News & Views

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# A wobbly road to drug resistance in melanoma: tRNA-modifying enzymes in translation reprogramming

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**Alterations in transcript-specific translation are emerging as a driver of cellular transformation and cancer etiology. A new study provides evidence for enhanced codon-dependent translation of hypoxia-inducible factor 1α in promoting glycolytic metabolism and drug resistance in melanoma cells. This specialized translation reprogramming relies, in part, on mTORC2-mediated phosphorylation of enzymes modifying the wobble position of the transfer RNA anticodon.**

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See also: **F Rapino *et al*** (June 2018)

Cancer cells rapidly adapt to extreme environmental conditions by changes in specific metabolic pathways (Ward & Thompson, 2012). The mechanisms that control this metabolic reprogramming by immediate changes in specific gene expression programs remain less clear. Now, an exciting new study by Rapino *et al* (2018) provides evidence for an unexpected role of enzymes that modify transfer RNA (tRNA) in this process. Specifically, they show that enzymes modifying uridine 34 ($U_{34}$) of tRNA are important for the maintenance and therapeutic resistance of an aggressive skin cancer known as melanoma. This tRNA modification is linked to codon-mediated translation regulation of hypoxia-inducible factor 1α (HIF1α), a transcription factor known for its role in metabolic adaptation to unfavorable growth conditions. From a clinical perspective, these exciting findings pave the way for targeting RNA-modifying enzymes in drug-resistant cancers. Of note, codon-mediated translation regulation of the KRAS and HRAS oncogenes has recently been implicated in tumorigenesis and drug resistance (Lampson *et al*, 2013; Ali *et al*, 2017) suggesting that alternative codon usage may be a more widespread feature of human cancers than previously thought.

Codon usage, tRNA abundance and modifications, and codon-anticodon base pairing are crucial for efficient and accurate translation of messenger RNAs (mRNAs) into proteins (Agris, 2004). How these basic decoding mechanisms are altered in cancer cells remains poorly understood. While $U_{34}$ tRNA-modifying enzymes have been implicated in metabolism, DNA damage response, and exocytosis (Esberg *et al*, 2006; Begley *et al*, 2007; Laxman *et al*, 2013), their role in cancer has not been extensively studied until now. By employing a combination of approaches and patient samples, Rapino *et al* (2018) demonstrate that key enzymes required for $U_{34}$ tRNA modifications are increased in melanomas exhibiting highly glycolytic phenotypes that are often associated with mutations in BRAF (one of the most commonly mutated genes in melanoma patients). Rapino and colleagues provide compelling evidence that $BRAF^{V600E}$ melanomas are "addicted" to high levels of $U_{34}$ tRNA-modifying enzymes. Together with an assortment of additional proteins, these enzymes catalyze a rather unconventional chemical modification involving methoxycarbonylmethyl formation at position 5 (termed $mcm^5U$) mediated by the multi-subunit Elongator (ELP) complex (Huang *et al*, 2005) and a subsequent thiolation at position 2 (termed $s^2U$) by cytosolic thiouridylases and the ubiquitin-related modifier 1 (URM1) pathway (Leidel *et al*, 2009) to generate 5-methoxycarbonylmethyl-2-thiouridine (termed $mcm^5s^2U$) at $U_{34}$ of the tRNA anticodon loop. This study further shows that resistance to targeted BRAF inhibition (vemurafenib) is overcome by a reduction in Elongator proteins ELP1 and ELP3 and the cytosolic thiouridylase subunit 1 and 2 (CTU1/CTU2).

An exciting question arises as to why a reduction in $U_{34}$ tRNA-modifying enzymes elucidates such a profound growth inhibition phenotype in highly metabolic melanomas. Rapino *et al* (2018) employ an elegant combination of proteomics and ribosome profiling experiments to identify a role for specific codons in rewiring translation mediated by $U_{34}$ tRNA-modifying enzymes in skin cancer cells. The authors show that the abundance of proteins enriched in codons, known to require thiolated tRNAs for decoding, such as Gln, Glu, and Lys codons, are significantly increased in $BRAF^{V600E}$-driven melanoma. Moreover, ELP3 depletion enhanced ribosome occupancy of mRNAs biased for codons decoded by $U_{34}$-modified tRNAs, without globally impacting ribosome footprinting density. Having identified that ELP3 influences decoding of specific codons during translation in melanoma, the authors

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next focus on HIF1α whose mRNA transcript was significantly enriched in codons that require $U_{34}$ tRNA modifications and whose protein, but not mRNA, was strongly decreased upon depletion of ELP3 or CTU1/2 (Fig 1). Mechanistically, the authors demonstrate that upon ELP3 reduction, ribosomes accumulate on HIF1α mRNA indicating a decrease in decoding during translation elongation. Rapino and colleagues identify that replacing codons that rely on $U_{34}$ tRNA-modifying enzymes with synonymous codons rescues HIF1α protein expression, defects in cell viability, and glucose metabolism observed upon ELP3 or CTU1 depletion. Consistent with previous reports (Nedialkova & Leidel, 2015), it was further shown that deficient decoding due to loss of $U_{34}$ tRNA-modifying enzymes results in accumulation of protein aggregates. Together, the findings presented in this study clearly demonstrate that in melanoma, accurate HIF1α mRNA translation requires $U_{34}$ tRNA-modifying enzymes (Fig 1). While the authors unquestionably show the importance of the $U_{34}$ tRNA-modifying enzymes in this process, they do not determine a direct change in tRNA modifications in their study. An exciting avenue for future research involves understanding how tRNA modifications within the anticodon loop interphase with the growing repertoire of RNA modifications (coined the epitranscriptome) to modulate translation elongation.

Given the observation that HIF1α is a determinant of response to BRAF inhibition in melanoma, the authors next probed the role of $U_{34}$ tRNA-modifying enzymes in acquired resistance to targeted BRAF therapy. This is an extremely relevant question as acquired resistance to BRAF inhibitors such as vemurafenib or dabrafenib is a characteristic of $BRAF^{V600E}$-driven melanoma. Interestingly, the authors demonstrate that reducing $U_{34}$ tRNA-modifying enzymes re-sensitized resistant $BRAF^{V600E}$ melanoma cells to vemurafenib and significantly reduced melanoma growth *in vivo*, which appears to rely on HIF1α. Finally, the authors show that increased levels of $U_{34}$ tRNA-modifying enzymes in resistant $BRAF^{V600E}$ melanoma

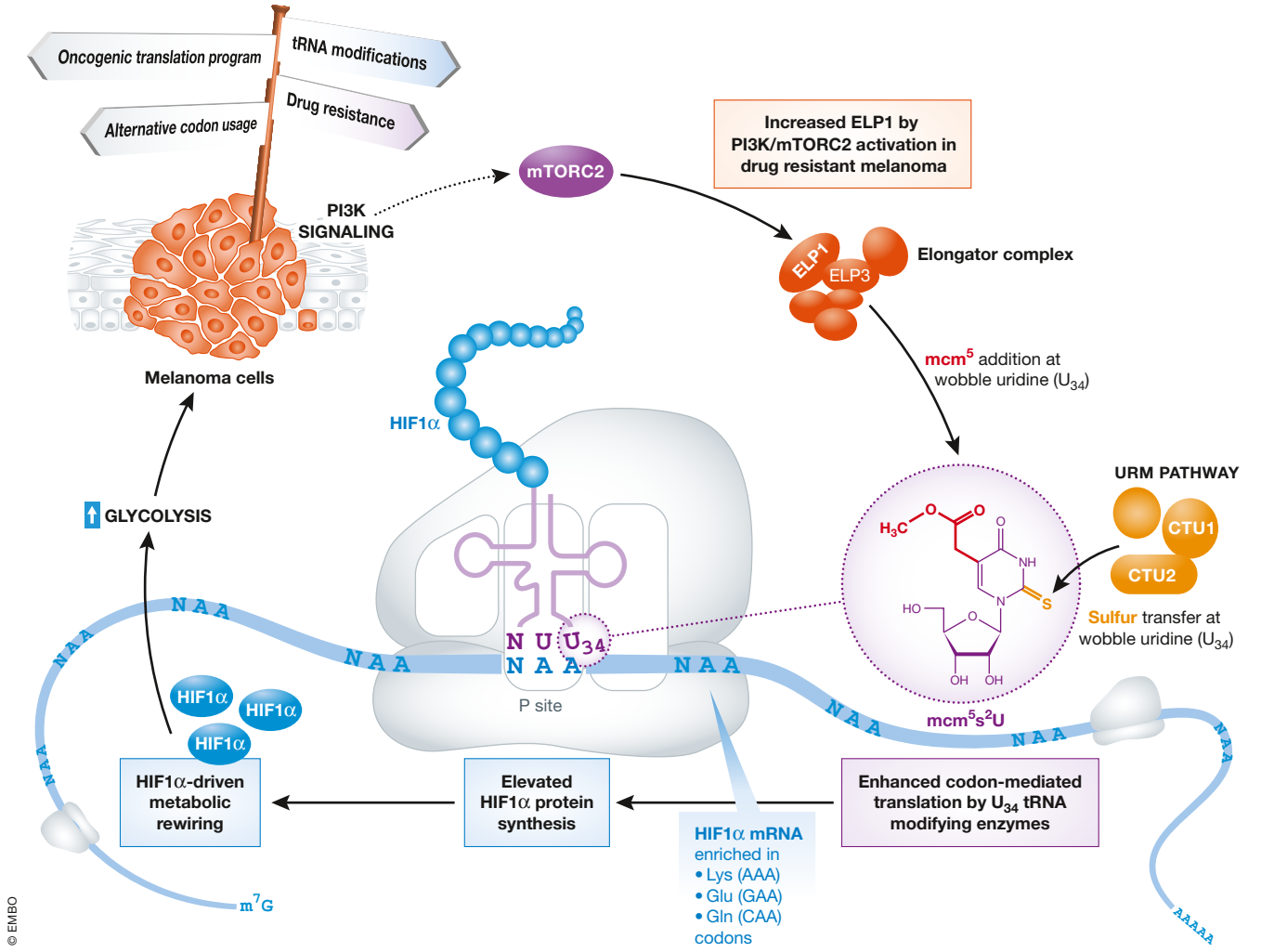


**Figure 1. Codon-dependent translation regulation of HIF1α couples glycolytic metabolism to drug resistance in melanoma.**
Cartoon depicts the proposed mechanism by which increased activation of $U_{34}$ tRNA-modifying enzymes promotes HIF1α protein synthesis, leading to enhanced glycolysis and conferring mTORC2-dependent resistance toward targeted BRAF inhibition.

are associated with elevated PI3K/mTOR signaling. Mechanistically, they illustrate that mTORC2 activity, but not mTORC1, enhances ELP1 phosphorylation at S1174, leading to elevated ELP1 levels and is crucial for the survival of HIF1α-dependent glycolytic melanoma cells. Altogether, these exciting findings provide a strong rationale for targeting RNA-modifying enzymes in drug-resistant melanoma and open a new portal into investigating the role of tRNA modifications and codon-mediated translation regulation in cancer pathogenesis.

Several questions arise, including whether the change in tRNA modification machinery identified in this study may be a harbinger of a more global effect of human oncogenes in controlling tRNA function to regulate specific gene expression programs to sustain malignant properties. Even though not addressed in this study, the tight interplay between $U_{34}$ tRNA-modifying enzymes and glycolysis in melanoma poses the question as to whether feedback loops exist whereby the products of metabolic pathways can, in turn, directly influence tRNA modifications to drive tumorigenesis. Notably, various RNA-modifying enzymes require cofactors such as ATP, vitamins, and metal ions to modulate their activity (Helm & Alfonzo, 2014), all of which are often considered by-products of cancer cell metabolism. This study will no doubt open several exciting avenues for future research and ignite the design of novel therapeutic strategies for "designer tRNAs" optimized for suppression of gene-specific translation downstream of oncogenic activation.

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