


LETTER TO THE EDITOR

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# HNRNPA2B1 promotes multiple myeloma progression by increasing AKT3 expression via m6A-dependent stabilization of ILF3 mRNA

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## Abstract

N6-methyladenosine (m6A) modification is the most prevalent modification in eukaryotic RNAs while accumulating studies suggest that m6A aberrant expression plays an important role in cancer. HNRNPA2B1 is a m6A reader which binds to nascent RNA and thus affects a perplexing array of RNA metabolism exquisitely. Despite unveiled facets that HNRNPA2B1 is deregulated in several tumors and facilitates tumor growth, a clear role of HNRNPA2B1 in multiple myeloma (MM) remains elusive. Herein, we analyzed the function and the regulatory mechanism of HNRNPA2B1 in MM. We found that HNRNPA2B1 was elevated in MM patients and negatively correlated with favorable prognosis. The depletion of HNRNPA2B1 in MM cells inhibited cell proliferation and induced apoptosis. On the contrary, the overexpression of HNRNPA2B1 promoted cell proliferation in vitro and in vivo. Mechanistic studies revealed that HNRNPA2B1 recognized the m6A sites of ILF3 and enhanced the stability of ILF3 mRNA transcripts, while AKT3 downregulation by siRNA abrogated the cellular proliferation induced by HNRNPA2B1 overexpression. Additionally, the expression of HNRNPA2B1, ILF3 and AKT3 was positively associated with each other in MM tissues tested by immunohistochemistry. In summary, our study highlights that HNRNPA2B1 potentially acts as a therapeutic target of MM through regulating AKT3 expression mediated by ILF3-dependent pattern.

**Keywords:** m6A, HNRNPA2B1, Multiple myeloma, MeRIP-Seq, ILF3, RNA stability, RIP-seq, AKT3

## To the Editor

N6-methyladenosine (m6A) modification is the most frequent RNA modifications in eukaryotic RNAs affecting gene expression, which is seldom investigated in MM [1–3]. Therefore, we checked the m6A genes in MM compared to normal plasma cells and the correlation of these genes with patient outcome including HNRNPA2B1, Mettl3, Mettl14, Wtap, etc., in MM patient

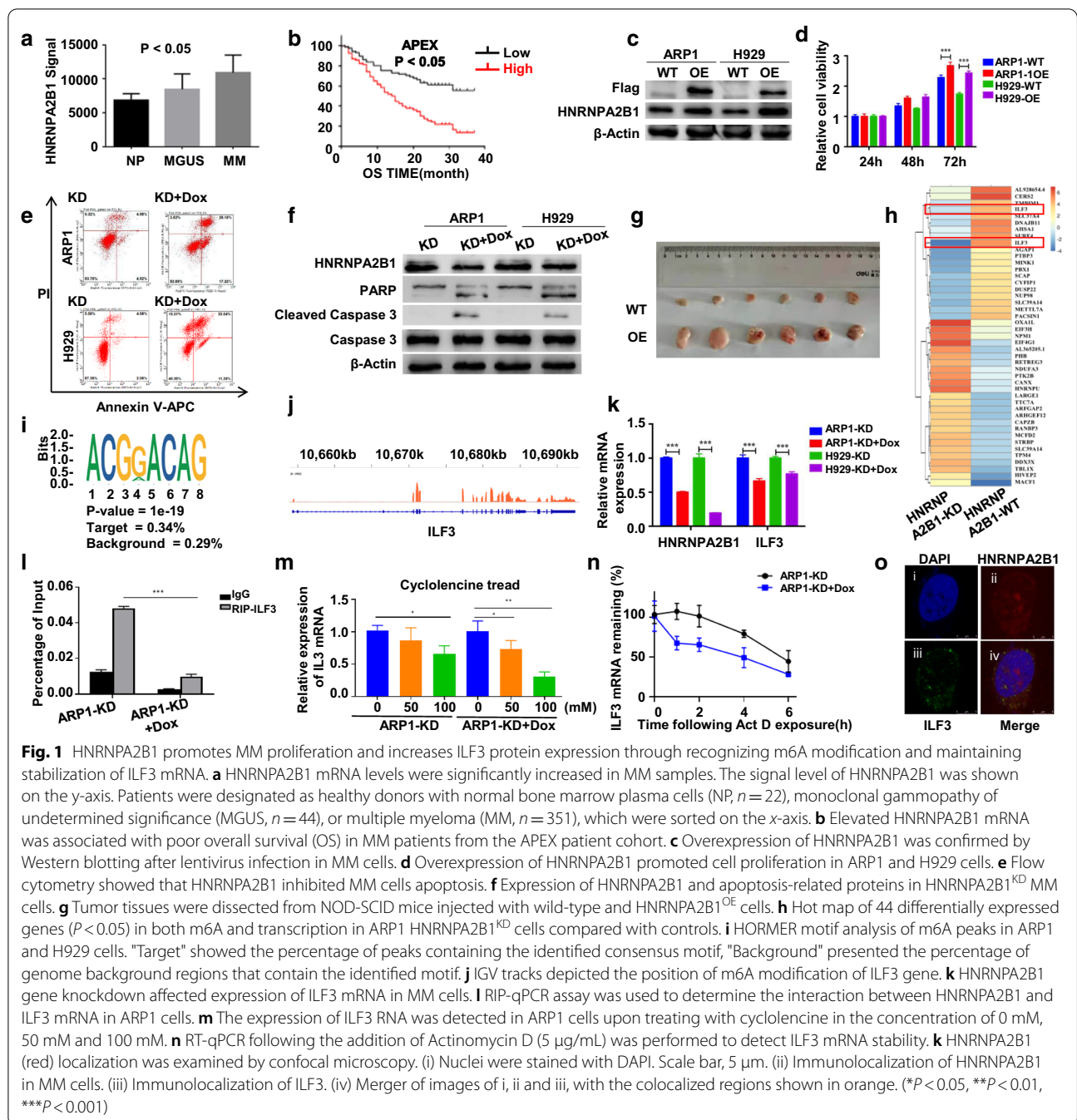
cohorts. Interestingly, HNRNPA2B1 was the exclusive gene, which was not only increased in MM samples but also associated with poor outcome in APEX, TT2 and HOVON65 patient cohorts (Fig. 1a, b, Additional file 1: Fig. S1a–h). HNRNPA2B1, RNA binding protein heterogeneous nuclear ribonucleoprotein A2B1, is a nuclear reader of m6A [4] and highly expressed in several cancers regulating the progression of cancer [5, 6] through multiple processes of mRNAs metabolism [7] including alternative splicing [8], cytoplasmic RNA trafficking [9], transcription and translation [10]. Here, we aimed to explore the potential functions and regulatory mechanism of HNRNPA2B1 in MM.

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Initially, we performed MTT assay that demonstrated the cellular proliferation was significantly increased in HNRNPA2B1 overexpression (HNRNPA2B1<sup>OE</sup>) cells and decreased in HNRNPA2B1 knockdown (HNRNPA2B1<sup>KD</sup>) cells (Fig. 1c, d, Additional file 1: Fig. S1i). Flow cytometry and WB analyses illustrated that knockdown of HNRNPA2B1 promoted cellular apoptosis (Fig. 1e, f). The nude mouse xenograft model by subcutaneous injection of HNRNPA2B1<sup>OE</sup> cells showed overexpression of

HNRNPA2B1 accelerating tumor growth in vivo (Fig. 1g and Additional file 1: Fig. S1j, k, l). These data indicated that HNRNPA2B1 promoted MM cellular growth in vitro and in vivo.

Next, m6A IP and RNA-seq analyses (MeRIP-Seq) found that ILF3 was downregulated ( $P < 0.05$ ) of both m6A and transcription in HNRNPA2B1<sup>KD</sup> cells compared with controls (Fig. 1h & Additional file 1: Fig. S2). The m6A consensus sequence (RRACH) motif [11] is

shown in Fig. 1i, in which the m6A modification of ILF3 was enriched in 3'-noncoding regions (Fig. 1j). Consistently, ILF3 expression was reduced in HNRNPA2B1<sup>KD</sup> cells (Fig. 1k, Additional file 1: Fig. S3a) while increased in HNRNPA2B1<sup>OE</sup> cells (Additional file 1: Fig. S3b, c). RNA immunoprecipitation (RIP)-qPCR assay revealed that ILF3 mRNA was enriched in the precipitates of HNRNPA2B1 antibody and silencing of HNRNPA2B1 decreased the abundance of the ILF3 transcript binding to HNRNPA2B1 (Fig. 1l). To further verify the role of m6A in regulation of ILF3 expression, methylation inhibitor cycloleucine was used that induced remarkable reduction of ILF3 in MM cells (Fig. 1m, Fig. S3d, e). Therefore, we can conclude that HNRNPA2B1 may stabilize ILF3 mRNA to play an important role. As expected, the stability of ILF3 was decreased in HNRNPA2B1<sup>KD</sup> cells (Fig. 1n, Additional file 1: Fig. S3f). The biological effects of HNRNPA2B1 might be related to its nucleocytoplasmic localization, as HNRNPA2B1 was distributed in both nuclear and cytoplasmic along with increased cytoplasmic localization of ILF3 (Fig. 1o, Additional file 1: Fig. S3g). The above results illustrated that HNRNPA2B1-induced expression of ILF3 was due to the enhanced stability of ILF3 mRNA transcripts upon recognition and bound of the m6A sites to HNRNPA2B1.

Notably, ILF3 expression was significantly elevated in plasma cells from MM patients (Fig. 2a) and associated with poor survival (Fig. 2b, Additional file 1: Fig. S4a, b). We established the ILF3 knockdown (ILF3<sup>KD</sup>) cells (Fig. 2c), which displayed decreased cell growth rate after induction compared to the non-induced cells (Fig. 2d). Apoptosis assay showed that ILF3 inhibited MM cellular apoptosis (Additional file 1: Fig. S4c, d, e). In addition, MTT result demonstrated that ILF3 knockdown by siRNA could reverse the cellular proliferation induced by increased HNRNPA2B1 suggesting that ILF3 is one of the most important m6A/HNRNPA2B1 targets in MM (Additional file 1: Fig. S4f, g).

Further RNA immunoprecipitation-sequencing (RIP-seq) analysis indicated that MAPK pathway was enriched (Fig. 2e) and AKT3 mRNA was significantly enriched by anti-ILF3 antibody in ARP1 cells. The ILF3 binding sites represented by peaks were enriched in AKT3 mRNA transcripts (Fig. 2f). In agreement with above results, decreased AKT3 was observed in ILF3<sup>KD</sup> cells (Fig. 2g, h) and RIP-qPCR using anti-ILF3 antibody confirmed a significantly reduced affinity of ILF3 to AKT3 mRNA in ARP1 ILF3<sup>KD</sup> cells compared to control (Fig. 2i). RNA decay assay showed a relatively lower stability of AKT3 transcripts in ILF3<sup>KD</sup> cells (Fig. 2j, Additional file 1: Fig. S5a) correspondingly. These data suggested that ILF3 promoted MM progression through stabilization of AKT3 transcripts.

Finally, we verified the effect of HNRNPA2B1 on AKT3. The expression of AKT3 was decreased in HNRNPA2B1<sup>KD</sup> cells (Fig. 2k, l), whereas the elevated expression of AKT3 was observed in HNRNPA2B1<sup>OE</sup> cells (Additional file 1: Fig. S5b, c). While AKT3 was interfered with siRNA (Fig. 2m), the cellular proliferation induced by HNRNPA2B1 was attenuated (Fig. 2n). Intriguingly, immunohistochemistry correlation analysis showed that HNRNPA2B1, ILF3 and AKT3 were highly increased in MM patients with statistical-correlated expression trend significantly compared to normal controls (Fig. 2o).

In summary, we demonstrate the m6A-dependent effect of HNRNPA2B1 on regulating AKT signaling pathway and the correlation between HNRNPA2B1 and MM cell growth. It is disclosed that the HNRNPA2B1/m6A/ILF3/AKT3 axis plays a key role in MM progression.

#### Abbreviations

m6A: N6-methyladenosine; HNRNPA2B1: Heterogeneous nuclear ribonucleoprotein A2B1; MM: Multiple myeloma; MeRIP-Seq: M6A IP seqing; ILF3: Interleukin enhancer-binding factor 3; RIP: RNA immunoprecipitation; AKT3: AKT serine/threonine kinase 3; ActD: Actinomycin D.

#### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13045-021-01066-6>.

**Additional file 1.** HNRNPA2B1 is a high-risk MM marker and promotes MM progression via enhancing ILF3-mediated expression of AKT3 in vitro and in vivo.

**Additional file 2.** Detailed materials and methods.

**Additional file 3.**

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#### Authors' contributions

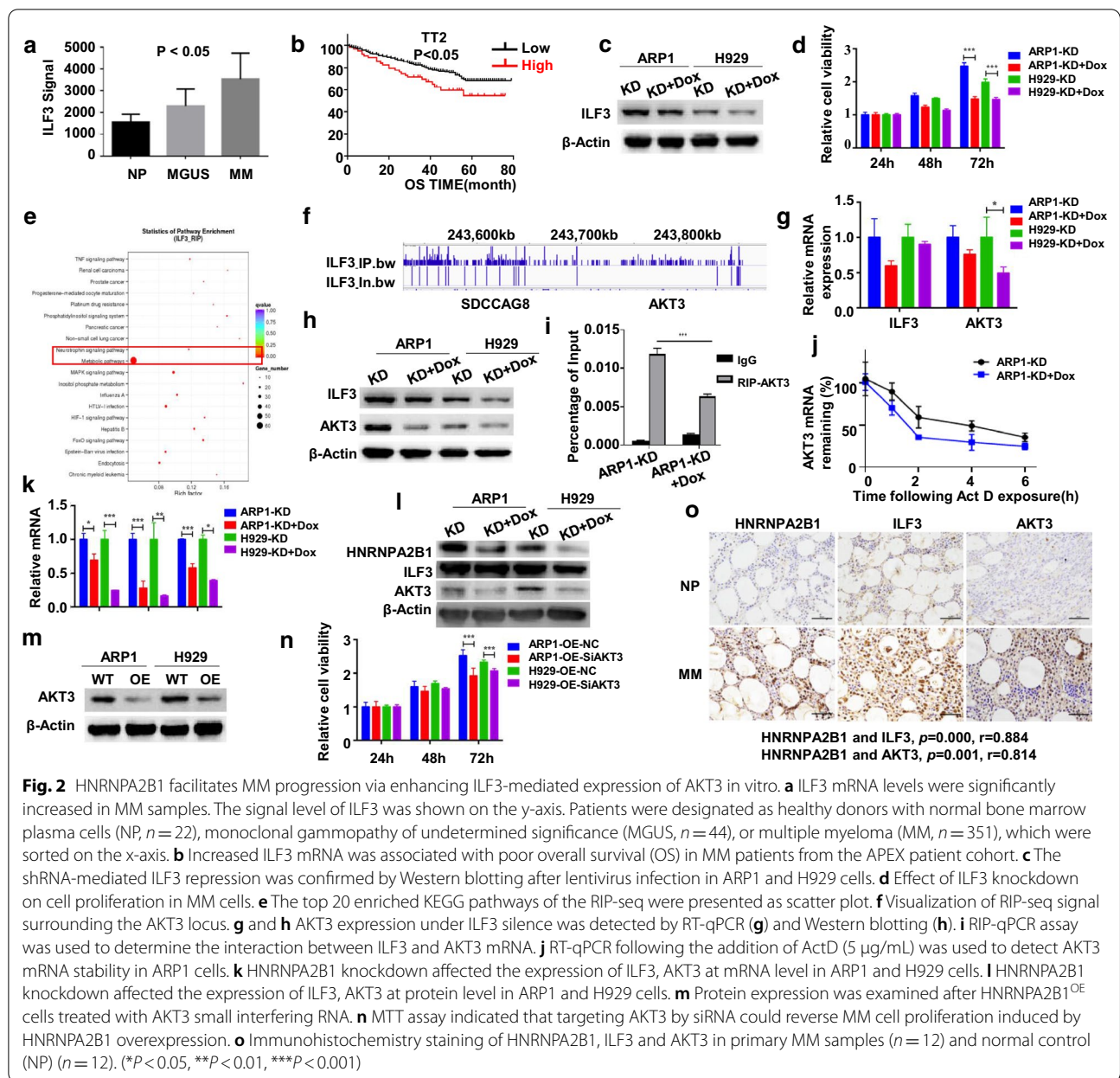
CG and YY designed the project, integrated the data and revised the manuscript; FJ, XT, CT, ZH, MK, CW, JZ and SG performed experiments, analyzed the data and drafted the manuscript; AJ, SJ, MB and FZ supervised this project and the manuscript; FZ offered part of the MM cohorts' database. All authors have read and approved the final version of the manuscript.

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#### Availability of data and materials

All supporting data are included in the manuscript and supplemental files. Additional data are available upon reasonable request to the corresponding author.



**Declarations**

**Ethics approval and consent to participate**

This study was approved by the Animal Ethics Committee of Nanjing University of Chinese Medicine (Ethics Registration No. 201905A003) in China.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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