

COMMENTARY



Wobble uridine tRNA modification: a new vulnerability of refractory melanoma

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ABSTRACT

The enzymes catalysing the modification of the wobble uridine (U₃₄) of tRNAs (U₃₄-enzymes) play an important role in tumor development. We have recently demonstrated that the U₃₄-enzymes are crucial in the survival of glycolytic melanoma cultures through a codon-specific regulation of HIF1α mRNA translation. Moreover, depletion of U₃₄-enzymes resensitizes resistant melanoma to targeted therapy. These results indicate that targeting U₃₄-enzymes represents a new therapeutic opportunity for melanoma patients.

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Reprogramming of mRNA translation has recently emerged as a central mechanism driving the adaptation of tumor cells during cancer progression and response to therapy.¹ mRNA translation is based on the pairing between codons of mRNAs and anticodons of tRNAs. Modifications of tRNA molecules in the anticodon, and particularly at the wobble position, directly impact their decoding capacity and affect translation elongation.² We previously showed that enzymes modifying the tRNA wobble uridine (U₃₄-enzymes) are essential during WNT-driven intestinal cancer initiation,³ they promote breast cancer metastasis,⁴ but they are dispensable for normal intestinal and breast tissue maintenance.³⁻⁵ In the current study,⁶ we explored whether wobble uridine tRNA modification contributes to specific mRNA translation rewiring during oncogene-induced transformation and/or adaptation of cancer cells to therapy.

Malignant melanoma is a highly aggressive form of skin cancer whose incidence is rising worldwide and with poor prognosis when not restricted by surgical exportation. Genomics studies of human melanoma have shown that the mitogen-activated protein kinase (MAPK) pathway is often deregulated in melanoma, with nearly 50% of the patients harbouring a single activating mutation of the *BRAF* gene, with V600E being the most common mutation (*BRAF*^{V600E}). Specific inhibitors of the oncoprotein *BRAF*^{V600E} have been used in the clinic and despite dramatic anti-tumor responses observed in the first weeks, insurgence of resistance deeply limits their efficacy.⁷ Among the various resistance mechanisms already described, metabolic reprogramming towards glycolysis has been shown to promote resistance towards *BRAF* inhibitors in melanoma patients.⁸

In our recent study,⁶ we found that U₃₄-enzymes are largely increased in human melanoma biopsies and primary melanoma lines compared to healthy melanocytes. Genetic deletion of *elp3* (*Elongator complex protein 3*), one of the

key U₃₄-enzyme, strongly impaired melanoma insurgence in zebrafish with a *BRAF*^{V600E}/*p53*^{-/-} background. Moreover, depletion of the U₃₄-enzymes ELP3 or CTU1/2 (Cytoplasmic tRNA 2-thiolation protein 1 and 2) induced cell death in patient-derived *BRAF*^{V600E} melanoma cultures. By combining comparative proteomics and metabolomics approaches, we demonstrated that highly glycolytic melanoma cultures are dependent on U₃₄-enzymes for survival. Specifically, we showed that the mRNA translation of the transcription factor Hypoxia-inducible factor 1-alpha (HIF1α), a key regulator of glycolysis in the cells, is supported by the U₃₄-enzymes. In fact, depletion of U₃₄-enzymes resulted in decreased amount of HIF1α protein levels due to abnormal accumulation of the ribosomes and defective translation elongation at the HIF1α transcript. Strikingly, we found that this effect is codon-specific, as a systematic replacement of the U₃₄-sensitive codons (namely AAA, GAA and CAA) in HIF1α mRNA into their insensitive synonymous counterpart (respectively AAG, GAG and CAG), abolished the need of U₃₄-enzymes during HIF1α translation: it normalized ribosome distribution along HIF1α transcript and it restored HIF1α protein expression upon depletion of U₃₄-enzymes. This indicates that U₃₄-enzymes regulate HIF1α translation in a codon-specific manner (Figure 1).

Previous studies have associated HIF1α expression and glycolytic switch to insurgence of resistance towards MAPK-based targeted therapies in melanoma.⁹ We found that resistant and metastatic malignant melanoma have higher levels of the U₃₄-enzymes and of HIF1α. Strikingly, when we depleted the U₃₄-enzymes in both *in vitro* and *in vivo* models of resistant melanoma, tumors were resensitized to targeted therapy. Moreover, this effect is, at least in part, due to HIF1α mistranslation. These results indicate that U₃₄-enzymes promote resistance towards targeted therapy, at least by promoting codon-specific HIF1α mRNA translation.

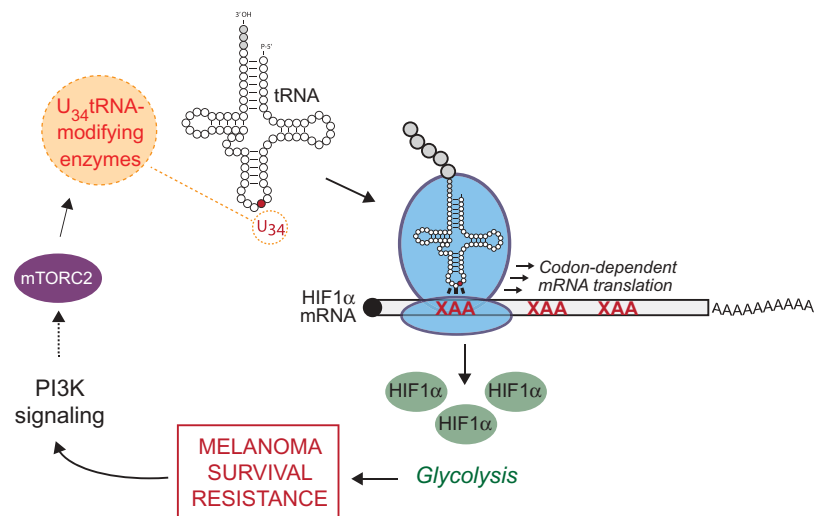


Figure 1. Mechanisms linking U₃₄-enzymes to melanoma cell survival and resistance to targeted therapy. Wobble uridine tRNA modifying enzymes (U₃₄-enzymes) are upregulated in malignant melanoma following activation of the Phosphoinositide-3-Kinase (PI3K) signalling pathway through a direct, mTOR Complex 2 (mTORC2)-dependent mechanism. U₃₄-enzymes promote mRNA translation of Hypoxia-induced factor 1-alpha (HIF1α) in a codon-dependent manner to sustain high levels of HIF1α and maintain a glycolytic metabolism, which confers resistance toward MAPK-based therapy.

Resistance in melanoma is often mediated by activation of the Phosphoinositide-3-Kinase (PI3K) pathway.⁷ In our study, we highlighted that the PI3K-mTOR Complex 2 (mTORC2) axis directly regulates the U₃₄-enzymes through phosphorylation and stabilization of the scaffold Elongator protein 1 (ELP1), suggesting that the levels of U₃₄-enzymes are dynamically regulated in time by the oncogenic PI3K-mTORC2 pathway,⁶ (Figure 1).

The efficacy of targeted therapies in melanoma is strongly limited by the insurgence of resistance, which causes relapses. In our study, we uncovered a novel resistance mechanism through upregulation of wobble uridine tRNA modification and codon-specific reprogramming of mRNA translation. We highlighted a new family of enzymes that is essential to promote adaptation to therapy and acquisition of resistance by regulation of selective mRNA translation.⁶ These data are highly significant for the patients because they suggest that targeting the U₃₄-enzymes in combination with MAPK-based therapies might become a new therapeutic option to prolong treatment efficacy and to prevent tumor relapse.

Further questions remain. First, although we have shown that HIF1α, whose mRNA is enriched in U₃₄-codons, relies on U₃₄-enzymes for its expression, it is still unclear how ribosome pausing at specific codons in mRNAs affects subsequent protein expression. One possible explanation is that a slow-down during translation elongation, as seen upon U₃₄-enzymes depletion, affects co-translational protein folding. This is supported by our data showing that the newly synthesized HIF1α is directed to the protein aggregates upon depletion of U₃₄-enzymes. Being able to predict the proteome that relies on U₃₄-enzymes for correct expression is essential to identify the pathological contexts where the U₃₄-enzymes play key roles. Second, we demonstrated that the PI3K-mTORC2 signalling pathway regulates the levels of the U₃₄-enzymatic complex Elongator. Understanding the precise mechanisms underlying this regulation merits further investigation. In which cellular compartment does this happen? What other

pathway(s) could be involved? How does this regulation translate into U₃₄-tRNA modifications and how does it impact on mRNA translation and the establishment of a specific proteome? Investigating how U₃₄-enzymes are regulated is crucial to predict their real impact in normal physiology and in pathophysiological conditions.

Modifications of tRNAs and their role in the regulation of specific translational reprogramming is a new and exciting field which has rapidly expanded in the last years. Our findings bring a new important piece of evidence demonstrating the role of U₃₄-enzymes in cancer adaptation to therapy by sustaining metabolic rewiring through codon-dependent regulation of HIF1α mRNA translation. Given the importance of HIF1α transcriptional activity in various malignant tumors,¹⁰ the targeting the U₃₄-enzymes might prove to be relevant not only for melanoma patients but also in a wide range of cancers.

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Disclosure of Potential Conflicts of Interest

No potential conflict of interest was reported by the authors.

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