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.

Reprogramming of mRNA translation has recently emerged as a central mechanism driving the adaptation of tumor cells during cancer progression and response to therapy. 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. We previously showed that enzymes modifying the tRNA wobble uridine (U34-enzymes) are essential during WNT-driven intestinal cancer initiation, they promote breast cancer metastasis, but they are dispensable for normal intestinal and breast tissue maintenance. In the current study, 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 (BRAFV600E). Specific inhibitors of the oncoprotein BRAFV600E have been used in the clinic and despite dramatic anti-tumor responses observed in the first weeks, insurgence of resistance deeply limits their efficacy. Among the various resistance mechanisms already described, metabolic reprogramming towards glycolysis has been shown to promote resistance towards BRAF inhibitors in melanoma patients.

In our recent study, 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-enzymes, strongly impaired melanoma insurgence in zebrafish with a BRAFV600E/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 BRAFV600E 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 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.
Resistance in melanoma is often mediated by activation of the Phosphoinositide-3-Kinase (PI3K) pathway. 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

The efficacy of targeted therapies in melanoma is strongly limited by the insufficiency 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 slowdown 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 relying 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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References
1. Truitt ML, Ruggero D. New frontiers in translational control of the cancer genome. Nat Rev Cancer. 2016;16:288–304. doi:10.1038/nrc.2016.27.
2. Nedialkova DD, Leidel SA. Optimization of codon translation rates via tRNA modifications maintains proteome integrity. Cell. 2015;161:1–13. doi:10.1016/j.cell.2015.05.022.
3. Ladang A, Rapino F, Heukamp LC, Tharun L, Shostak K, Hermand D, Delaunay S, Klevernic I, Jiang Z, Jacques N, et al. ELP3 drives Wnt-dependent tumor initiation and regeneration in the intestine. J Exp Med. 2015;212:2057–2075. doi:10.1084/jem.20142288.
4. Delaunay S, Rapino F, Tharun L, Zhou Z, Heukamp L, Termathe M, Shostak K, Klevernic I, Florin A, Desmecht H, et al. ELP3 links tRNA modification to IRES-dependent translation of LEF1 to sustain metastasis in breast cancer. J Exp Med. 2016;213:2503–2523. doi:10.1084/jem.20151916.
5. Rapino F, Delaunay S, Zhou Z, Chariot A, Close P. tRNA modification: is cancer having a wobble? Trends Cancer. 2017;3:249–252. doi:10.1016/j.trecan.2017.02.004.
6. Rapino F, Delaunay S, Rambow F, Zhou Z, Tharun L, De Tullio P, Sin O, Shostak K, Schmitz S, Piepers J, et al. Codon-specific translation reprogramming promotes resistance to targeted therapy. Nature. 2018;558:605–609. doi:10.1038/s41586-018-0115-1.
7. Homet B, Ribas A. New drug targets in metastatic melanoma. J Pathol. 2014;232:134–141. doi:10.1002/path.4259.
8. Abildgaard C, Guldberg P. Molecular drivers of cellular metabolic reprogramming in melanoma. Trends Mol Med. 2015;21:164–171. doi:10.1016/j.molmed.2014.12.007.
9. Parmenter TJ, Kleinschmidt M, Kinross KM, Bond ST, Li J, Kaadle MR, Rao A, Sheppard KE, Hugo W, Pupo GM, et al. Response of BRAF-mutant melanoma to BRAF inhibition is mediated by a network of transcriptional regulators of glycolysis. Cancer Discov. 2014;4:423–433. doi:10.1158/2159-8290.CD-13-0646.
10. Denko NC. Hypoxia, HIF1 and glucose metabolism in the solid tumor. Nat Rev Cancer. 2008;8:705–713. doi:10.1038/nrc2468.