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COMMENTARY

Emerging role of mRNA epitranscriptomic regulation in chemoresistant cancer cells

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ABSTRACT
Cancer persister cells remain a significant barrier to effective anti-cancer therapy. We found that melanoma persister cells undergo a reversible reprogramming of mRNA translation. A subset of mRNAs, harboring N6-methyladenosine in their 5′-untranslated regions, is translationally up-regulated in an eIF4A-dependent manner. Targeting eIF4A prevents the emergence of resistant clones.

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The most important factor that limits the success of targeted therapy against cancer is the emergence of drug resistance. Most of the focus in understanding resistance has been on genetic-driven resistance, including mutations that enable bypassing target inhibition through impaired binding of the drug or activation of alternative signaling pathways. In contrast to the resistance-conferring genetic mutations, emerging evidence raises the importance of reversible, non-genetic mechanisms in therapy evasion.[1] Even with the most effective cancer therapies that lead to complete response, a small number of residual cancer cells, that is undetectable by clinical or radiological exploration (Figure 1a), persists throughout the treatment.[2,3] These cancer persister cells can serve as a reservoir for the development of diverse acquired genetic resistance when the treatment is maintained (Figure 1b).[1] Therefore, targeting cancer persister cells appears as an attractive strategy to increase the effectiveness of existing therapies before the acquisition of *de novo* genetic mutations that lead to resistance.

Despite the milestone discovery of the cancer persister cell in human non-small cell lung cancer in 2010,[3] the biological mechanisms of this population of cells are still poorly defined. Several mechanisms have been proposed to explain the ability of the cancer persister cells to tolerate drugs *in vitro*. Activation of the histone lysine demethylase 5A and 5B (KDM5A/B) mediated by insulin-like growth factor 1 receptor (IGF1R) renders lung cancer cells persist the treatment of epithelial growth factor receptor (EGFR) inhibitors.[3] In the following study, the authors found that trimethylation of lysine 9 on histone H3 (H3K9me3) represses long-interspersed repeat elements 1 (*LINE-1*), and counterbalance the expression interferon-stimulated genes to promote persister cell survival.[4] A dependency on glutathione peroxidase 4 (GPX4) was also found in multiple cancer persister cell models, and it was shown that loss of GPX4 function induces ferroptosis in persister cells and correlates with the prevention of tumor relapse.[5] In contrast to most of the studies focusing on epigenetic and transcriptional level, our recent findings highlight the involvement of an mRNA epitranscriptomic mechanism in $BRAF^{V600E}$ mutated melanoma persister cell tolerance to anti-BRAF and anti-MEK targeted therapies.

mRNA methylation, particularly N6-methyladenosine (m^6A), is a post-transcriptional modification that largely impacts the translatome.[6] It has been shown that m^6A modification close to the stop codon or in the 3′-untranslated region (3′UTR) of mRNA can promote the mRNA degradation. However, m^6A modification close to 5′-untranslated region (5′UTR) of mRNA recruits translation initiation factors (i.e. eukaryotic translation initiation factor 4A, eIF4A) and thus promote the mRNA translation.[6] m^6A is deposited by a methyltransferase complex consisting of methyltransferase like-3 (METTL3), methyltransferase like-4 (METTL14) and wilms tumor 1-associated protein (WTAP). The identification of these enzymes enables the discovery of the dynamically regulated function of m^6A in controlling stem cell differentiation, cellular heat-shock response, DNA damage and tumorigenesis.[6] Using *in vitro* melanoma persister cell model, we found that a substantial -m^6A-associated mRNA translational remodeling is correlated with the melanoma persistent state upon anti-BRAF and anti-MEK treatments.[7] These m^6A-associated mRNAs are up-regulated at the translational level in spite of the global down-regulation of mRNA translation activity in melanoma persister cells (Figure 1c). Using m^6A RNA immunoprecipitation followed by RNA sequencing, we showed that these mRNAs specifically harbor m^6A modifications in their 5′UTRs.[7] Knocking down the methyltransferase (i.e. *METTL3* or *WTAP*) abrogates the association of these mRNAs with the polysomes, and sensitizes melanoma cells to anti-BRAF and anti-MEK treatments. Using polysome profiling, we showed that the up-regulation of this subset of mRNAs requires the RNA helicase eIF4A, whose inhibition selectively kills the melanoma persister cells compared to treatment-naïve cells. In addition, eIF4A inhibitor decreases the association of m^6A-modified mRNAs with polysomes and prevents the emergence of drug resistance.[7]

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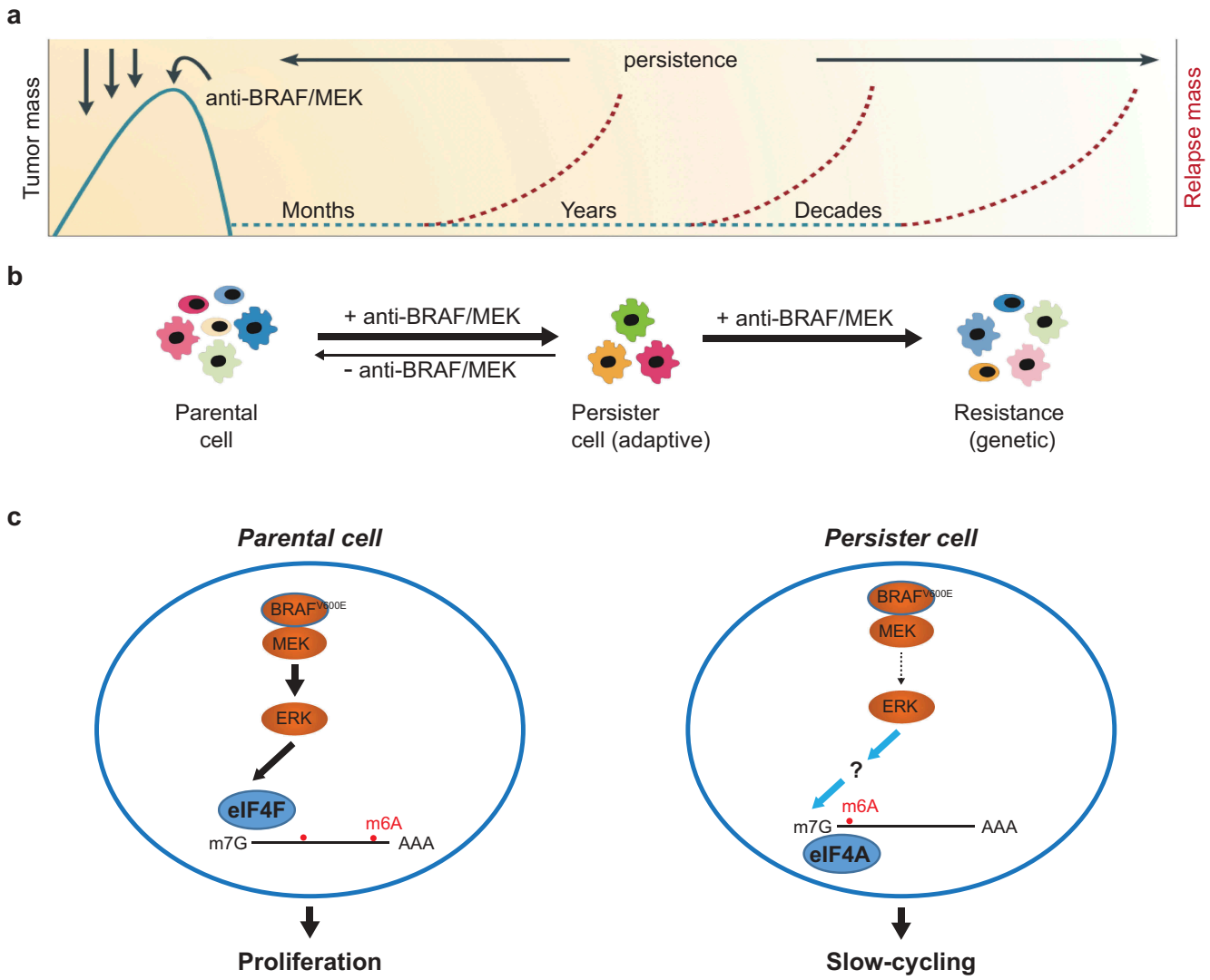

Figure 1. Translational reprogramming in chemoresistant melanoma cells.

(**a**) Clinical relevant view of the cancer cell persistence during the therapy. Initial response to targeted therapy rendering clinically undetectable tumor mass can sustain during months to years until tumor relapse with acquired genetic mutations. (**b**) Schematic view of the melanoma persister cell model. The persister cells could give rise to sensitive cells when the drug was removed and constitute a reservoir that eventually acquire irreversible genetic alterations leading to drug resistance upon continued treatment. (**c**) Eukaryotic translation initiation factor 4A (eIF4A)-mediated mRNA translational remodeling in melanoma persister cells. mRNAs harboring N6-methyladenosine (m^6A) modification in their 5′-untranslated regions (5′UTRs) are selectively translated in melanoma persister cells and may lead to a slow-cycling cellular state. In contrast, treatment-naïve cells are dependent on eukaryotic translation initiation factor 4F (eIF4F) mediated translation leading to a high proliferative cellular state. m7G represents mRNA N7-methylguaosine cap structure. AAA represents mRNA poly(A) tail. $BRAF^{V600E}$: BRAF site 600 valine (V) to glutamic acid (E) mutation. MEK: Mitogen-activated protein kinase. ERK: extracellular regulated protein kinases.

The origin of cancer persister cells still remains to be fully established. Nevertheless, it likely involves the transition of cellular state through a slow-cycling drug persistent state. More recently, it has shown that very rare melanoma cells that transiently display high expression of AXL receptor tyrosine kinase (AXL) prior to drug exposure were more likely to survive than treatment-naïve cells.[8] These studies support the idea that cancer persister cells are not a pre-existing subpopulation rather may arise stochastically from a dynamically fluctuating cell population. This is consistent with a recent single-cell study of melanoma persister cells.[9] This study defined four melanoma persistent states, including starvation-like state, neuro crest stem cell-like state, invasive and pigmented state. In particular, all co-existing persistent states are quiescent, indicating that diversity of persistence mechanisms share similar pre-requisite slow-cycling phenotype. Our current findings point to the possible link between mRNA translational remodeling, mRNA epitranscriptomic regulation, and slow-cycling cellular phenotype. It will be important to further determine whether mRNA epitranscriptomic mediated translational remodeling is the cause or the consequence of the pre-requisite slow-cycling phenotype (Figure 1c). Notwithstanding, a recent study has shown that mRNA translation is involved in non-heritable resistance to apoptosis induced by TNFα-related apoptosis-inducing ligand (TRAIL), even though they did not quest the role of m^6A modification in this context.[10] Therefore, mRNA translation may serve as a general mechanism by which a subpopulation of cancer cells remains persistent upon drug exposure and eventually evolve *bona fide* resistant mutations.

Our findings suggested that mRNA translation modulation by eIF4A inhibition may be explored as a potential

therapeutic strategy for targeting melanoma persister cells in combination with anti-BRAF and anti-MEK treatments.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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