Targeting METTL14 and RNA methylation to treat osteosarcoma

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Osteosarcoma (OS) is one of the most common and aggressive primary bone malignancies in both adults and children. During the past 4 decades, the substantial improvements in surgery and neoadjuvant chemotherapy have significantly increased the survival rate of OS patients. However, with currently available treatment regimens, more than 80% of the OS patients with metastasis and/or relapse do not survive over 5 years,[1] which underscores the unmet medical need to discover novel effective therapeutic target(s). The poor understanding of cellular and molecular mechanisms during osteosarcomagenesis has seriously prevented the development of new treatment approaches.

In eukaryotes, the gene expression is strictly regulated at multiple layers, including genetics, epigenetics, and epitranscriptomics, to maintain the normal bioprocess, while dysregulation of gene expression may lead to pathogenesis and tumorigenesis. The covalent chemical modifications in RNA have been revealed since 1950s and emerged as a crucial layer to manipulate gene expression at post-transcriptional level. To date, although more than 170 distinct chemical marks have been identified in RNAs, N^6-methyladenosine (m^6A), methylated adenosine at the N^6 position, is the most abundant internal modification in messenger RNA (mRNA). Since the initial discovery in 1974, the studies focusing on m^6A modification have been stuck for several decades due to the lack of quantification and sequencing approaches. Until 2011, identification of the first m^6A demethylase, fat mass and obesity-associated protein (FTO), indicates that m^6A is dynamic and reversable and resurged the broad interest in RNA modifications. The m^6A is always enriched in the RRACH (R=A or G; H = A, C, or U) context around stop codon region, and its machinery includes "writers", "erasers" and "readers" that deposit, remove, and recognize it, respectively.[2] Specifically, the m^6A methylation is co-transcriptionally installed in mRNA by a multicomponent complex composed of a stable core subunit, the methyltransferase-like 3 (METTL3)/METTL14 heterodimer, and additional regulators. The m^6A modification can be reversed by a-ketoglutarate (a-KG)-dependent and Fe(II)-dependent demethylases, FTO and AlkB homolog 5 (ALKBH5). The characterization of m^6A readers, such as YT521-B homology (YTH) domain family 1-3 (YTHDF1-3), YTH domain containing 1-2 (YTHDC1-2), and insulin-like growth factor 2 mRNA-binding proteins 1-3 (IGF2BP1-3), has provided insightful knowledge into the underlying mechanisms of m^6A-mediated gene expression regulation, including nuclear export, mRNA stability, alternative splicing, and translation efficiency. Therefore, m^6A represents the best characterized RNA modification thus far. Recently, accumulating evidence have suggested that both the m^6A abundance and its machinery are usually dysregulated in various cancers and such dysregulation is responsible for tumor initiation, progression, drug resistance, metastasis, and refractory.[3] More exciting, the novel small compounds targeting m^6A machinery have been developed and utilized to treat tumors, especially leukemia.[4] However, the biological functions and molecular mechanisms of m^6A and its regulators remain poorly understood in OS. It's still elusive about the role of m^6A machinery during osteosarcomagenesis.

In a recent issue of eBioMedicine, Li and colleagues determined the global m^6A abundance in OS tumors, systemically revealed the crucial tumor-promoting role of METTL14 during OS progression and metastasis, and highlighted that METTL14 might act as a promising therapeutic target for OS treatment.[5] Here, the authors observed that the overall m^6A abundance was significantly up-regulated in OS tumors in contrast to their corresponding controls, and such increased m^6A modification in mRNA was mainly attributed to the robust upregulation of METTL14, but not other m^6A regulators. Furthermore, the higher expression of METTL14 predicted the more adverse survival of OS patients. Via both *in vitro* and *in vivo* studies, the authors demonstrated that METTL14 played a critical oncogenic role in OS via facilitating cell growth/proliferation, promoting lung metastasis, and maintaining the stemness of cancer

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stem cells (CSCs). To further clarify the underlying cellular/molecular mechanism, they conducted transcriptome-wide m^6A-modified RNA immunoprecipitation sequencing (MeRIP-seq) and RNA sequencing (RNA-seq) in OS cells upon *METTL14* depletion, demonstrated Meningioma 1 (MN1) as a functionally essential and m^6A-dependent downstream target of METTL14. Mechanistically, METTL14 could directly install m^6A modification in *MN1* mRNA, and IGF2BP2 specifically recognized the m^6A marks and potentiated the mRNA stability and translation efficiency of *MN1* in OS. Additionally, previous studies have supported that forced expression of MN1 conferred resistance to all-trans-retinoic acid (ATRA)-mediated anti-tumor activity in acute promyelocytic leukemia (APL),[6] while it's still underdetermined whether such effect is involved in OS. In this study, the authors also demonstrated that METTL14/m^6A/IGF2BP2 axis-induced upregulation of MN could lead to the chemotherapy resistance to ATRA in OS, and suggested the potent therapeutic efficiency of ATRA in treating OS patients with endogenous low expression of MN1.

Overall, this proof-of-concept article has comprehensively investigated the oncogenic role of m^6A writer METTL14, elucidated its molecular mechanism via targeting MN1, and indicated that METTL14 might function as a prognostic predictor and a promising therapeutic target for OS patients. For further bench-to-bedside research, there are still a series of fundamental and clinical questions to be answered: (1) Given that several previous studies have reported the opposite (tumor-suppressor) role of METTL14 in OS,[7,8] further studies will be warranted to elucidate the divergent functions of m^6A modification and its writer in distinct OS subtypes; (2) It's also necessary to evaluate whether METTL14 is required for the survival and growth of normal human osteoblasts; (3) ATRA is not the standard first-line chemotherapy agent to treat OS and the IC_{50} values of ATRA in killing OS cells are pretty high (> 50 μM), indicating ATRA might be not the ideal drug for OS therapy. It will be of significance to assess whether genetic depletion and/or pharmacological inhibition of METTL14 can sensitize OS cells to standard chemotherapy, including doxorubicin, cisplatin, and methotrexate.

Contributors

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Declaration of interests

The authors declare no conflict of interest.

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