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m6A-dependent regulation of Cap-dependent translation

Smolin E.*, Buyan A., Lyabin D., Kulakovskiy I., Eliseeva I.
Institute of Protein Research, RAS, Pushchino, Russia
** smolinegoralexeyevich@gmail.com*

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Motivation and Aim: The role of m6A methylation in mRNA of the regulation of translation is currently well-covered. When METLL3 is knockdown (i. d. methylated level decrease), total protein synthesis becomes more sensitive to mTOR inhibitors. This implies that under normal conditions a significant part of mRNA is methylated, and under cap-dependent translation inhibition conditions (e. g., stress inhibiting mTOR kinase activity) it allows mRNA to be effectively translated. However, the issue of the mechanism for working and regulation of the process is still open to settle. According to the general assumption, the eIF3 factor is binding with m6A in the 5'-UTR mRNA, which results in the ribosome planting on mRNA without the cap-structure [1]. However, it is not known how the whole transcriptome show changes in response to the inhibition of the mTOR signal pathway in methylase-free cells (METTL3 or METTL14).
Methods and Algorithms: The HEK293T cell line was employed in this study. For this genes knockdowns, the transfection of siRNA using Lipofectamin was employed. The protein expression level was evaluated by the analysis of the Western-blot. HEK293T cells, together with HEK293T knockdown METTL3/METTL14, were cultured in the absence and presence of Torin1 for 2 hours, after which cells were collected for NGS library preparation [2].
Deletion of adapters, filtering of reads that were too short, and deduplication were performed using CutAdapt (v2.10) and seqkit (v0.10.1). Mapping and read counting were conducted using STAR (v2.7.6), the standard human genome assembly hg38, by means of GENCODE basic gene annotation (v.38). Gene filtering was applied based on the number of reads from RNA-Seq and Ribo-Seq, with the thresholds of 5 counts per million (cpm) for RNA-Seq (the minimum library size of 2.2 million reads) and 15 cpm for Ribo-Seq (the minimum library size of 0.7 million reads). The read normalization and differential translation were computed using DESeq2 (v1.38.1), with the normalization performed by means of PSP73; GFP spike-ins for RNA-Seq, and Ribo spike-ins for Ribo-Seq. The analysis of RNA m6A methylation utilized data from DART-Seq, m6A-REF-Seq, miCLIP, MINES, and eTAM-Seq, originally obtained from HEK293T cells [3, 4].
Results: Experiments were conducted on HEK293T cell lines with the knockdown of methylases METTL3/14 (hereafter referred to as KD) and on wild-type (WT) cells under normal conditions and upon mTOR inhibition. Differential translation analysis identified several sets of genes exhibiting varying sensitivity to mTOR signaling pathway inhibition. The largest group, subsequently termed as “Down”, comprised 3849 mRNAs, demonstrating decreased translation levels in both WT and KD cells. As it was anticipated, this gene group was enriched with mRNAs containing TOP motifs [5], which are the primary targets of mTOR. Additionally, a group of genes (termed as

Neutral) was identified, where translation remained unchanged upon mTOR inhibition in both cell lines. Of particular interest is the group of genes (termed as KD Down), where translation levels of corresponding mRNAs decreased exclusively in cells with the knockdowns of METTL3/14 methylases.

To determine the proportion of methylated transcripts in each of the studied groups, the processed data from quantitative mapping of m6A modification (eTAM-Seq) were utilized. The analysis revealed an increasing proportion of methylated transcripts in the sequence Down < KD Down < Neutral. Based on it, it can be hypothesized that the sensitivity of different mRNA groups to mTOR inhibition depends on the degree of mRNA methylation.

Conclusion: We have demonstrated that the knockdown proteins of the methyltransferase complex METTL3 and METTL14 in HEK293T cells leads to the reduction in overall translation levels when the mTOR signaling cascade is inhibited. Analysis of the distribution of m6A modification in mRNA showed that the cumulative methylation level increases in the sequence Down < KD Down < Neutral. Thus, the most sensitive mRNAs to mTOR inhibition (the Down group) have the lowest methylation level. The elevated methylation levels are observed in the KD Down and Neutral groups. In wild-type cells, translation of such mRNAs is resistant to mTOR inhibition. However, when the amount of methyltransferase in the cell is reduced, leading to decreased mRNA methylation, translation is inhibited only in the KD Down group. It can be hypothesized that there is a certain threshold level of the mRNA methylation necessary for translation stability against mTOR inhibition. In wild-type cells, methylation below the threshold is a characteristic of the members of the Down group, whose translation is inhibited upon mTOR inhibition. With the reduced methyltransferase activity, the methylation level of the KD Down group members falls below the threshold, resulting in their translation being inhibited. Meanwhile, the members of the Neutral group, which a characterized by a higher methylation level, the reduction in methylation does not reach the threshold value, so their translation continues to be resistant to mTOR inhibition.

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