

T-ALL: ALL a matter of Translation?

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Cancer studies have historically focused on changes in the transcriptome of tumor cells to reveal the molecular pathways responsible for cellular transformation. In the last 20 years, however, it has become clear that deregulation of translation also plays a crucial role in cancer development and progression. Major players in cancer biology, such as the PI3K pathway, tumor suppressor protein p53 and MYC, can deregulate expression of components of the translation machinery and can influence the spectrum of mRNAs which are most efficiently translated in the cell. In addition, somatic lesions affecting ribosome biogenesis, translation initiation and elongation factors have been described in a wide variety of human cancers.¹ The cancer promoting action of such lesions is supported by the observation that patients with congenital mutations in ribosomal proteins or ribosome biogenesis factors develop ribosomopathies, diseases with specific pathological characteristics that often lead to an increased risk of developing hematopoietic malignancies and solid tumors.²

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy caused by accumulation of multiple co-operating oncogenic lesions in developing T cells. It has been well-established that prominent roles in T-ALL pathogenesis are played by: i) ectopic expression of oncogenic transcription factors such as TAL1, TLX1 and TLX3; ii) deregulation of the CDKN2A and CDKN2B cell cycle regulators; iii) constitutive activation of NOTCH1 signaling; and iv) hyperactivation of cytokine signaling by defects, e.g. in IL7R and JAK kinases.^{3,4} Recent next generation sequencing based screens in T-ALL have further expanded the spectrum of defects involved in the pathogenesis

of this disease. First, it became clear that epigenetic regulators like PHF6, SUZ12, EZH2, TET1 and KDM6A are also involved in T-ALL pathogenesis.⁵ Second, defects in essential components of the translation machinery have been revealed. We described mutations affecting the ribosomal proteins L5/uL18 (RPL5/uL18) and L10/uL16 (RPL10/uL16) in 10% of pediatric T-ALL patients.⁶ In addition, ribosomal protein L22/eL22 (RPL22/eL22) is inactivated in another 10% of T-ALLs⁷ and rare mutations in the ribosomal protein L11/uL5 (RPL11/uL5) gene were found in relapsed T-ALL patients (Figure 1).⁸ The oncogenic capacity of ribosomal protein defects in T-ALL is illustrated by the observation that loss of one copy of *RPL22/eL22* accelerates tumor formation in a mouse model of T-cell lymphoma.⁷ Whereas acquiring mutations in ribosomal proteins is the most direct way to influence generation and function of mature ribosomes in a cell, other mutations in T-ALL may also affect these processes. For example, up to 40% of all T-ALL cases carry loss-of-function defects in the *PHF6* gene.⁹ While the exact role of PHF6 in T-ALL is not yet understood, it has been proposed that it functions as an epigenetic regulator. PHF6 localizes in the nucleolus, inhibits rRNA synthesis, and its interactors, identified by mass spectrometry, include several ribosomal proteins.^{10,11} These observations suggest a role for PHF6 in ribosome biogenesis, and loss of PHF6 function in T-ALL could thus represent an alternative way to interfere with proper formation of ribosomes (Figure 1).

Besides the identification of mutations in ribosomal proteins, there are additional indications for an essential role of translational deregulation in T-ALL. Translation of most cellular

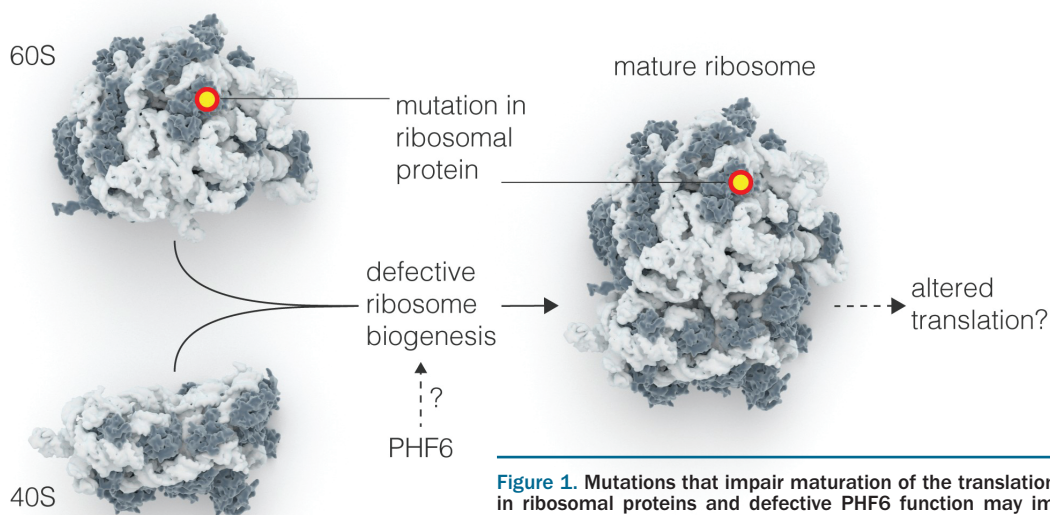


Figure 1. Mutations that impair maturation of the translation machinery in T-ALL. Mutations in ribosomal proteins and defective PHF6 function may impair formation of mature ribosomes (ribosome biogenesis) in T-ALL. Note that ribosome biogenesis defects have only been experimentally proven so far for mutations in ribosomal proteins, the effects of PHF6 are more speculative. Effects on altered translation are also speculative.

mRNAs depends on a cap structure in the 5' part of the mRNA (cap-dependent translation). However, for other mRNAs, translation is initiated by alternative structures such as an internal ribosomal entry site (cap-independent translation). It was shown that T-ALL development in mice can be accelerated by overexpression of eIF4A and eIF4E, two proteins that are part of the protein complex that initiates cap-dependent translation downstream of mTORC1.^{12,13} The strict dependence of T-ALL cells on cap-dependent translation is further supported by the observations that silvestrol, a drug inhibiting eIF4A, can impair proliferation of mouse and human T-ALL leukemia cells, both *in vitro* and *in vivo*,¹² and that interference with eIF4E by shRNA knockdown or administration of the eIF4E inhibitor 4EGI-1 reduces the viability of T-ALL blasts *in vitro*.¹³ As far as we are aware, no specific genetic lesions in *eIF4A*, *eIF4E* or other components of the protein complex supporting cap-dependent translation have been described in T-ALL, suggesting that the role of other (known) genetic lesions in T-ALL in driving cap-dependent translation has been underestimated. An obvious candidate is the NOTCH1 pathway which is hyperactivated in 60% of T-ALL cases by mutations in the NOTCH1 receptor itself and/or by inactivation of its ubiquitinating ligase FBXW7.³ Whereas hyperactive NOTCH1 signaling most likely drives T-cell transformation by influencing several pathways, NOTCH1 hyperactivates the PI3K-AKT-mTORC1 pathway and may thus support cap-dependent translation. Indeed, NOTCH1 activity in T-ALL cells leads to HES1 mediated downregulation of PTEN, a critical negative regulator of the PI3K pathway. Moreover, NOTCH1 can activate AKT via the LCK tyrosine kinase in T cells. Furthermore, numerous signaling molecules

upstream of PI3K, including the interleukin 7 receptor alpha chain (IL7RA) and the pre-T-cell receptor alpha (PTCRA), are up-regulated upon activation of NOTCH1 signaling in T-ALL lymphoblasts.¹⁴ In addition to impairing degradation of NOTCH1, FBXW7 deletion may further enhance cap-dependent translation by directly stabilizing mTOR, another known substrate of FBXW7.¹⁵ Inactivation of the *PTEN* tumor suppressor, an event occurring in 20% of T-ALLs, is another way for T-ALL cells to hyperactivate the PI3K-AKT-mTORC1 axis. Finally, hyperactivation of receptor tyrosine kinases can also stimulate mTORC1 activity and cap-dependent translation (Figure 2). Interestingly, whereas constitutive activation of the receptor tyrosine kinase TRK leads to increased mTORC1 signaling in lymphoid cells, pre-leukemic cells with hyperactive TRK tend to acquire activating mutations in *NOTCH1* and loss of *PTEN*. This leads to T-ALL during clonal evolution, resulting in even stronger activation of mTORC1 and more pronounced dependence on cap-dependent translation.¹³

How the defects in the translation machinery or cap-dependent translation described above drive T-ALL is the next important question. The Wendel laboratory recently showed that inhibition of eIF4A function with the drug silvestrol in T-ALL cells impairs the translation of components essential for T-ALL pathogenesis, such as NOTCH1, MYC, RUNX1 and BCL11B, suggesting that cells with hyperactive cap-dependent translation may shift towards a T-ALL-promoting translation program (Figure 2).¹² It is not clear at this point if the defects in the ribosome are acting via a similar mechanism or one that is completely different. Whereas the *RPL10/uL16* mutations impair ribosome biogenesis and translational fidelity,^{6,16} a thorough characterization of the

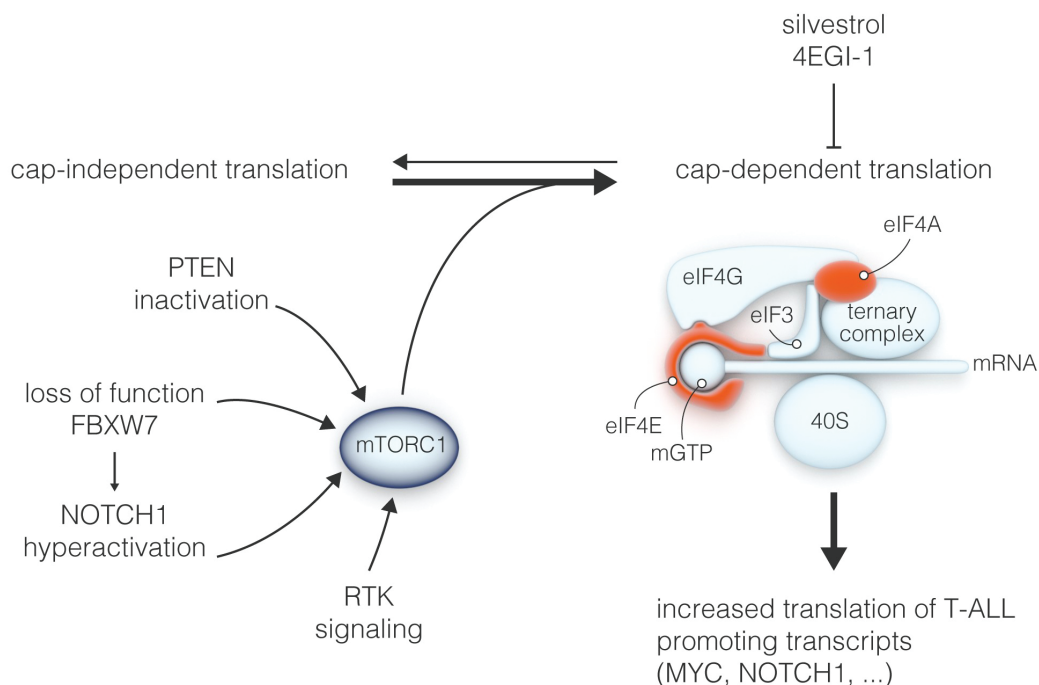


Figure 2. T-ALL cells depend on cap-dependent translation. Several molecular lesions in T-ALL (PTEN or FBXW7 inactivation, NOTCH1 or RTK hyperactivation) promote cap-dependent translation in T-ALL cells, resulting in more efficient translation of T-ALL promoting genes. Cap-dependent translation can be inhibited by drugs like silvestrol and 4EGI-1, that target eIF4A and eIF4E in the protein complex initiating cap-dependent translation. RTK: receptor tyrosine kinase.

effects of these ribosomal defects on the cellular translation profile in T-ALL has still not been made. Moreover, it remains to be established whether these defects are promoting leukemia by altering the translational profile of the ribosome (Figure 1) or by affecting translation-independent extra-ribosomal functions that have been assigned to these proteins.

Is the occurrence of defects in the ribosome and in the regulation of translation a specific characteristic of T-ALL? As far as we are aware, residue R98 in RPL10/uL16, a strong mutational hotspot in T-ALL, is not targeted by mutations in other cancer types, including other acute leukemias such as B-ALL or AML. At this point, we do not understand the reason for the unique occurrence of this mutation in T-ALL. In contrast, *RPL22/eL22* and *RPL5/uL18* were identified in the pan-cancer project as genes that are recurrently mutated in various cancer types. The dependence on cap-dependent translation is also not unique for T-ALL cells. Silvestrol and 4EGI-1 were shown to have therapeutic effects in xenograft models for various leukemias and solid tumors.¹⁷⁻¹⁹ It still needs to be determined, however, if this addiction to cap-dependent translation drives cell type specific translation programs in different tumor types.

In conclusion, in addition to the extensive list of defective processes and molecular aberrations already known in T-ALL, a central role of defective translation machinery and regulation was recently revealed in this disease. These findings offer novel opportunities for T-ALL therapy. Current treatment regimens consist of intensive schemes of chemotherapy and are associated with a multitude of long-term side-effects, while failing to induce long-term remission in 25% of pediatric and 50% of adult patients. For patients with one or several lesions driving cap-dependent translation, such as *PTEN*, *NOTCH1* or *FBXW7* mutations, drugs like 4EGI-1 or silvestrol could be considered. In contrast to what one might expect, off-target toxicity of these compounds seems limited. 4EGI-1 shows no toxicity on human CD34⁺ cells,²⁰ and numerous studies showing therapeutic effects of these drugs in *in vivo* application in mouse tumor xenografts support the concept that there is a therapeutic window for treatment in humans.

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Tyrosine kinase inhibitors in BCR-ABL positive acute lymphoblastic leukemia

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Philadelphia chromosome (Ph)/BCR/ABL-positive acute lymphoblastic leukemia (ALL) is the most common genetic abnormality associated with adult ALL and has been shown to confer the worst prognosis to both children and adults.^{1,2} Approximately 3%-5% children and 25%-

40% adults with ALL have a malignant clone expressing the Ph chromosome. The presence of the Ph chromosome in adults increases with age.^{1,2}

Ph-positive (Ph+) ALL patients often present with an aggressive leukemia that is resistant to standard therapies