**METTL3 Regulates Ossification of the Posterior Longitudinal Ligament via the lncRNA XIST/miR-302a-3p/USP8 Axis**

Xiaoqiu Yuan†, Lei Shi†, Yongfei Guo, Jingchuan Sun, Jinhao Miao, Jianguo Shi and Yu Chen*

Spine Center, Department of Orthopaedics, Changzheng Hospital, Naval Medical University, Shanghai, China

The prevalence of ossification of the posterior longitudinal ligament (OPLL) is increasing, and currently there is no effective medical treatment for OPLL. Methyltransferase like 3 (METTL3), one of the components of the N6-methyladenosine (m6A) methyltransferase complex, regulates gene expression via modification of mRNA. Although METTL3 has been implicated in a variety of diseases, its role in OPLL remains to be elucidated. Primary ligament fibroblasts were used in this study. To investigate the role of METTL3 in OPLL, METTL3 was silenced or overexpressed. m6A RNA methylation was measured by commercially available kits. Luciferase reporter assay was performed to investigate the binding of miR-302a-3p and METTL3, and the binding of miR-302a-3p and USP8. Quantitative RT-PCR and western blots were used to evaluate mRNA and protein expression, respectively. OPLL increases METTL3 and its m6A modification. Overexpressing METTL3 significantly promoted osteogenic differentiation of primary ligament fibroblasts. Mechanism study showed that METTL3 increased m6A methylation of long non-coding RNA (lncRNA) X-inactive specific transcript (XIST). Further study showed that lncRNA XIST regulates osteogenic differentiation of primary ligament fibroblasts via miR-302a-3p, which targets ubiquitin-specific protease 8 (USP8). METTL3 enhanced osteogenic differentiation of primary ligament fibroblasts via the lncRNA XIST/miR-302a-3p/USP8 axis. The findings highlight the importance of METTL3-mediated m6A methylation of XIST in OPLL and provide new insights into therapeutic strategies for OPLL.

**Keywords:** ossification of the posterior longitudinal ligament, long non-coding RNA, methyltransferase like 3, m6A methylation, miR-302a-3p, X-inactive specific transcript

**INTRODUCTION**

Ossification of posterior longitudinal ligament (OPLL), is a process of fibrosis, calcification, and OPLL of spine (Belanger et al., 2005; Shi et al., 2019). OPLL is most commonly seen in Asian (Saetia et al., 2011), with a prevalence of 0.4 to 3.0% (Matsunaga and Sakou, 2012). Although a number of researches about OPLL have been carried out, no effective treatments are available and surgical decompression is the main choice. However, surgical decompression faces high risk and serious complications such as cerebrospinal fluid leakage and spinal cord injury (Saetia et al., 2011).
The pathogenesis of OPLL remains to be elucidated. Various molecular signaling pathways have been implicated in OPLL development such as oxidative stress, inflammation, and mitogen-activated protein kinases (MAPK) pathways (Yuan et al., 2019). Other study showed the ubiquitin-proteasome system, a major pathway for protein degradation, also contributes to OPLL development and progression (Tsuru et al., 2018). Sun et al. (2018) have shown that ubiquitin-specific protease 8 (USP8) stabilizes gap junction protein connexin43 (Cx43) through regulating its polyubiquitination, which has been shown to regulate OPLL via the NF-κB signaling pathway (Yuan et al., 2019). These studies suggest that USP8 may involve in the development process of OPLL. 

N^6-methyladenosine (m^6A) in mRNA, catalyzed by the methyltransferase like 3 (METTL3)-containing methyltransferase complex, has emerged as a regulatory mechanism which controls gene expression (Ping et al., 2014; Zaccara et al., 2019). Dysregulation of METTL3 has been associated with the pathogenesis of Alzheimer's disease (AD) (Huang et al., 2020). Cai et al. (2018) showed that METTL3 promotes breast cancer through inhibiting let-7g. Other studies also support that METTL3 plays a role in inflammatory response, rheumatoid arthritis, and cardiovascular disease (Song et al., 2019; Wang et al., 2019b). However, its role in the development process of OPLL remains unclear.

Increasing studies suggest that m^6A methylation regulates non-coding RNAs, such as miRNAs and IncRNAs (Ma et al., 2019). Patil et al. (2016) showed that m^6A RNA methylation promoted long non-coding RNA (IncRNA) X-inactive specific transcript (XIST)-mediated transcriptional repression. It also has been shown that XIST can be modified by METTL3 and recognized by the methylated reader YTH domain containing 1 (YTHDC1), thereby contributing to XIST-mediated X chromosome inactivation (Coker et al., 2020). Although methylation modification has been implicated in the occurrence and development of many diseases, its role in OPLL is largely unclear. Therefore, the role of METTL3 and its effects on m^6A modification of XIST in OPLL and the underlying mechanism were investigated.

**MATERIALS AND METHODS**

**Patient Information**

A total of 20 non-OPLL and 20 OPLL patients undergoing anterior cervical decompression surgery were included. The OPLL patients with 8 cases of local type, 10 cases of segmental type, and 2 cases of mixed type, included 10 males and 10 females, with a mean age of 45.9 ± 8.6 years. By contrast, non-OPLL patients with 14 cases of cervical fracture and 6 cases of cervical disk herniation, including 10 males and 10 females, with a mean age of 48.5 ± 9.4 years. The study protocol was approved by the Ethics Committee of Changzheng Hospital. Informed consent was obtained.

**Culture of Primary Ligament Fibroblasts**

Posterior longitudinal ligament specimens were collected during the Posterior longitudinal ligament surgery (Yang et al., 2011). Ligaments were dissected from a non-ossified site, minced, washed, and cultured in DMEM at 37°C. Osteogenic differentiation was induced by DMEM containing FBS (10%), ascorbic acid (25 μg/ml), β-glycerophosphate (10 mM) and dexamethasone (10 nM) for 2 weeks.

**Immunocytochemistry**

Human primary ligament fibroblasts were characterized via immunocytochemical staining of a fibroblast marker, vimentin (Reuther et al., 2003). Cells were cultured on microscopic glasses to confluence, washed, fixed, and permeabilized. After 1 h blockage with 5% goat serum, cells were incubated with mouse anti-vimentin (Beyotime Biotechnology, AF0318, 1:500) for 12 h at 4°C overnight.

**Immunofluorescence (IF) Microscopy**

Human primary ligament fibroblasts were fixed, permeabilized, and blocked. Cells were then incubated with anti-METTL3 antibody (Abcam, ab195352, 1:1000) or Goat Anti-rabbit IgG (H + L) antibody (Beyotime Biotechnology, A0423, 1:500). DAPI was used to stain nuclei. Cells were observed using a confocal microscopy (Heerbrugg, Switzerland).

**Gene Silencing/Overexpression**

Plasmid construction, lentivirus production and titration were done according to previous publication (Cribbs et al., 2013). Briefly, shRNAs targeting METTL3 (shRNA#1, GCTGCACCTTCAGACGAATT; shRNA#2, GGATAACCTGCAAGTATGTT; shRNA#3, GCTCAACATACCCGTACTA) or XIST (shRNA#1, GCTTCTAACTAGCTGAATT; shRNA#2, GCATGCATCTTGGAGATTT; shRNA#3, CCATGCATCTTGGAATT) were synthesized and inserted into pLKO.1 vector (Origene). To overexpress METTL3 or ubiquitin-specific protease (USP8), the coding sequence was inserted into pLVX-Puro plasmids (Clontech). HEK293T cells were co-transfected with pLKO.1 or pLVX-Puro, and packaging plasmids. Viruses were collected 2 days later.

**miRNA Inhibitor and Mimic**

miR-302a-3p inhibitor (5′-UCACCAAACAUUGAAGCAGCUA-3′), mimic (5′-UAAGUGUCUAUGAUGUGUGAG-3′) or negative control (NC, 5′-CAGUACUUUGUGUAUCAA-3′) were synthesized by Invitrogen (Beijing China). Inhibitor/mimic was used at 100 pmol for 24 h.

**m^6A Assay**

m^6A level was measured using m^6A RNA Methylation Assay Kit (Abcam).

**RNA Immunoprecipitation (RIP) Assays**

Magna RIP RNA-Binding Protein Immunoprecipitation kit (Millipore) was used. Total RNA was used as input control. IgG was used as isotype control. RNAs were isolated for qRT-PCR assay.
Alizarin Red S Staining
Third passage of human primary ligament fibroblasts were seeded at 4 × 10^4 cells/35 mm and cultured for 3 weeks in osteogenic-condition medium. Cells were then fixed and stained by 1% Alizarin Red S (Sigma-Aldrich, Shanghai).

RNA Extraction and Quantitative PCR
RNA was collected with TRIzol. One µg RNA was used for quantitative PCR using SYBR PCR mastermix (Thermo). Results were analyzed using 2^(-ΔΔCt) method. Primer sequences were listed in Table 1.

Western Blotting
Third passage of human primary ligament fibroblasts were lysed and proteins were resolved, blotted to PVDF membranes, blocked in 5% skim milk and incubated with primary antibodies (USP8, Abcam, ab228572; collagen I, Abcam, ab90395; ALP, Abcam, ab83259; METTL3, Abcam, ab195352, 1:1000; GAPDH, Cell Signaling, #5174, 1:2000). Membranes were then incubated with secondary antibodies and incubated with ECL (Pierce) for imaging.

Nuclear-Cytoplasmic Fractionation
Fractionation was performed with PARIS™ Kit (Invitrogen) according to manufacturer’s protocol. RNA of each part was collected using TRIzol.

Dual Luciferase Reporter Assay
XIST sequences carrying putative miR-302a-3p binding site, METTL3-3’UTR or USP8-3’UTR sequences were inserted into the pGL3 vector (Promega, Madison, WI, United States). Potential miR-302a-3p binding site was mutated. In brief, XIST, METTL3, and USP8 WT or MUT plasmids and miR-302a-3p mimic or inhibitor were co-transfected into 293T cells for measurement of luciferase activity.

Statistical Analysis
Data were shown as mean ± SD from triplicates. Statistics were conducted by GraphPad Prism (San Diego, CA, United States) using Student t-test between two groups if they were normally distributed and the variation was comparable or ANOVA test among three or more groups if the variation were comparable. The correlation of the two genes was examined by Spearman correlation test. P-values less than 0.05 were defined as statistically significant.

RESULTS
METTL3 and Its m^6^A Methylation Are Significantly Increased in OPLL Patients
To investigate the role of METTL3 in OPLL, we first checked the expression of METTL3 and its m^6^A levels in ligament tissues from OPLL and non-OPLL control patients. Data suggested that OPLL not only significantly upregulated the m^6^A level but also significantly increased METTL3 level compared with that of controls (Figures 1A,B). Because previous study showed that in addition to METTL3, other methyltransferases including Wilms’ tumor 1-associating protein (WTAP) and METTL14 also regulate m^6^A modification, we measured the expression of WTAP and METTL14 as well (Cheng et al., 2019). Results showed that at the mRNA level, OPLL slightly increased the expression of METTL14, but did not affect the expression of WTAP (Figures 1C,D). Then, primary ligament fibroblasts were isolated from controls and patients with OPLL and characterized by positive immunohistochemical staining of Vimentin (Figure 1E). We also measured the expression of METTL3 in primary ligament fibroblasts. As shown in Figure 1F, ligament fibroblasts of OPLL patients exhibited a significant higher expression of METTL3. These findings indicate that OPLL increases METTL3 and its m^6^A modification.
METTL3 Promotes Ossification of Primary Ligament Fibroblasts

To explore the role of METTL3 in ossification, METTL3 was successfully silenced or overexpressed in primary ligament fibroblasts (Figures 2A,B), and the cells underwent Alizarin Red S staining of extracellular calcium deposits. Results showed that overexpression of METTL3 significantly increased osteogenic differentiation, while silencing of METTL3 significantly suppressed osteogenic differentiation (Figure 2C). The expression of other osteogenic differentiation-related marker proteins, including alkaline phosphatase (ALP) and collagen type I (COL1), was also measured (Zhu et al., 2019). Western blotting analysis showed that overexpression of METTL3 significantly increased ALP and COL1, while silencing of METTL3 significantly decreased ALP and COL1 (Figure 2D). These findings suggest that METTL3 enhances ossification of primary ligament fibroblasts.

METTL3 Promotes Osteogenic Differentiation of Primary Ligament Fibroblasts Through Upregulation of lncRNA XIST1

To investigate how METTL3 regulates ossification, we first measured the expression of multiple lncRNAs, which were increased in OPLL tissues compared with non-OPLL tissues screened by using lncRNA microarray (Yuan et al., 2019). Results showed that LINC00657 and XIST were significantly increased by overexpressing METTL3, but decreased by METTL3 silencing. Manipulation of METTL3 did not affect the expression of POLDIP2, OIP5-AS1, MALAT1, NEAT1, or SLC26A4-AS1 (Figure 3A). Then, we checked the expression of LINC00657 and XIST in ligament tissues from controls and patients with OPLL, and found that OPLL significantly increased the expression of both LINC00657 and XIST (Figures 3B,C). Correlation analysis showed that METTL3 was significantly correlated with XIST, but not LINC00657 (Figures 3D,E). Next, we checked the levels of XIST m^6^-A and XIST in primary ligament fibroblasts silenced or overexpressed METTL3. Results suggested that silencing METTL3 in primary ligament fibroblasts significantly decreased m^6^-A modification of XIST, while overexpressing METTL3 significantly increased m^6^-A modification of XIST (Figure 3F). Then, XIST was successfully silenced in primary ligament fibroblasts (Figure 3G). Alizarin Red S staining results showed that silencing XIST significantly diminished METTL3 overexpression-promoted osteogenic differentiation of primary ligament fibroblasts (Figure 3H). We also investigated the effect of silencing XIST on the expression of ALP and COL1, and found that silencing XIST significantly diminished METTL3 overexpression-induced ALP and COL1 expression (Figure 3I). The data indicate that METTL3 promotes osteogenic ossification through upregulation of lncRNA XIST.

LncRNA XIST Regulates Osteogenic Differentiation of Primary Ligament Fibroblasts via miR-302a-3p

To figure out how lncRNA XIST regulates osteogenic differentiation, we first checked the distribution of XIST and found that XIST is abundant in the cytoplasm of primary
ligament fibroblasts (Figure 4A). Then, we performed a bioinformatic analysis and identified potential binding sites for miR-302a-3p, miR-302b-3p, miR-302c-3p, miR-302d-3p, and miR-302e in XIST sequences (Figure 4B). Next, the expression of those 5 miRNAs in XIST-silencing primary ligament fibroblasts from patients with OPLL was measured and data showed that silencing of XIST significantly increased the expression of miR-302a-3p, but not the others (Figure 4C). The RIP and qRT-PCR confirmed that the expression of XIST or miR-302a-3p associated with AGO2 (Figure 4D). Then, WT or mutated XIST and miR-302a-3p mimics or NC were transfected into 293T cells to perform a luciferase assay, as shown in Figure 4E, transfection of miR-302a-3p mimics suppressed the luciferase activity of WT XIST but not mutant XIST. We next transfected miR-302a-3p mimics, inhibitors or NC to primary ligament fibroblasts and checked the expression of XIST. Results showed that miR-302a-3p mimic significantly decreased the expression of XIST, while miR-302a-3p inhibitor significantly increased the expression of XIST (Figure 4F). Correlation analysis showed that XIST levels was significantly negatively correlated to miR-302a-3p (Figure 4G). Alizarin Red S staining results showed that silencing XIST significantly suppressed osteogenic differentiation of primary ligament fibroblasts, which was ameliorated by overexpressing miR-302a-3p inhibitor (Figure 4H). Silencing XIST significantly decreased ALP and COL1 expression, which was ameliorated by overexpressing miR-302a-3p inhibitor (Figure 4I). These results indicate that IncRNA XIST regulates osteogenic differentiation of primary ligament fibroblasts via miR-302a-3p.

miR-302a-3p Inhibits Osteogenic Differentiation of Primary Ligament Fibroblasts by Targeting USP8

To further investigate how miR-302a-3p inhibits osteogenic differentiation of primary ligament fibroblasts, we first performed a bioinformatic analysis and found that there was a potential binding site of miR-302a-3p in the 3’UTR of USP8 (Figure 5A). Luciferase report assay results showed that overexpressing miR-302a-3p remarkably suppressed luciferase activity of USP8-3’UTR-WT while miR-302a-3p inhibitor promoted luciferase activity of USP8-3’UTR-WT, but had no effect on the luciferase activity of USP8-3’UTR-Mutant (Figure 5B). We then conducted real time PCR and western blotting to measure the effect of miR-302a-3p on the expression of USP8, and results indicated that overexpressing miR-302a-3p sharply downregulated USP8 while miR-302a-3p inhibitor significantly increased USP8 (Figures 5C,D). Then, we checked the expression of USP8 in ligament tissues from controls and patients with OPLL, and found that OPLL significantly increased the expression of USP8 (Figure 5E). Correlation analysis showed that USP8 mRNA was negatively correlated with miR-302a-3p level (Figure 5F). Next, USP8 was successfully overexpressed in primary ligament fibroblasts (Figures 5G,H). The effects of miR-302a-3p and USP8
on osteogenic differentiation of primary ligament fibroblasts were investigated, and Alizarin Red S staining results showed that miR-302a-3p mimic significantly suppressed osteogenic differentiation of primary ligament fibroblasts, which was ameliorated by overexpressing USP8 (Figure 5I). Results also showed that miR-302a-3p mimic significantly decreased ALP and COL1 expression, which was ameliorated by overexpressing USP8 (Figure 5J). These findings suggest that USP8 mediates the inhibitive effect of miR-302a-3p on ossification.

**miR-302a-3p Targets METTL3 to Regulate Osteogenic Differentiation**

Bioinformatic analysis of METTL3-3′UTR sequence also showed a potential miR-302a-3p binding sites (Figure 6A). Therefore, we also performed a luciferase assay for miR-302a-3p and METTL3 interaction. Results suggested that overexpressing miR-302a-3p remarkably repressed luciferase activity of METTL3-3′UTR-WT, while miR-302a-3p inhibitor dramatically increased the luciferase activity of METTL3-3′UTR-WT, but had no effect on the luciferase activity of METTL3-3′UTR-Mutant (Figure 6B). qPCR and immunoblotting results showed that miR-302a-3p mimic significantly decreased METTL3, while miR-302a-3p inhibitor significantly increased METTL3 (Figure 6C). Correlation analysis showed that METTL3 mRNA was negatively correlated with miR-302a-3p level (Figure 6D). We then performed Alizarin Red S staining to study the effect of miR-302a-3p and METTL3 on osteogenic differentiation of primary ligament fibroblasts. Results indicated that overexpressing miR-302a-3p suppressed...
osteogenic differentiation of primary ligament fibroblasts, which was ameliorated by overexpressing METTL3 (Figure 6E). We further demonstrated that overexpressing miR-302a-3p downregulated ALP and COL1 expression, which was abolished by overexpressing METTL3 (Figure 6F). These findings suggest that miR-302a-3p targets METTL3 to regulate osteogenic differentiation.

**DISCUSSION**

In the current study, we demonstrated that METTL3 and its m^6^A modification are increased in OPLL patients. Overexpression of METTL3 significantly promoted ossification of primary ligament fibroblasts. Our findings also suggest that METTL3 increased m^6^A methylation of IncRNA XIST, and IncRNA XIST affect ossification of primary ligament fibroblasts via the miR-302a-3p/USP8 axis. Data also indicate that there is a negative feedback loop between METTL3 and miR-302a-3p. Identifying the important role of METTL3/XIST/miR-302a-3p/USP8 signaling may have great relevance to the pathogenesis of OPLL, and may facilitate the development of new drugs for OPLL treatment.

N^6^-methyladenosine has been recognized as a popular post-transcriptional RNA modification (Zhang et al., 2019). In addition, m^6^A modifications also regulate non-coding RNAs, such as miRNAs and IncRNAs (Ma et al., 2019). m^6^A is dynamically regulated by m^6^A demethylases and a methyltransferase writer complex which contains METTL3, METTL14, WTAP, KIAA1429, and RBM15/15B (Ping et al., 2014; Cheng et al., 2019). Recent studies have shown that among the methyltransferase writer complex, METTL3 is in charge of catalyzing m^6^A formation (Wang...
et al., 2016; Wang et al., 2019a). Coker et al. found that METTL3 promotes m⁶A modification of XIST, which is recognized by m⁶A reader YTHDC1, leading to X chromosome inactivation (Coker et al., 2020). Liu et al. indicated that METTL3 promotes m⁶A modification of lncRNA THOR and cancer cell proliferation (Liu et al., 2020). Another study indicated that lncRNA pncRNA-D suppresses cyclin D1 and arrests cell cycle through RNA m⁶A modification (Yoneda et al., 2020). In the current study, we found that METTL3 increased m⁶A methylation and expression levels of lncRNA XIST, leading to the promotion of osteogenic differentiation of primary ligament fibroblasts. These findings revealed a new role of METTL3 in OPLL, showing that METTL3 promotes ossification of primary ligament fibroblasts through upregulation of lncRNA XIST.

Long non-coding RNAs are above 200 nucleotides and do not code protein (Kung et al., 2013). It has been reported that lncRNAs play important roles in various biological processes through transcriptional and post-transcriptional gene regulation (Yoon et al., 2013). In the meantime, increasing evidence suggests that lncRNAs can act as sponges either for miRNAs (Cesana et al., 2011). For example, Wu et al. (2017) have shown that lncRNA-PAGBC can sponge miR-133b and miR-511, and Wang et al. (2014) indicated that lncRNA AK048451 binds miR-489 and acts as a miR-489 sponge. Our findings suggest that silencing of XIST significantly increased miR-302a-3p and suppressed ossification of primary ligament fibroblasts indicated by weaker Alizarin Red S staining and lower expression of ALP and COL1. However, whether lncRNA XIST acts as a miR-302a-3p sponge in primary ligament fibroblasts is still unclear and needs further investigation. We also proved that miR-302a-3p directly targets USP8, which stabilizes Cx43 that has been shown to regulate OPLL via the NF-κB signaling pathway (Yuan et al., 2019). Moreover, the expression of ALP and COL1 was also positively regulated by Cx43/NF-κB signaling.

Our findings not only increase our knowledge of METTL3/XIST in osteogenic differentiation of primary ligament fibroblasts, but also broaden our understanding of the pathogenesis of OPLL.
Interestingly, our data also suggested that miR-302a-3p can target METTL3, and METTL3 was negatively correlated with miR-302a-3p level. Overexpressing miR-302a-3p significantly suppressed osteogenic differentiation of primary ligament fibroblasts which was ameliorated by overexpressing METTL3, indicating a negative feedback loop between METTL3 and miR-302a-3p. These findings indicate a very important role of METTL3/XIST in osteogenic differentiation of primary ligament fibroblasts, and thus, improve our understanding of the pathogenesis of OPLL. There are certainly some limitations in this study. For example, this study was mainly performed in fibroblasts. Further studies using animal models and clinical samples will provide more relevant data. Although further studies are needed, this study identifies a new molecular mechanism underlying OPLL.

Taken together, the present study revealed a new role of METTL3/XIST, showing that METTL3 promotes osteogenic differentiation of primary ligament fibroblasts and OPLL through miR-302a-3p and its target USP8 by regulating upregulation of IncRNA XIST.

**ETHICS STATEMENT**

The studies involving human participants were reviewed and approved by the Changzheng Hospital. The patients/participants provided their written informed consent to participate in this study.

**AUTHOR CONTRIBUTIONS**

XY and LS conceived and designed the work. LS, YG, and JCS performed the research and collected and analyzed the data. JM and JGS collected human tissue samples. JM, JGS, and YC provided technical assistance. XY and YC wrote the manuscript. All authors read and approved the final manuscript.

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**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2021.629895/full#supplementary-material
REFERENCES

Belanger, T. A., Roh, J. S., Hanks, S. E., Kang, J. D., Emery, S. E., and Bohlin, H. H. (2005). Ossification of the posterior longitudinal ligament. Results of anterior cervical decompresion and arthrodesis in sixty-one North American patients. J. Bone Joint Surg. Am. 87, 610–615.

Cai, X., Wang, X., Cao, C., Gao, Y., Zhang, S., Yang, Z., et al. (2018). HRXIP-elevated methyltransferase METTL3 promotes the progression of breast cancer via inhibiting tumor suppressor let-7g. Cancer Lett. 415, 11–19.

Cesana, M., Cacchiarelli, D., Legnini, I., Santini, T., Sthandier, O., Chinappi, M., et al. (2011). A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. Cell 147, 358–369.

Cheng, X., Li, M., Rao, X., Zhang, W., Li, X., Wang, L., et al. (2019). KIAA1429 regulates the migration and invasion of hepatocellular carcinoma by altering m6A modification of ID2 mRNA. Onco Targets Ther. 12, 3421–3428.

Coker, H., Wei, G., Moiniort, B., Mohammed, S., Nesterova, T., and Brockdorff, N. (2020). The role of the Xist 5′ m6A region and RBM15 in X chromosome inactivation. Wellcome Open Res. 5:31.

Cribbs, A. P., Kennedy, A., Gregory, B., and Brennan, F. M. (2013). Simplified production and concentration of lentiviral vectors to achieve high transduction in primary human T cells. BMC Biotechnol. 13:98. doi: 10.1186/1472-6750-13-98.

Huang, H., Camats-Perna, J., Medioreis, R., Anggono, V., and Widagdo, J. (2020). Altered expression of the m6A methyltransferase METTL3 in Alzheimer's Disease. eNeuro 7:ENEURO.0125-20.2020.

Kung, J. T., Colognori, D., and Lee, J. T. (2013). Long noncoding RNAs: past, present, and future. Genetics 193, 651–669.

Liu, H., Xu, Y., Zhao, B., Shi, T., Lai, J., and Li, Z. (2020). A novel N6-methyladenosine (m6A)-dependent fate decision for the IncRNA THOR. Cell Death Dis. 11:613.

Ma, S., Chen, C., Li, X., Liu, J., Zhou, Q., and Zhang, G., et al. (2019). The interplay between m6A RNA methylation and noncoding RNA in cancer. J. Hematol. Oncol. 12:121.

Matsunaga, S., and Sakou, T. (2012). Ossification of the posterior longitudinal ligament of the cervical spine: etiology and natural history. Spine (Phila Pa 1976) 37, E309–E314.

Patil, D. P., Chen, C. K., Pickering, B. F., Chow, A., Jackson, C., Gutmann, M., et al. (2016). m(6)A RNA methylation promotes XIST-mediated transcriptional repression. Nature 537, 369–373.

Ping, X. L., Sun, B. F., Wang, L., Xiao, W., Yang, X., Wang, W. J., et al. (2014). Mammalian WTAP is a regulatory subunit of the N6-methyladenosine methyltransferase. Cell Res. 24, 177–189.

Reuther, T., Kohl, A., Komposch, G., and Tomakidi, P. (2003). Morphogenesis and proliferation in mono- and organotypic co-cultures of primary human periodontal ligament fibroblasts and alveolar bone cells. Cell Tissue Res. 312, 189–196.

Saetia, K., Cho, D., Lee, S., Kim, D. H., and Kim, S. D. (2011). Ossification of the posterior longitudinal ligament: a review. Neurosurg. Focus 30:E3.

Shi, L., Shi, G., Li, T., Luo, Y., Chen, D., Miao, J., et al. (2019). The endoplasmic reticulum stress response participates in connexin 43-mediated ossification of the posterior longitudinal ligament. Am. J. Transl. Res. 11, 4113–4125.

Song, H., Feng, X., Zhang, H., Luo, Y., Huang, J., Lin, M., et al. (2019). METTL3 and ALKBH5 oppositely regulate m(6)A modification of TFEB mRNA, which dictates the fate of hypoxia/reoxygenation-treated cardiomyocytes. Autophagy 15, 1419–1437. doi: 10.1080/15548627.2019.1586246

Sun, J., Hu, Q., Peng, H., Peng, C., Zhou, L., Lu, J., et al. (2018). The ubiquitin-specific protease USP8 deubiquitates and stabilizes Cx43. J. Biol. Chem. 293, 8275–8284. doi: 10.1074/jbc.ra117.013115.

Tsuru, M., Ono, A., Umezawa, H., Takeuchi, M., and Nagata, K. (2018). Ubiquitin-dependent proteolysis of CXCL7 leads to posterior longitudinal ligament ossification. PLoS One 13:e0196204. doi: 10.1371/journal.pone.0196204.

Wang, H., Xu, H., Huang, M., Liu, J., Gu, Y., Ma, L., et al. (2019a). Mettl3-mediated mRNA m(6)A methylation promotes dendritic cell activation. Nat. Commun. 10:1898.

Wang, J., Yan, S., Hu, L., Wang, S., and Xu, D. (2019b). METTL3 attenuates LPS-induced inflammatory response in macrophages via NF-kappaB signaling pathway. Med. Inflamm. 2019:3120391.

Yang, H. S., Lu, X. H., Chen, D. Y., Yuan, W., Yang, L. L., Chen, Y., et al. (2011). Mechanical strain induces Cx43 expression in spinal ligament fibroblasts derived from patients presenting ossification of the posterior longitudinal ligament. Eur. Spine J. 20, 1459–1465. doi: 10.1007/s00586-011-1767-9.

Yoneeda, R., Ueda, N., Urashinski, H., Hirasaki, M., and Kurokawa, R. (2020). Long noncoding RNA pncRNA-D reduces cyclin D1 gene expression and arrests cell cycle through RNA m(6)A modification. J. Biol. Chem. 295, 5626–5639. doi: 10.1074/jbc.R119.011556.

Yoon, J. H., Abdelmohsen, K., and Gorospe, M. (2013). Posttranscriptional gene regulation by long noncoding RNA. J. Mol. Biol. 425, 3723–3730. doi: 10.1016/j.jmb.2012.11.024.

Yuan, X., Guo, Y., Chen, D., Luo, Y., Chen, D., Miao, J., et al. (2019). Long non-coding RNA MALAT1 functions as miR-1 sponge to regulate Connexin 43-mediated ossification of the posterior longitudinal ligament. Bone 107, 305–314. doi: 10.1016/j.bone.2019.06.019.

Zaccara, S., Ries, R. J., and Jaffrey, S. R. (2019). Reading, writing and erasing mRNA methylation. Nat. Rev. Mol. Cell Biol. 20, 608–624. doi: 10.1038/s41580-019-0168-5.

Zhang, C., Fu, J., and Zhou, Y. (2019). A review in research progress concerning m6A methylation and immunoregulation. Front. Immunol. 10:922. doi: 10.3389/fimmu.2019.00922.

Zhu, Y., Wang, Y., Jia, Y., Xu, J., and Chai, Y. (2019). Catalpol promotes the viability and proliferation of periodontal ligament fibroblasts and alveolar bone cells. Am. J. Transl. Res. 11, 4113–4125.

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