METTL3 as a Predictive and Prognostic Molecular Marker in Breast Cancer and Melanoma

College of Pharmacy, Chosun University

Hong Seok Choi

Methyltransferase-like 3 (METTL3) is the catalytic subunit of the N^6-adenosine methyltransferase complex responsible for the N^6-methyladenosine (m^6A) modification of mRNA in mammalian cells. The expression of METTL3 is increased in several cancers; however, the underlying mechanisms that regulate METTL3 expression are unclear. We explored the regulatory roles of peptidyl prolyl *cis-trans* isomerase NIMA-interacting 1 (PIN1) in METTL3 stability and the m^6A modification of mRNA. PIN1 interacted with METTL3 in a phosphorylation-dependent manner and prevented its ubiquitin-dependent proteasomal and lysosomal degradation. PIN1-stabilized METTL3 increased the m^6A modification of *TAZ* and epidermal growth factor receptor (*EGFR)* mRNA, resulting in efficient translation. Knocking out PIN1 reduced polysome assembly and TAZ and EGFR mRNA in polysome fractions. Furthermore, inhibiting the MEK1/2 kinases and PIN1 reduced the m^6A-dependent translation of *TAZ* and *EGFR* via the destabilization of METTL3, thereby inhibiting breast cancer cell proliferation and the induction of cell cycle arrest at the G0/G1 phases. The METTL3 knockout further reduced the colony formation induced by overexpressing PIN1 in MCF7 cells. In an orthotopic mouse model, the enhanced growth of tumors formed in PIN1-overexpressing 4T1 cells was suppressed by the knockout of METTL3, supporting the positive role of PIN1 in METTL3-induced tumorigenesis. In the clinical context, PIN1 and METTL3 expression were significantly increased in breast tumors compared to normal tissues. METTL3 expression increased with tumor progression and was positively correlated with PIN1 expression in breast cancer tissues. Taken together, our data highlight the regulatory role of PIN1 in mRNA translation and suggest that the PIN1/METTL3 axis may be an alternative therapeutic target for breast cancer.

In a different clinical setting, acquired resistance often limits therapeutic efficacy of the BFAF (V600E) kinase inhibitor PLX4032 in patients with advanced melanoma. Epitranscriptomic modification of mRNAs by N^6-methyl adenosine (m^6A) modification contributes to melanoma pathogenesis; however, its role in acquired PLX4032 resistance remains unexplored. Here, we showed that m^6A methyltransferase METTL3 expression is upregulated in A375R cells, a PLX4032-resistant subline of A375 melanoma cells, compared with the parental cells. Moreover, METTL3 increased the m 6 A modification of epidermal growth factor receptor (EGFR) mRNA in A375R cells, which promoted its translation efficiency. In turn, increased EGFR expression facilitated rebound activation of the RAF/MEK/ERK pathway in A375R cells, inducing PLX4032 resistance. In contrast, knockout of METTL3 in A375R cells reduced EGFR expression and restored PLX4032 sensitivity. PLX4032 treatment following METTL3 knockout induced apoptosis and reduced colony formation in A375R cells and reduced A375R cell-derived tumor growth in BALB/c nude mice. These findings indicate that METTL3 promotes rebound activation of the RAF/MEK/ERK pathway through EGFR upregulation and highlight a critical role for METTL3-induced m 6 A modification in acquired PLX4032 resistance in melanoma, implicating METTL3 as a potential candidate for targeted chemotherapy.