Review of: "WTAP-mediated m6A modification of lncRNA NORAD promotes intervertebral disc degeneration"

Juan Li

Potential competing interests: The author(s) declared that no potential competing interests exist.

This is a study which showed the role of lncRNANORAD m^{6}A modificationsin intervertebral disc degeneration (IVDD). Previous research has shown that m^{6}A is the most prevalent RNA modification at the posttranscriptional level and involved in various diseases and cellular processes. The underlying mechanism of m^{6}A regulation in IVDD remains elusive. The principal finding was that methylation of the lncRNA NORAD significantly increases in senescent nucleus pulposus cells (NPCs) by m^{6}A sequencing. WTAP is increased in senescent NPCs due to an epigenetic increase in H3K4me3 of the promoter mediated by KDM5a, and significantly promotes NORAD m^{6}A modification. Furthermore, YTHDF2-mediated decay of NORAD is enhanced in senescent NPCs, and then deficiency of NORAD results in less sequestraion of PUMILIO proteins, further investigation found that fewer of the RNA-binding proteins PUM1/2 were sequestered when NORAD was downregulated, in turn increasing the degradation of targeted mRNAs of E2F3, thus promoting the senescence of NPCs.

There are many highlights in this article that deserve attention and appreciation:

1. Recent studies have revealed that m6A modification regulates multiple musculoske-letal disorders. In this study, The author first revealed that the lncRNA NORAD could be modified by m^{6}A due to an increase in WTAP, which was regulated by KDM5a-mediated H3K4me3 modification of the promoter. NORAD was downregulated during disc degeneration and NPC senescence, while overexpression of NORAD inhibited NPC senescence *in vitro*. The research showed that NORAD m^{6}A modification or the NORAD/PUMILIO/E2F3 axis could serve as a potential therapeutic target to inhibit the senescence of NPCs and development of IVDD.
2. In this study, Authors focused on the regulation of m^{6}A modification in the senescence of NPCs and found that WTAP was obviously upregulated and interacted with METTL3 and METTL14 to increase the formation of methyltransferase complexes in senescent NPCs.
3. It next investigated whether the upregulation of WTAP in NPCs was mediated by histone modification, and found that KDM5a mediated H3K4me3 modification of the WTAP promoter facilitates its transcription, thus promoting m^{6}A modification of the lncRNA NORAD.
4. To clarify the binding reader of m^{6}A-modified NORAD, It performed RNA-pulldown and CLIP assays and confirmed that YTHDF2 recognized methylated NORAD as a reader, resulting in its decreased stability and expression.
5. In this study, The author confirmed by RNA pull-down and RIP-qPCR that NORAD sequestered PUM1/2 and inhibited their pro-decay function in NPCs, which was attenuated in senescent NPCs due to the increase in m6A-mediated decay of NORAD. It further found, in senescent NPCs, that PUM1/2 targeted and degraded more transcripts of E2F3, a key regulator of the cell cycle and proliferation, thus contributing to NPC senescence.

On the other hand, there are a number of concerns with this study which include the following:

1. To explicate the regulatory mechanism of the m^{6}A modification, the expression of methylases and demethylases in NPCs were analyzed by western blot analysis, the results of which showed that expression of the methylase WTAP was significantly increased, in accordance with the sequencing results. In this part, there is a problem that the selection of methylation modification enzymes is not comprehensive. ALKBH5 and WTAP have opposite effects on m^{6}A modification, and thedemethylation modification of ALKBH5 should also receive equal attention.
2. In Part 2, WTAP-mediated m^{6}A modification of NORAD contributedto NPC senescence. To highlight the uniqueness of WTAP for NORAD m^{6}A modification, the effects of METTL3, ALKBH5 and METTL14 on NORAD expression are also shown.
3. In Part 3, WTAP upregulation is induced by the epigenetic alteration of H3K4me3. In addition to H3K4me3, H3K27me3 and H3K9me3 also undergo corresponding changes at the WTAP promoter in senescent NPCs, which should not be ignored by the authors.

In summary, The study puts forward a mechanism in the process of NPC senescence mediated by m^{6}A regulation, revealing that interruption of NORAD m^{6}A modification or targeting of the NORAD/PUMILIO/E2F3 axis could alleviate the progression of IVDD, and further provides a potential epigenetic therapeutic strategy to treat IVDD.

Juan Li

Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School