOR serine-threonine protein kinase with Rictor, mSIN1, Protor-1, Deptor and mLST8. These interacting proteins are not well characterised but have been implicated in either stabilising each other or the complex. Alternatively, they act as repressors, such as is the case for Deptor (1).

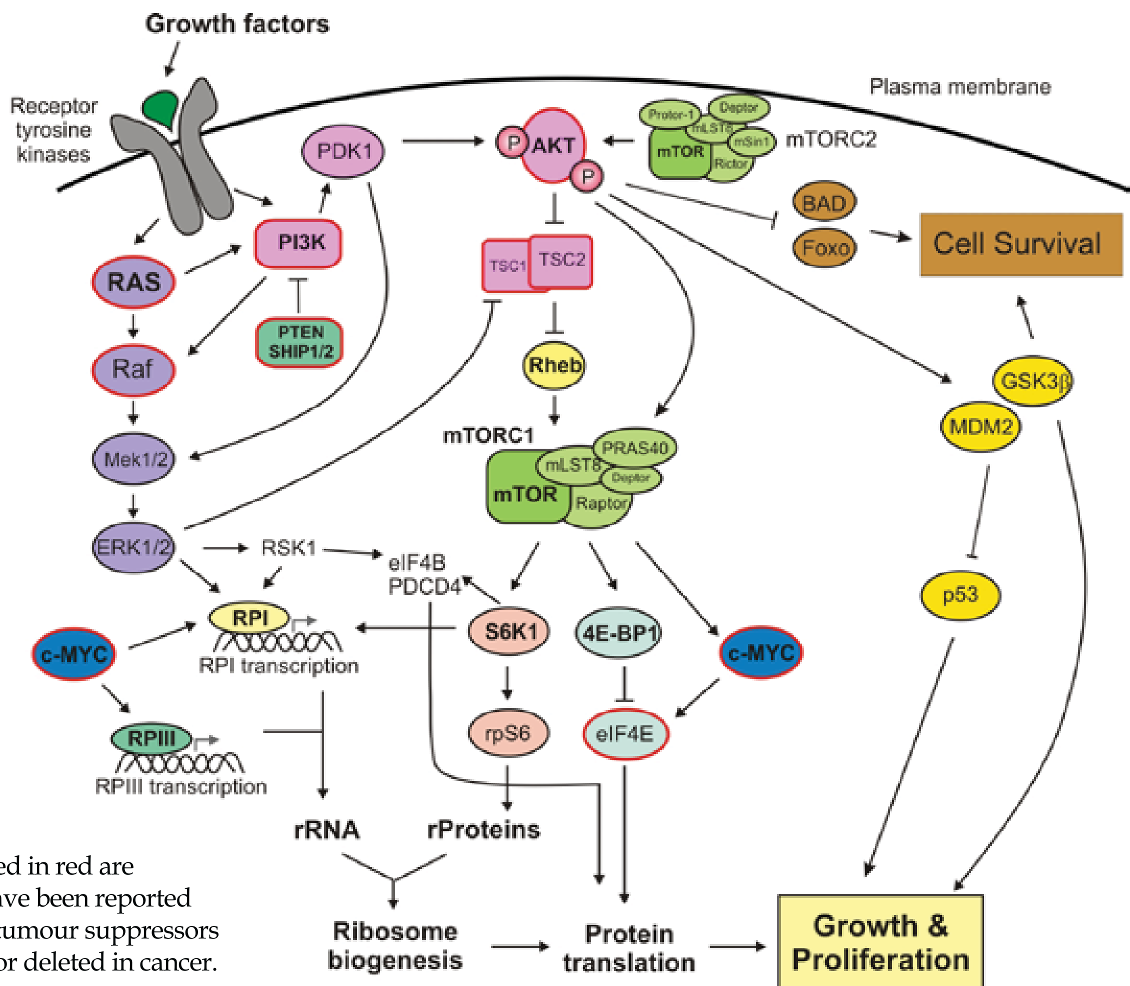

Fig. 1. Signalling network controlling ribosome synthesis and function. The PI3K/AKT/mTORC1 signalling hub regulates ribosome synthesis and function. In addition, by interacting with the MYC and RAS signalling pathways at multiple levels it forms a super signalling network that exquisitely controls protein synthesis and cell growth. Circled in red are oncogenes that have been reported to be mutated or tumour suppressors that are mutated or deleted in cancer.

Activated AKT is released from the plasma membrane and phosphorylates multiple nuclear and cytoplasmic targets, resulting in pleiotropic effects on cellular homeostasis, including the control of normal cell growth, proliferation, migration, metabolism, survival and angiogenesis (**Fig. 1**). Thus, deregulation of AKT signalling affects multiple processes often referred to as 'hallmarks of cancer'. One key mechanism by which AKT mediates such control on cell growth, proliferation and metabolism is via activation of the mTORC1 complex (reviewed in ref. 2). mTORC1 is known to act as a master regulator of protein synthesis in response to multiple inputs. Like mTORC2, mTORC1 includes the subunits mTOR, mLST8 and Deptor; however, instead of Rictor, it has Raptor and PRAS40 and is sensitive to inhibition by rapamycin and its analogues (RAD001 and CCI-779). Again like mTORC2, the functions of the mTOR interacting proteins have not been fully defined. However, Raptor has been reported to promote mTORC1 activity by regulating the assembly of the complex and recruiting substrates. Deptor and PRAS40 are negative regulators with PRAS40 competing with the mTORC1 substrates for interaction with Raptor. Deletion of mLST8 had no effect on mTORC1 activity *in vivo* (1).

AKT activates mTORC1 via both phosphorylation of TSC2 and PRAS40 (reviewed in ref. 2). Phosphorylation of TSC2 inhibits its GTPase-activating protein activity, resulting in activation of RheB and hence mTORC1. AKT phosphorylation of the negative mTORC1 regulator, PRAS40, releases it from the complex and thus relieves its repression. Once mTORC1 is activated, it is also able to phosphorylate PRAS40 and thus dissociate it from its complex.

mTORC1 Regulation of Protein Synthesis: A Story of Efficiency versus Capacity

Activation of mTORC1 results in stimulation of protein synthesis and thus cell growth via targeting multiple critical effectors of both the efficiency of translation by existing ribosomes and the synthesis of new ribosomes (**Fig. 1**). Whilst modulating translation efficiency is a powerful mechanism for rapidly increasing protein synthesis, it may eventually be limited by the number of functional ribosomes. Thus regulation of translation efficiency is often utilised by the cell for a short-term gain in translation or for specific translation of distinct mRNA species, while increasing the number of ribosomes, through increased ribosomal gene transcription, is required for the sustained increase in protein synthesis required for muscle hypertrophy and in diseases such as cancer. mTORC1 mediates these effects via two key substrates, eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1) and S6 kinase 1 (S6K1), which in turn modulate similar and different aspects of protein synthesis (reviewed in refs. 1,3,4).

i) Translation Efficiency

mTORC1 modulation of translation efficiency occurs at many levels and involves a number of key players. For example, mTORC1 can dictate whether specific mRNAs are preferentially translated. mTORC1 phosphorylation of 4E-BP1 is required for efficient 5′ cap dependent translation of mRNA. Phosphorylation of 4E-BP1 relieves its association and thus inhibition of eIF4E. Active eIF4E binds the mRNA 5′ cap to initiate a sequential cascade of recruitment of initiation factors (eIF4G, eIF4A) to form the eIF4F complex, followed by the addition of eIF3, 40S ribosome and then the ternary complex (eIF2, Met-tRNA and GTP), ultimately resulting in initiation of translation. Translation initiation is also enhanced by mTORC1 phosphorylation of S6K1.

mTORC1 activation of S6K1 has been implicated in promoting the translation of specific mRNA species. mTORC1 is required for optimal translation of mRNAs with 5′ terminal oligopyrimidine tract (5′TOP) sequences, the majority of which encode for proteins required for ribosome synthesis or are regulators of translation. While initial observations implicated the phosphorylation of ribosomal protein S6 (rpS6) by S6K1 in this process, mTORC1-dependent regulation of these mRNAs was observed in S6K1 knockout mice (5), indicating the existence of alternate mechanisms for the regulation of 5′TOP mRNA translation.

In addition to 5′TOP mRNA, activated S6K1 also enhances the translation of mRNAs with complex secondary structures in their 5′ untranslated region (UTR), which usually exhibit a poor efficiency of translation. Such mRNAs tend to encode for proteins that promote cellular growth and proliferation, including cyclin D1, c-MYC and HIF-1α. Mechanistically, it is thought that S6K1 achieves this regulation by phosphorylation of eIF4B and PDCD4, which enhances eIFA's helicase activity and consequently unwinds the mRNAs, making them more accessible to the translation initiation complex.

S6K1 can also improve translation elongation via phosphorylating and inhibiting eukaryotic elongation factor (eEF) kinase 2 (eEFK2). Consequently, eEF2 is now able to bind the ribosome. Another S6K1 substrate, SKAR, is a scaffold protein localised at exon junctions, which when phosphorylated, recruits activated S6K1 to newly generated mRNAs. Consequently, this promotes accuracy and efficiency of gene expression for spliced mRNAs.

ii) Translation Capacity

Recent studies in our laboratory (6) and others (7,8) have shown that mTORC1 is required to coordinate the synthesis of both ribosomal proteins and ribosomal RNA (rRNA), the two major constituents of the ribosome. As described above, mTORC1/S6K1 signalling enhances translation of 5′TOP mRNAs of which a number include ribosomal proteins. In addition, we have demonstrated that S6K1 indirectly enhances transcription of the 45S ribosomal genes (rDNA) that encode the 18S, 28S and 5.8S rRNA. Transcription of the rDNA by RNA polymerase I (Pol I of RPI) is coordinated by a group of transcription or associated factors and is a key rate-limiting step for ribosome biogenesis. It is likely that S6K1 either directly or indirectly alters the activity of the pre-initiation complex or of downstream processes such as elongation. Such factors include UBF, the SL-1 complex, PAF53, TCOF and RRN3, of which a number are regulated by phosphorylation or contain putative phosphorylation sites (9,10). To date, none of these have been shown to be phosphorylated

directly by S6K1; however, studies in yeast suggest that not all the mammalian regulators/modulators of rDNA transcription have been identified (9,10), indicating that novel S6K1 substrates intimately involved in rDNA transcription may await discovery.

The AKT/mTORC1, RAS and MYC Super Network: Master Regulator of Ribosome Biogenesis

Whilst various growth-promoting stimuli converge on the PI3K/AKT/mTORC1 signalling hub, this hub is itself subject to regulation by the other key growth control pathways downstream of RAS and MYC, resulting in the generation of a super signalling network controlling both ribosome synthesis and function. For example, ERK or AKT activation combined with increased MYC expression results in hyperactivation of rDNA transcription (Hannan, Pearson *et al.*, unpublished data). How is such superactivation achieved? With respect to ERK, it seems that it impinges on the same factors as those regulated by MYC. For example, expression array analysis demonstrates that MYC regulates the transcription of a suite of factors termed the 'Pol I regulon' directly involved in Pol I transcription, including Pol I subunits and the core transcription factors UBF, SL-1 and RRN3 (11). Concomitantly, RAS activation of ERK signalling regulates phosphorylation and activation of at least some of these key regulon factors, such as the core Pol I transcription factors, UBF and RRN3 (9,10). In effect, MYC increases the amount of the available transcription factors, while ERK increases their activity.

AKT also synergises with MYC to regulate Pol I transcription, and intriguingly, at least part of this activation is independent of downstream regulators such as mTORC1. Furthermore, AKT is required for maximal activation of c-MYC in malignant cells, such as Em-MYC lymphoma cells. Interestingly, the 'Pol I regulon' components PolR1C, TAF1C and TCOF all contain putative AKT phosphorylation sites according to Motif Scan. While the *in vivo* phosphorylation of these sites by AKT remains to be established, the theme that is beginning to emerge is that c-MYC transcriptionally up-regulates the expression of important rDNA transcription factors whose activity is enhanced by phosphorylation on key residues directly or indirectly in response to ERK, AKT and mTORC1 signalling.

The AKT/mTORC1, RAS and MYC Super Network: Ribosome Function and Cancer

Consistent with the observation that the PI3K/AKT/mTORC1, RAS and MYC super network is the key regulator of ribosome biogenesis and growth, its dysregulation has been shown to play a pivotal role in cancer initiation and progression (12,13). There are numerous examples of activating mutations, deletions or amplifications of key signalling molecules within this network (**Fig. 1**). For example, activating mutations in the PI3K PI3CA gene or inactivating mutations in the negative regulator PTEN account for approximately 30% of human sporadic tumours, while activation of c-MYC by amplification or translocation occurs in over 10% of human cancers and RAS mutations are present in 35-45% of human colorectal and 90% of pancreatic cancers.

Since there are currently few clinically relevant small molecule inhibitors that efficiently target MYC or RAS, our data raise the possibility that targeting either the fundamental process of ribosome biogenesis or the PI3K/AKT/mTORC1 pathway may be a viable approach in tumours driven by RAS and MYC. To date, PI3K, dual PI3K/mTORC1, mTOR and AKT inhibitors are being utilised to treat cancers in extensive clinical trials (14) and, in some cases, with promising results. The most common examples are derivatives of the mTORC1 inhibitor rapamycin, such as CCI-779, which has been shown to increase the overall survival of poor prognosis patients with advanced renal cell carcinoma. Similarly, the AKT inhibitor perifosine, currently in a Phase II clinical trial, resulted in a significant response in 35% of patients with relapsed or relapsed/refractory Waldenström's macroglobulinemia (15).

Intriguingly, in a subset of tumors, components of multiple arms of the growth control super network cluster together, implying a synergy in cancer initiation or progression. For example, 56% of patients with colon adenocarcinomas had mutations in *KRAS*, *BRAF* and/or *PIK3CA* (16). These observations raise the possibility that targeting multiple aspects of the growth control super network may be required in some tumour types. Conversely, the development of inhibitors directly affecting ribosome synthesis and function may obviate the need for combination approaches and provide broad-spectrum efficacy in a high percentage of tumours driven by dysregulation in growth control pathways. Indeed, as described in Sanij *et al.* in this Showcase on Research, Cylene Pharmaceuticals have developed potent and specific inhibitors of Pol I that exhibit antitumour activity in pre-clinical models.

In summary, the data presented here suggest that at least part of the mechanism by which these pathways drive malignancies is via their ability to modulate ribosome biogenesis and function (17-20). Intriguingly, genetic confirmation of this comes from the recent observations that mutations at the level of the ribosome result in cancer susceptibility syndromes. For example, mutations in ribosomal proteins RpS19, RpS17, RpS24, RpL35a, RpS7, RpL5 and RpL11 have been associated with the human disease Diamond Blackfan anemia that is characterised by hypoplastic anemia with a predisposition to leukemia (21). Furthermore, mutations in RpS14 are associated with 5q-syndrome and a predisposition to acute myeloid leukemia (21). Thus, dysregulation of ribosome biogenesis can no longer be interpreted as a passive readout for malignant transformation and is increasingly being seen as a viable therapeutic target for the treatment of an increasing range of tumours that now can be termed ribosomopathies.

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