

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

Mary McMahon¹  & Davide Ruggero^{1,2} 

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 1 α 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 (U₃₄) 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 1 α (HIF1 α), 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 U₃₄ 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 U₃₄ 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 U₃₄ 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 mcm⁵U) mediated by the multi-subunit Elongator (ELP) complex (Huang *et al*, 2005) and a subsequent thiolation at position 2 (termed s²U) by cytosolic thiouridylases and the ubiquitin-related modifier 1 (URM1) pathway (Leidel *et al*, 2009) to generate 5-methoxycarbonylmethyl-2-thiouridine (termed mcm⁵s²U) at U₃₄ 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 U₃₄ 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 U₃₄ 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 U₃₄-modified tRNAs, without globally impacting ribosome footprinting density. Having identified that ELP3 influences decoding of specific codons during translation in melanoma, the authors

1 School of Medicine and Department of Urology, Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA, USA. E-mail: mary.mcmahon@ucsf.edu

2 Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA, USA. E-mail: davide.ruggero@ucsf.edu
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next focus on HIF1 α whose mRNA transcript was significantly enriched in codons that require U₃₄ 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 HIF1 α mRNA indicating a decrease in decoding during translation elongation. Rapino and colleagues identify that replacing codons that rely on U₃₄ tRNA-modifying enzymes with synonymous codons rescues HIF1 α 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 U₃₄ tRNA-modifying enzymes results in accumulation of protein aggregates. Together, the findings presented in this study clearly demonstrate that in melanoma, accurate HIF1 α mRNA translation requires U₃₄ tRNA-modifying enzymes (Fig 1). While the authors unquestionably show the importance of the U₃₄ 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 HIF1 α is a determinant of response to BRAF inhibition in melanoma, the authors next probed the role of U₃₄ 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 U₃₄ tRNA-modifying enzymes re-sensitized resistant BRAF^{V600E} melanoma cells to vemurafenib and significantly reduced melanoma growth *in vivo*, which appears to rely on HIF1 α . Finally, the authors show that increased levels of U₃₄ tRNA-modifying enzymes in resistant BRAF^{V600E} melanoma

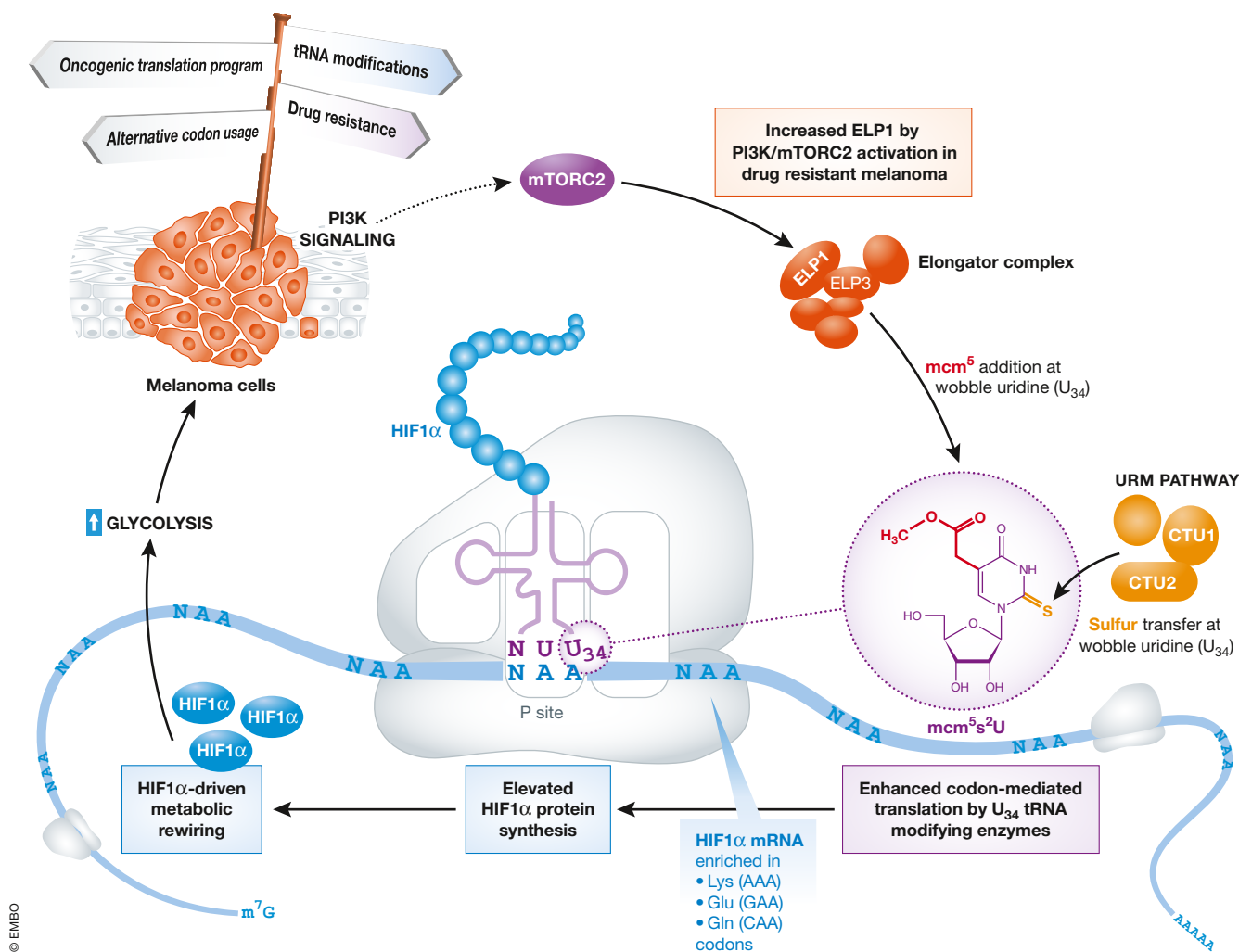


Figure 1. Codon-dependent translation regulation of HIF1 α couples glycolytic metabolism to drug resistance in melanoma. Cartoon depicts the proposed mechanism by which increased activation of U₃₄ tRNA-modifying enzymes promotes HIF1 α protein synthesis, leading to enhanced glycolysis and conferring 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 HIF1 α -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 U₃₄ 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.

References

- Agris PF (2004) Decoding the genome: a modified view. *Nucleic Acids Res* 32: 223–238
- Ali M, Kaltenbrun E, Anderson GR, Stephens SJ, Arena S, Bardelli A, Counter CM, Wood KC (2017) Codon bias imposes a targetable limitation on KRAS-driven therapeutic resistance. *Nat Commun* 8: 15617
- Begley U, Dyavaiah M, Patil A, Rooney JP, DiRenzo D, Young CM, Conklin DS, Zitomer RS, Begley TJ (2007) Trm9-catalyzed tRNA modifications link translation to the DNA damage response. *Mol Cell* 28: 860–870
- Esberg A, Huang B, Johansson MJO, Bystrom AS (2006) Elevated levels of two tRNA species bypass the requirement for elongator complex in transcription and exocytosis. *Mol Cell* 24: 139–148
- Helm M, Alfonzo JD (2014) Posttranscriptional RNA modifications: playing metabolic games in a cell's chemical legoland. *Chem Biol* 21: 174–185
- Huang B, Johansson MJO, Bystrom AS (2005) An early step in wobble uridine tRNA modification requires the elongator complex. *RNA* 11: 424–436
- Lampson BL, Pershing NLK, Prinz JA, Lacsina JR, Marzluff WF, Nicchitta CV, MacAlpine DM, Counter CM (2013) Rare codons regulate KRas oncogenesis. *Curr Biol* 23: 70–75
- Laxman S, Sutter BM, Wu X, Kumar S, Guo XF, Trudgian DC, Mirzaei H, Tu BP (2013) Sulfur amino acids regulate translational capacity and metabolic homeostasis through modulation of tRNA thiolation. *Cell* 154: 416–429
- Leidel S, Pedrioli PGA, Bucher T, Brost R, Costanzo M, Schmidt A, Aebersold R, Boone C, Hofmann K, Peter M (2009) Ubiquitin-related modifier Urm1 acts as a sulphur carrier in thiolation of eukaryotic transfer RNA. *Nature* 458: 228–232
- Nedialkova DD, Leidel SA (2015) Optimization of codon translation rates via tRNA modifications maintains proteome integrity. *Cell* 161: 1606–1618
- Rapino F, Delaunay S, Rambow F, Zhou Z, Tharun L, De Tullio P, Sin O, Shostak K, Schmitz S, Piepers J, Ghesquière B, Karim L, Charlotiaux B, Jamart D, Florin A, Lambert C, Rorive A, Jerusalem G, Leucci E, Dewaele M *et al* (2018) Codon-specific translation reprogramming promotes resistance to targeted therapy. *Nature* 558: 605–609
- Ward PS, Thompson CB (2012) Metabolic reprogramming: a cancer hallmark even Warburg did not anticipate. *Cancer Cell* 21: 297–308