Immunology Research and Perspectives

https://irp.cultechpub.com/irp

E-ISSN: 3154-7826

Vol.2, No.2, June 2026

DOI: 10.64229/950cwp20

Article

Investigating the Impact of METTL3 on Reinforcing Cell Adhesion by Enhancing Immunity through m6A-Mediated Stabilization of Integrin β1 mRNA in Osteosarcoma

Awais Ali, Syed Luqman Ali*

Department of Biochemistry, Abdul Wali Khan University, Mardan, Pakistan

*Corresponding author: Syed Luqman Ali, syedluqmanali5@gmail.com

Abstract

Osteosarcoma, a prevalent primary malignancy, continues to present significant challenges for current treatment strategies. However, a promising breakthrough emerges with PR-619, a novel inhibitor of deubiquitinating enzymes, showcasing remarkable anti-tumor effectiveness across various cancers. Recent advances in N6-methyladenosine (m6A) mRNA methylation reveal its critical role in human diseases, notably cancer. In our investigation, we've unveiled heightened METTL3 (Methyltransferase 3 (N6-Adenosine-Methyltransferase Complex Catalytic Subunit)) expression was identified in osteosarcoma, particularly in cases with bone metastasis, and was significantly associated with advanced disease progression and poor prognosis. Functional analyses demonstrated that METTL3, through an m6A-HuR-dependent mechanism, regulates Integrin β1 (ITGB1) expression, thereby enhancing its interaction with Collagen I and promoting osteosarcoma cell adhesion and motility, ultimately facilitating bone metastasis. Notably, METTL3-ITGB1-mediated adhesion remodeling may also influence immune-tumor interactions within the bone tumor microenvironment by altering extracellular matrix dynamics and immune cell accessibility. These findings elucidate a previously unrecognized epitranscriptomic mechanism underlying osteosarcoma metastasis and highlight METTL3 as a potential therapeutic target warranting further investigation with potential implications for potential effects on the tumor microenvironment that require future investigation.

Keywords

METTL3, Osteosarcoma M6A, Anti-tumor, ITGB1, Cell adhesion

Article History

Received: 20 November 2025 Revised: 27 July 2026

Accepted: 21 August 2026 Available Online: 24 August 2026

1. Background

Osteosarcoma is a rare but aggressive primary malignant bone tumor, predominantly affecting children, adolescents, and young adults, with a smaller secondary incidence peak in older adults [1-3]. It is not a leading cause of global cancer-related mortality, but it represents the most common primary bone malignancy worldwide [4,5]. Osteosarcoma is characterized by a strong propensity for metastasis, particularly to the lungs and bone. With the introduction of multimodal treatment strategies, including surgery and combination chemotherapy, outcomes for patients with localized disease have improved, and the current 5-year overall survival rate is approximately 60%-70% [1,6]. However, prognosis remains poor for patients with metastatic disease at diagnosis or relapse, with 5-year survival rates declining to below 30%, underscoring the urgent need to elucidate the molecular mechanisms underlying osteosarcoma progression and metastasis [7,8].

In recent years, the spotlight has turned to epigenetic modifications in RNA, capturing global scientific interest. Over 100 distinct chemical alterations have been uncovered within various RNA types, spanning messenger RNAs (mRNAs), transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), and non-coding RNAs. Among these, N6-methyladenosine (m6A) stands out as one of the most abundant internal post-transcriptional modification in eukaryotic mRNA [9]. Similar to DNA and histones, it undergoes reversible methylation, m6A methylation is a dynamic and reversible process regulated by specific methyltransferases ("writers"), demethylases ("erasers"), and m6A-binding proteins ("readers") that collectively determine RNA fate [10]. m6A plays an essential role in adaptable mRNA metabolism, impacting RNA processing, transport between the nucleus and cytoplasm, translation, and decay [11,12]. Beyond its canonical functions in RNA biology, accumulating evidence indicates that m6A modification critically regulates immune signaling pathways and immune cell function. m6A has been shown to modulate innate immune responses by controlling the expression of interferon-stimulated genes and inflammatory cytokines, while also shaping adaptive immunity through effects on T-cell differentiation, activation, and effector function. Dysregulation of m6A machinery has therefore been implicated in tumor immune evasion and cancer-immune microenvironment interactions [13,14]. In cancer biology, m6A RNA methylation has been associated with tumor initiation, cancer stemness, cell proliferation, invasion, and metastatic progression in multiple malignancies, including hepatocellular carcinoma, colorectal cancer, and pancreatic cancer [15,16]. However, the precise epitranscriptomic mechanisms through which m6A regulates osteosarcoma bone metastasis remain poorly understood. In the present study, we identify METTL3 as a key epitranscriptomic regulator driving bone metastasis in osteosarcoma by enhancing ITGB1/Collagen I-mediated adhesion through m6A-dependent stabilization of ITGB1 mRNA. These findings provide novel mechanistic insight into organ-specific metastasis in Osteosarcoma and establish a functional link between m6A regulation, tumor adhesion, and metastatic progression.

Recent studies have increasingly recognized that tumor cell-intrinsic molecular mechanisms can profoundly influence the tumor immune microenvironment. In particular, alterations in extracellular matrix organization and integrin-mediated adhesion are known to regulate immune cell infiltration, antigen presentation dynamics, and immune cell trafficking within tumors. Integrin β1 (ITGB1), a key receptor mediating interactions with collagen I in the bone matrix, not only supports tumor cell adhesion and metastatic colonization but also contributes to the structural remodeling of the extracellular matrix that can either facilitate or restrict immune cell access to tumor sites. Meanwhile, m6A RNA methylation has emerged as an important regulator of immune signaling pathways, including interferon responses, cytokine expression, and immune cell activation. Therefore, understanding how METTL3-dependent m6A modifications regulate integrin signaling may provide insight into how tumor cells modulate their surrounding immune microenvironment and evade immune surveillance.

2. Methodology

2.1 Patients and Tissue Specimens

Patients included in this retrospective study were recruited from Astana Medical University, Astana, Kazakhstan, where they underwent diagnosis and surgical treatment for osteosarcoma. Between 2008 and December 2014, primary osteosarcoma tissues and matched adjacent non-tumorous bone tissues were collected during surgical resection as part of routine clinical management. The study cohort consisted of 15 patients with localized osteosarcoma and 16 patients with osteosarcoma presenting with bone metastasis. Clinical specimens were collected using standardized institutional protocols and subsequently processed for molecular and histopathological analyses [1]. For each patient, primary tumor tissues and matched adjacent non-tumorous bone tissues were obtained during standard surgical resection of osteosarcoma lesions, following established orthopedic oncology protocols to preserve tissue integrity. No osteosarcoma-related surgical procedures were involved in this study All human tissue specimens included in this retrospective study were obtained from patients who underwent surgical treatment at Astana Medical University between 2008 and 2014 after written informed consent had been obtained in accordance with institutional and national ethical regulations in force at the time of tissue collection. Tissue samples were fully anonymized before subsequent molecular analyses. The present retrospective data analysis and manuscript preparation were conducted following approval by the Institutional Ethics Committee of Abdul Wali Khan University Mardan (Approval No. AWKUM/2025/4877). The study complied with the ethical principles of the Declaration of Helsinki and all applicable

institutional guidelines governing research involving human specimens [8].

2.2 Cell Lines and Cell Culture

Human osteosarcoma cell lines (143B and MG-63) were obtained from the American Type Culture Collection and cultured according to the supplier's recommendations. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in a humidified incubator containing 5% CO_2. All subsequent transfection, molecular, and functional assays were performed using these osteosarcoma cell lines.

Cells were seeded at assay-specific densities according to the requirements of each experimental procedure. For assays requiring long-term colony formation or low-density growth assessment, cells were seeded at 2×10^2 cells per well to allow individual colony initiation and expansion. For functional assays evaluating migration, invasion, proliferation, or molecular analyses, higher cell densities were used as appropriate to ensure sufficient cell numbers for reliable measurements.

2.3 Plasmids and Transfections

Lipofectamine 2000 (Invitrogen, Carlsbad, CA) was used to introduce shRNAs that target METTL3 and ITGB1 into cells. These shRNAs were acquired from Peshawar, Pakistan. Table S1 lists the relevant shRNA sequences. Using primers listed in Table S2, the coding sequences for METTL3 and HuR were amplified by PCR and then added to the pCDNA3.1(+) expression vector. PC3 and LNCaP are examples of stable cell lines that were produced using previously defined protocols. Each insert was cloned into the pMIR-REPORT and pGL3-Basic vectors to create further constructs, such as ITGB1-CDS-WT, ITGB1-CDS-Mut (which included thymine replacements for the m A-site), ITGB1-5′UTR-WT, and ITGB1-5′UTR-Mut (which contained equivalent m A-related alterations). Promega provided the pRL-SV35 plasmid that was utilized as an internal control.

2.4 Immuno-Histochemical Analysis

Immunohistochemistry was used to evaluate METTL3 protein expression and subcellular localisation in clinical osteosarcoma tissues. METTL3 immunoreactivity scores were calculated using a previously approved grading system.

2.5 *In Vitro* Cell Adhesion Assay

An 88-well culture plate's triplicate wells were pre-coated with either a BMEC monolayer or 1 μg/cm² type I collagen, and they were then cultured at 37 °C for the whole night. The wells were then blocked with 1% BSA in DMEM for 2.5 hours at 37 °C after being washed three times with serum-free media. Each well was then filled with a total of 2×10^2 cells, which were allowed to adhere for 20 to 35 minutes. After removing non-adherent cells with PBS, 100 μl of serum-free DMEM containing 100 μl of MTS reagent was added. After the plate was incubated at 37 °C for an extra 3.5 hours, absorbance at 480 nm was measured.

2.6 Quantitative Reverse Transcription-PCR

Target transcripts were amplified using primer sets specified in Table S2 after total cellular RNA was extracted using TRIzol in compliance with published protocols. The comparative $2^{-\Delta\Delta CT}$ technique was used to quantify relative mRNA expression levels, with GAPDH acting as the internal reference for normalisation.

2.7 Western Blotting

Western blotting was carried out in accordance with accepted practices [14]. HRP-conjugated anti-mouse and anti-rabbit IgG secondary antibodies (Abcam) were used to detect the signal after primary antibodies against METTL3, HuR, ITGB1, and GAPDH (all from Abcam) were used [17].

2.8 m6A Dot Blot Assay

As directed by the manufacturer, mRNA was separated from total RNA using the Dynabeads® mRNA Purification Kit. After denaturing the pure mRNA for four minutes at 94 °C to remove secondary structures, it was quickly chilled on ice and then crosslinked to a membrane twice using a Stratalinker 2200. After blocking the membrane for an hour at room temperature while gently shaking it, the membrane was incubated with an anti-m A antibody for an entire night at 5 °C. Goat anti-rabbit IgG-HRP was then incubated for 1.5 hours at room temperature. After that, the membrane was created using Hyperfilm ECL for signal visualisation and sealed in plastic [18].

2.9 Dual-Luciferase Reporter Assay

Each well of a 96-well plate was filled with 2×10^2 cells, which were then transfected with 100 ng of the ITGB1-luc reporter construct and 12 ng of pRL-CMV to account for transfection variability. The Dual-Luciferase Assay system (Promega, Madison, WI, USA) was used to measure luminescence from both reporters after a 46-hour incubation period. Triplicate measurements were made, and the results are shown as mean values with matching standard deviations [19].

2.10 m6A qPCR

LNCaP and PC3 cells were first harvested for total RNA extraction, providing a comprehensive pool of cellular transcripts for downstream analysis. The isolated RNA was then subjected to controlled chemical fragmentation in order to generate shorter, uniformly sized RNA pieces suitable for immunoprecipitation. Following fragmentation, the Magna MeRIP m6A Kit (Merck Millipore) was used to enrich for RNA molecules containing m6A modifications. This process involved incubating the RNA fragments with a highly specific anti-m6A antibody, allowing selective immunoprecipitation of methylated transcripts while non-methylated RNA remained in the supernatant. After washing and elution, the m6A-enriched RNA fraction was collected for downstream analysis. To quantify the abundance of specific m6A-modified transcripts, the purified RNA was subjected to reverse-transcription quantitative PCR (RT-qPCR), enabling precise measurement of the relative levels of modification-bearing mRNAs and providing insight into the epitranscriptomic regulation occurring in these cells.

2.11 RNA Binding Protein Immunoprecipitation (RIP)

Using the RIPTM RNA-Binding Protein Immunoprecipitation Kit (Millipore) and the provided methodology, the RNA immunoprecipitation process was carried out. In order to capture target complexes, magnetic beads were conjugated with 6 μg of an antibody against METTL3 (ab195352, Abcam) and treated with cellular extracts for a whole night at 4 °C. Following six washing stages, proteinase K was applied to the bound material, then phenol-chloroform extraction was used to purify the RNA. Using qPCR, the interaction between METTL3 and ITGB1 transcripts was evaluated, and the results were normalized to the matching input proportion.

Using the RIPTM RNA-Binding Protein Immunoprecipitation Kit (Millipore) and the associated protocol, the RIP technique was carried out. To separate METTL3-associated ribonucleoprotein complexes, cellular lysates were treated overnight at 4 °C with magnetic beads attached to 6 μg of a METTL3-targeting antibody (ab195352, Abcam). After six rounds of washing, the material was digested by proteinase K, and RNA was extracted using a phenol-chloroform method. qPCR was used to measure the correlation between METTL3 and ITGB1 transcripts, and signals were corrected in relation to the input percentage.

2.12 RNA Stability Assay

RNA stability in Osteosarcoma cells was evaluated by applying 5 mg/ml of actinomycin D (A9415, Sigma-Aldrich). Cells were collected after predetermined incubation periods, and total RNA was extracted for qPCR and reverse transcription. Since actinomycin D inhibits the production of nascent mRNA, the degradation constant (K_decay) was calculated using the formula $\ln(C/C_0) = K\_decay \cdot t$, where C represents the residual mRNA at time t, t is the inhibition period, and C represents the starting transcript level. The decay rate was obtained via exponential fitting of C/C_0 with time, and the half-life ($t_{1/2}$) was computed using $\ln(1/2) = -K\_decay \cdot t_{1/2}$. RNA turnover dynamics may be precisely characterized thanks to this analytical method.

2.13 *In Vivo* Metastasis Assay

The Experimental Animal Centre in Guangdong Province, China, provided 25-week-old male SCID mice that were kept in a pathogen-free environment. The animals were divided into five groups of four at random: PC3-shNC, PC3-shMETTL3-1, PC3-shMETTL3-2, LNCaP-vector, and LNCaP-METTL3. 1×10^2 cells were injected into the right tibia's cortical area to create bone lesions. X-ray imaging was used to assess tumour development following an 8-week surveillance period. Following the animals' euthanasia, the removed tumours were prepared for hematoxylin-eosin staining in order to facilitate histological evaluation.

2.14 Statistical Analysis

Every experiment was conducted in triplicate, and the results are shown as the mean ± standard deviation. One-way analysis of variance was used to evaluate group differences, and SPSS 18.0 was used for statistical processing. Statistical significance was deemed to be shown by a P value less than 0.05.

3. Results

3.1 METTL3 is Overexpressed in Osteosarcoma Tissues and Correlated with Osteosarcoma BM and Osteosarcoma

METTL3 protein abundance was assessed by immunohistochemistry in 18 localised Osteosarcoma specimens, 18 Osteosarcoma patients with bone metastases, and their corresponding surrounding tissues. Consistent cytoplasmic and nuclear staining with varying intensities was produced by the METTL3 antibody (Figure 1A). Twelve of the thirty-two neighbouring tissues (46.2%) and twenty-five of the thirty-three Osteosarcoma samples (94.3%) had METTL3 positive. When comparing malignant tissues to their corresponding non-tumorous counterparts, staining intensity was significantly higher (6.12 ± 1.56 vs. 4.78 ± 2.00, P = 0.002; Figure 1B). The greatest signal was found in Osteosarcoma with bone metastases (7.25 ± 2.33, P = 0.026 vs. localised osteosarcoma). Table 1 provides a summary of the details. Reduced METTL3 expression was linked to longer overall survival, according to survival analysis (P = 0.019; Figure 1C). Western blotting and qRT-PCR analysis of METTL3 levels in five Osteosarcoma cell lines and normal osteosarcoma epithelial cells (RWPE-1) revealed significantly greater expression in Osteosarcoma cells, especially in PC3 (Figures 1D and 1E). These results validated the use of PC3 and LNCaP for further research.

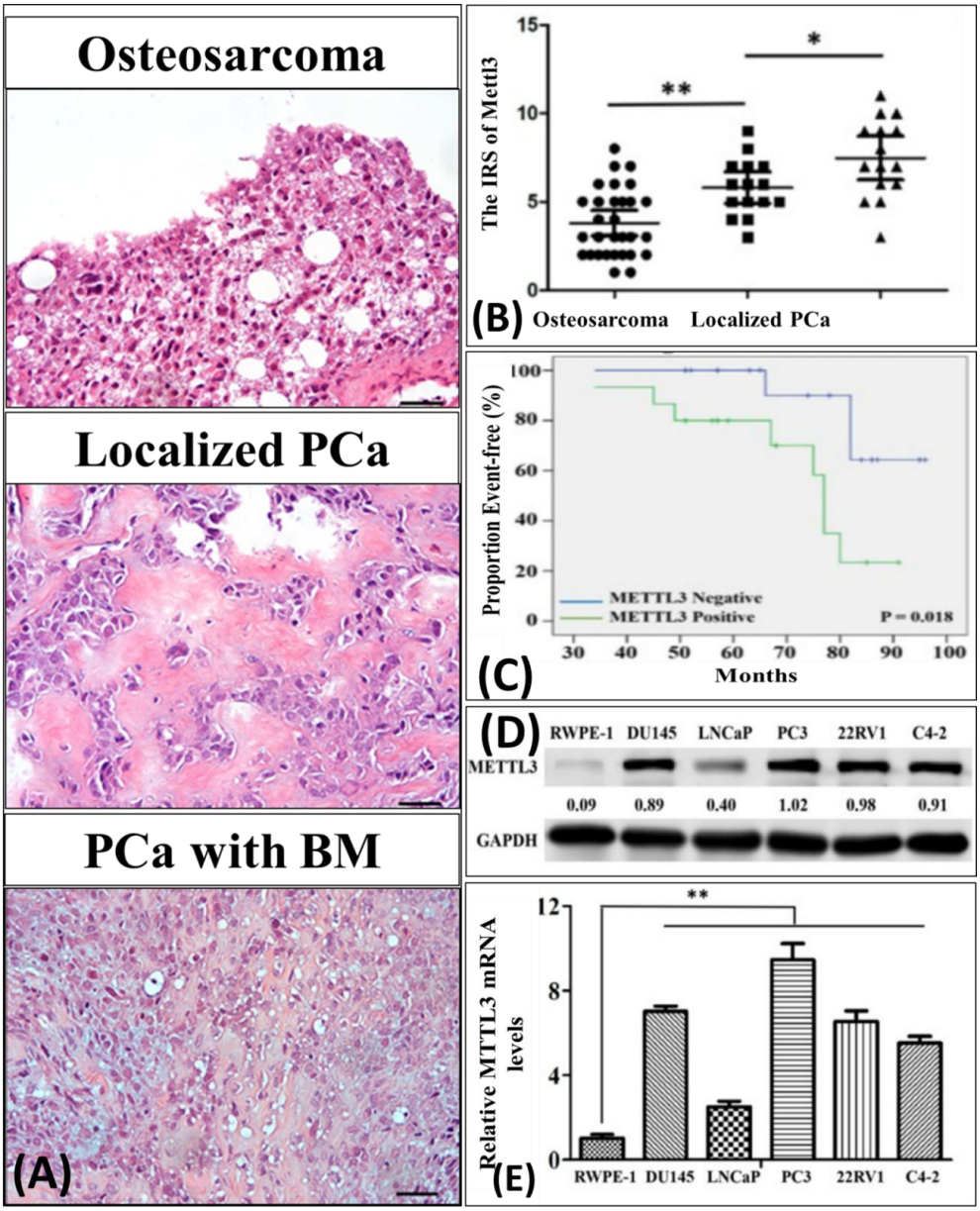

Figure 1. Elevated METTL3 expression is associated with bone metastasis and poor prognosis in osteosarcoma. (A) Representative immunohistochemical staining of METTL3 in localized osteosarcoma, Osteosarcoma with bone metastasis, and adjacent non-tumorous bone tissues. (B) Quantification of METTL3 immunoreactivity scores among tissue groups. (C) Kaplan-Meier survival analysis showing reduced overall survival in patients with high METTL3 expression. (D-E) Western blot and qRT-PCR analysis of METTL3 expression in osteosarcoma cell lines compared with normal control cells. GAPDH was used as an internal control. *Statistical significance: *P < 0.05, **P < 0.01.

METTL3 expression was evaluated in human osteosarcoma cell lines (143B and MG-63/U2OS) and normal osteoblastic cells. The selected osteosarcoma cell lines exhibited significantly higher METTL3 expression and were therefore used for subsequent functional analyses. Stable METTL3-overexpressing and METTL3-silenced osteosarcoma cell models were established to investigate the role of METTL3 in regulating cell adhesion, migration, ITGB1 expression, m6A modification, and bone metastatic potential. All subsequent *in vitro* and *in vivo* experiments were performed using these osteosarcoma cell lines

Table 1. METTL3 expression in osteosarcoma tissues and their matched non-cancerous osteosarcoma counterparts was assessed by immunohistochemical labelling.

Tissue Group	Number of Samples (n)	Percentage (%)
Osteosarcoma with bone metastasis	18	54.5%
Localized osteosarcoma	15	45.5%
Total osteosarcoma cases	33	100%
Paracarcinoma tissues	35	–

METTL3 was found in both the cytoplasm and the nucleus, according to the staining, with Osteosarcoma samples showing considerably stronger and more frequent signals than nearby tissues. This differential pattern indicates that METTL3 is more prevalent in malignant lesions than in the surrounding normal tissue.

3.2 METTL3 Increases Adhesion of Osteosarcoma Cells to Collagen I and Osteosarcoma Motility

To create stable LNCaP-METTL3 and PC3-shMETTL3 cell lines, lentiviral constructs encoding METTL3 or shMETTL3 were created. LNCaP cells showed more than a 235-fold rise in METTL3 transcripts, while PC3 cells showed a 70-85% drop, according to qRT-PCR verification (Figure 2A, 2B). METTL3 significantly affected the adherence of cancer cells to type I collagen, the main component of the bone matrix linked to skeletal colonisation. In comparison to control cells, METTL3 depletion decreased PC3 binding, but METTL3 overexpression increased LNCaP attachment to collagen I (Figure 2C). Regardless of METTL3 status, adhesion to laminin, vitronectin, and fibronectin remained similar (Figure 2D). Additionally, METTL3 influenced Osteosarcoma cell motility, with overexpression promoting migration and silencing inhibiting it. Furthermore, in collagen I-enriched environments, elevated METTL3 expression enhanced Osteosarcoma cell survival and proliferation, but METTL3 knockdown reduced these effects (Figure 2E).

Stable METTL3-overexpressing and METTL3-silenced osteosarcoma cell lines were established to investigate the functional role of METTL3. Validation by qRT-PCR and western blot confirmed successful modulation of METTL3 expression. Functional assays demonstrated that METTL3 overexpression significantly enhanced osteosarcoma cell adhesion to Collagen I, migration, and proliferation, whereas METTL3 knockdown markedly suppressed these phenotypes, indicating that METTL3 promotes the aggressive behavior of osteosarcoma cells.

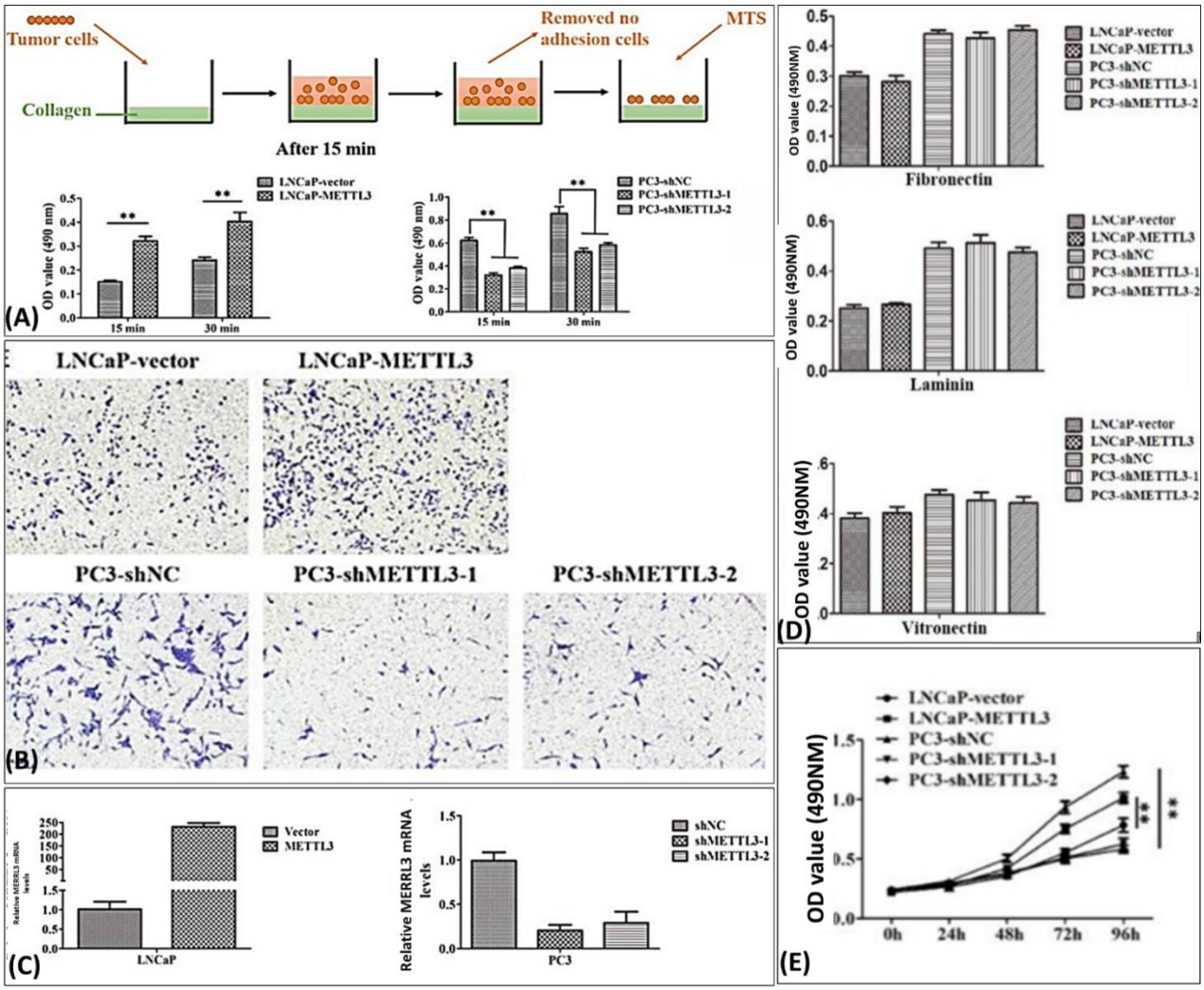

Figure 2. METTL3 promotes extracellular matrix adhesion, migration, and proliferation of prostate cancer cells. (A) Collagen I adhesion assay showing enhanced adhesion following METTL3 overexpression and reduced adhesion after knockdown. (B) Transwell assay demonstrating increased migration with METTL3 overexpression and decreased migration following METTL3 silencing. (C) qRT-PCR validation of METTL3 expression in LNCaP and PC3 cells. (D) Adhesion of METTL3-modified cells to fibronectin, laminin, and vitronectin. (E) MTS assay showing METTL3-dependent changes in cell viability/proliferation. **P < 0.01.

3.3 ITGB1 Mediates the Adhesion Ability of Osteosarcoma Cells by METTL3 Expression

By assessing adhesion-related molecules using Western blotting and quantitative reverse transcription PCR, this study investigated the role of METTL3 in regulating osteosarcoma cell interactions with the extracellular matrix (ECM). The results showed that METTL3 selectively influences integrin expression: while other integrins associated with bone metastasis, including α2, αv, and β3, remained unchanged, METTL3 overexpression in LNCaP cells significantly elevated ITGB1 levels, whereas METTL3 knockdown in PC3 cells markedly reduced ITGB1 expression. Analysis of Osteosarcoma patient samples from the TCGA (n = 96) and GTEx GSE40272 datasets further confirmed a strong and consistent correlation between METTL3 and ITGB1 expression, underscoring the clinical relevance of this regulatory relationship. Functionally, because ITGB1 is the primary receptor for Collagen I, silencing ITGB1 with two independent siRNAs diminished METTL3-driven adhesion and invasive capacity in LNCaP-METTL3 cells, and blocking ITGB1 with specific antibodies similarly prevented METTL3-induced adhesion and migration. Together, these findings demonstrate that METTL3 promotes Osteosarcoma cell adhesion, migration, and recruitment to Collagen I predominantly through the upregulation of ITGB1. Our results indicate that METTL3-mediated m6A modification contributes to the regulation of ITGB1 expression in osteosarcoma cells, primarily through a mechanism involving HuR-dependent stabilization of ITGB1 mRNA (Figure 3).

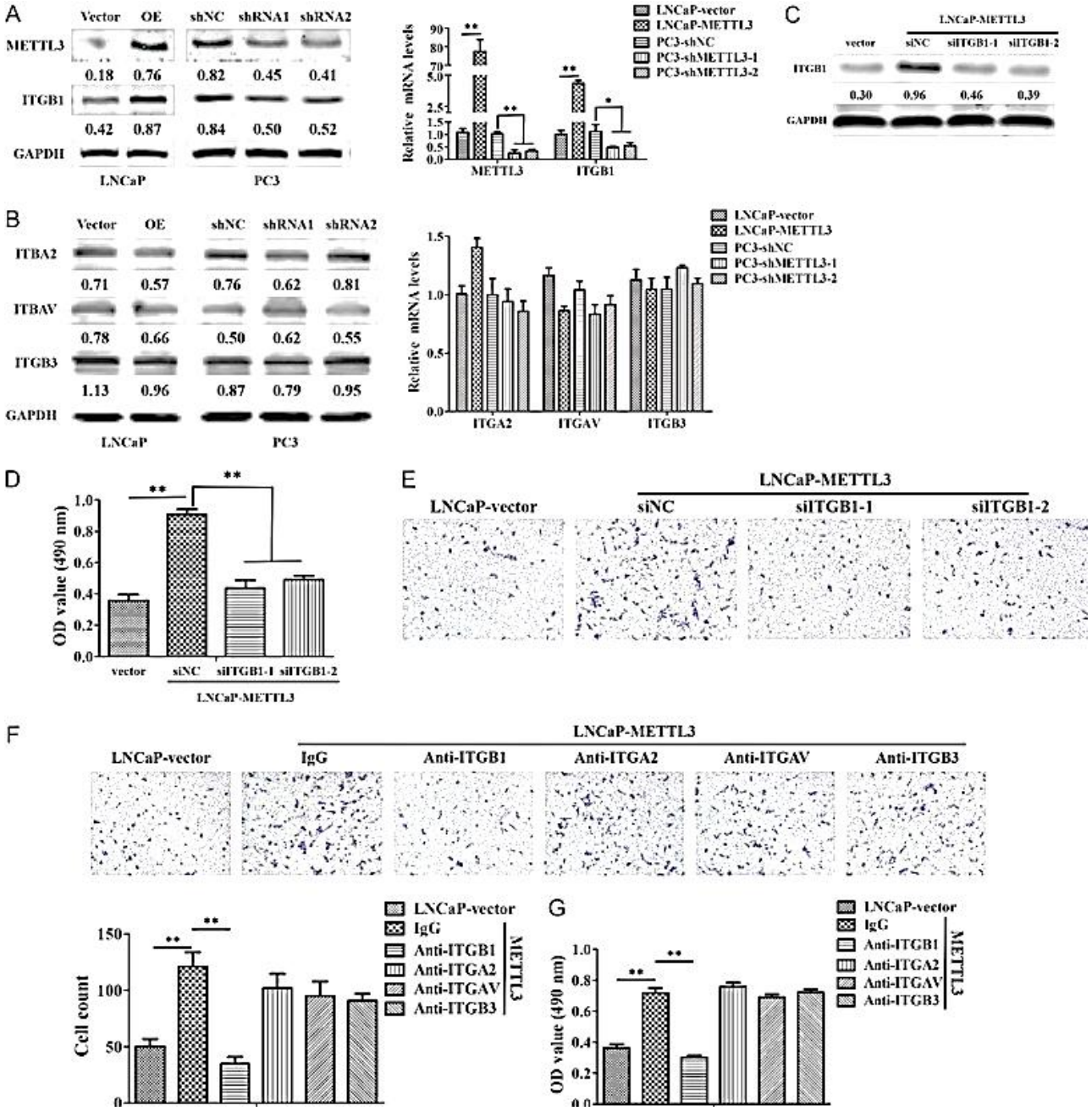

Figure 3. ITGB1 mediates METTL3-dependent adhesion and migration in prostate cancer cells. (A) Western blot and qRT-PCR analysis of METTL3 and ITGB1 expression following METTL3 overexpression or knockdown. (B) Expression of other integrin subunits in METTL3-modified cells. (C) Western blot validation of ITGB1 knockdown in LNCaP-METTL3 cells. (D-E) ITGB1 silencing reduces METTL3-enhanced cell adhesion and migration. (F-G) Blocking ITGB1 with a specific antibody inhibits METTL3-induced migration and adhesion. *P < 0.05, **P < 0.01.

3.4 METTL3 Regulated ITGB1 Expression in an m6A-Dependent Manner

METTL3-positive osteosarcoma cells differ from METTL3-silenced cells in that they have increased adherence to Collagen I as a result of increased ITGB1 expression. METTL3 mainly modulates the stability and translation of ITGB1, according to treatment with CHX and MG132. Dot blot analysis revealed a decrease in global m6A levels after METTL3 knockdown, and gene-specific m6A qPCR verified lower m6A modification on ITGB1 transcripts. A clear correlation between METTL3 and ITGB1 was shown by luciferase reporter assays; PC3-shMETTL3 cells transfected with wild-type ITGB1 3'UTR and CDS showed decreased activity; this effect was eliminated when putative m6A sites were altered. On the other hand, METTL3 overexpression improved luciferase activity and raised m6A levels on ITGB1 mRNA. Additionally, ITGB1 mRNA stability was greatly impacted by METTL3-mediated m6A alteration, which increased it with overexpression and decreased it upon knockdown. These results demonstrate how important METTL3-dependent m6A alteration is for controlling ITGB1 expression (Figure 4).

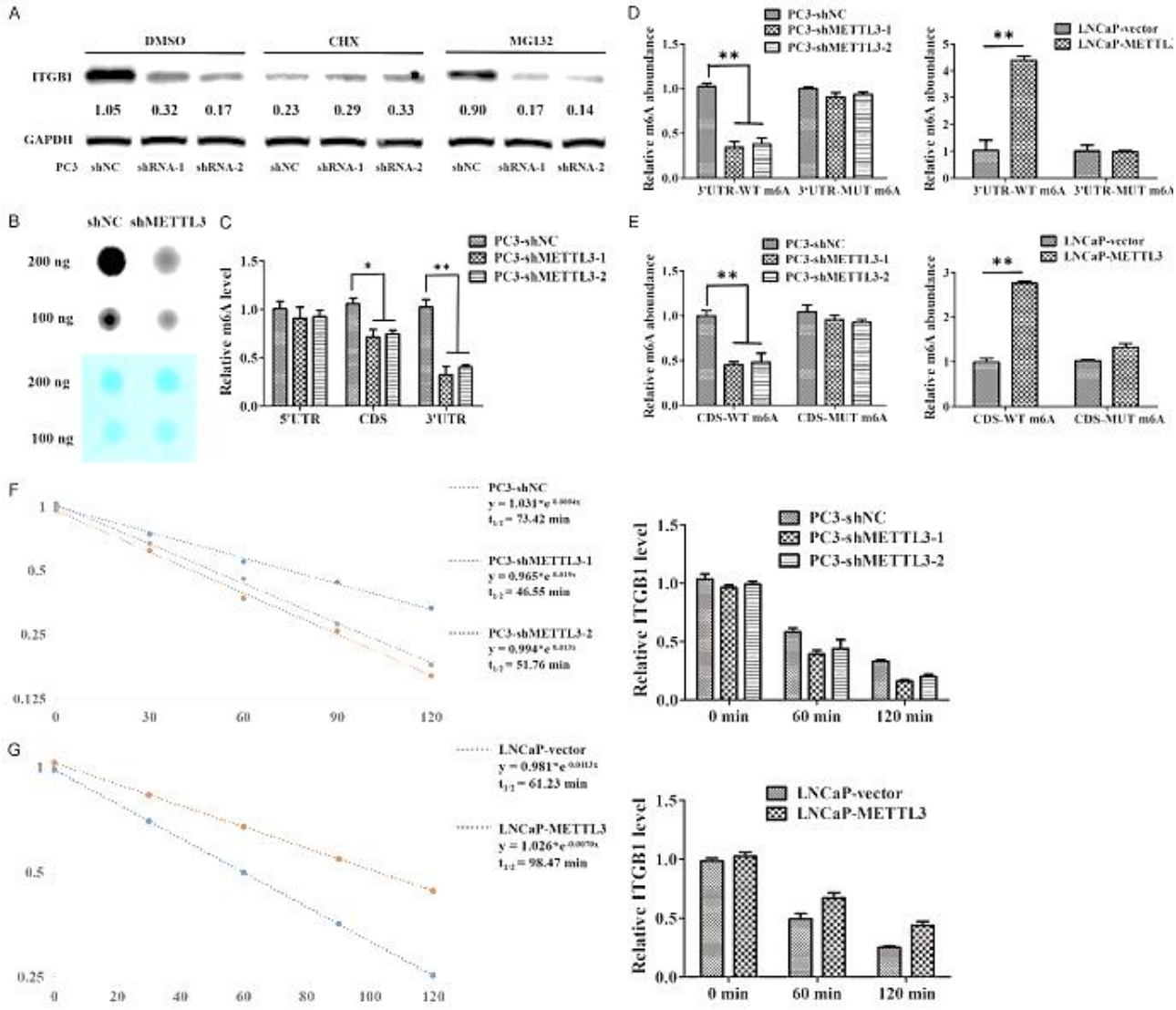

Figure 4. METTL3 regulates ITGB1 expression through m6A modification. (A) Cycloheximide and MG132 treatment assays showing the effects of METTL3 knockdown on ITGB1 protein stability. (B) Dot blot analysis demonstrating altered global m6A levels following METTL3 depletion. (C) m6A-RIP-qPCR showing reduced m6A modification of ITGB1 mRNA after METTL3 knockdown. (D-E) m6A-qPCR analysis of the ITGB1 3' UTR and CDS, including wild-type and mutant m6A sites, in PC3 and LNCaP cells. (F-G) ITGB1 mRNA stability assays demonstrating altered transcript decay following METTL3 modulation. *P < 0.05, **P < 0.01.

3.5 HuR Enhances ITGB1 mRNA Stability Via an m6A-Dependent Manner

It is known that m6A-binding proteins, such as YTHDF2 and YTHDF1, control the destiny of mRNA, with YTHDF1 promoting translation and YTHDF2 encouraging destruction. However, RIP tests demonstrating HuR binding to ITGB1 transcripts in PC3 and LNCaP cells indicated that silencing HuR, rather not YTH family members, dramatically decreased ITGB1 mRNA stability. In Osteosarcoma tissues and cell lines, HuR was significantly overexpressed. While its overexpression had the opposite consequences, its knockdown lowered ITGB1 mRNA and protein levels, decreased adherence to Collagen I, and impeded cell motility. The HuR-ITGB1 relationship was impaired by METTL3 inhibition, demonstrating that METTL3-dependent m6A modification controls HuR-mediated stabilisation of ITGB1 transcripts in Osteosarcoma cells (Figure 5).

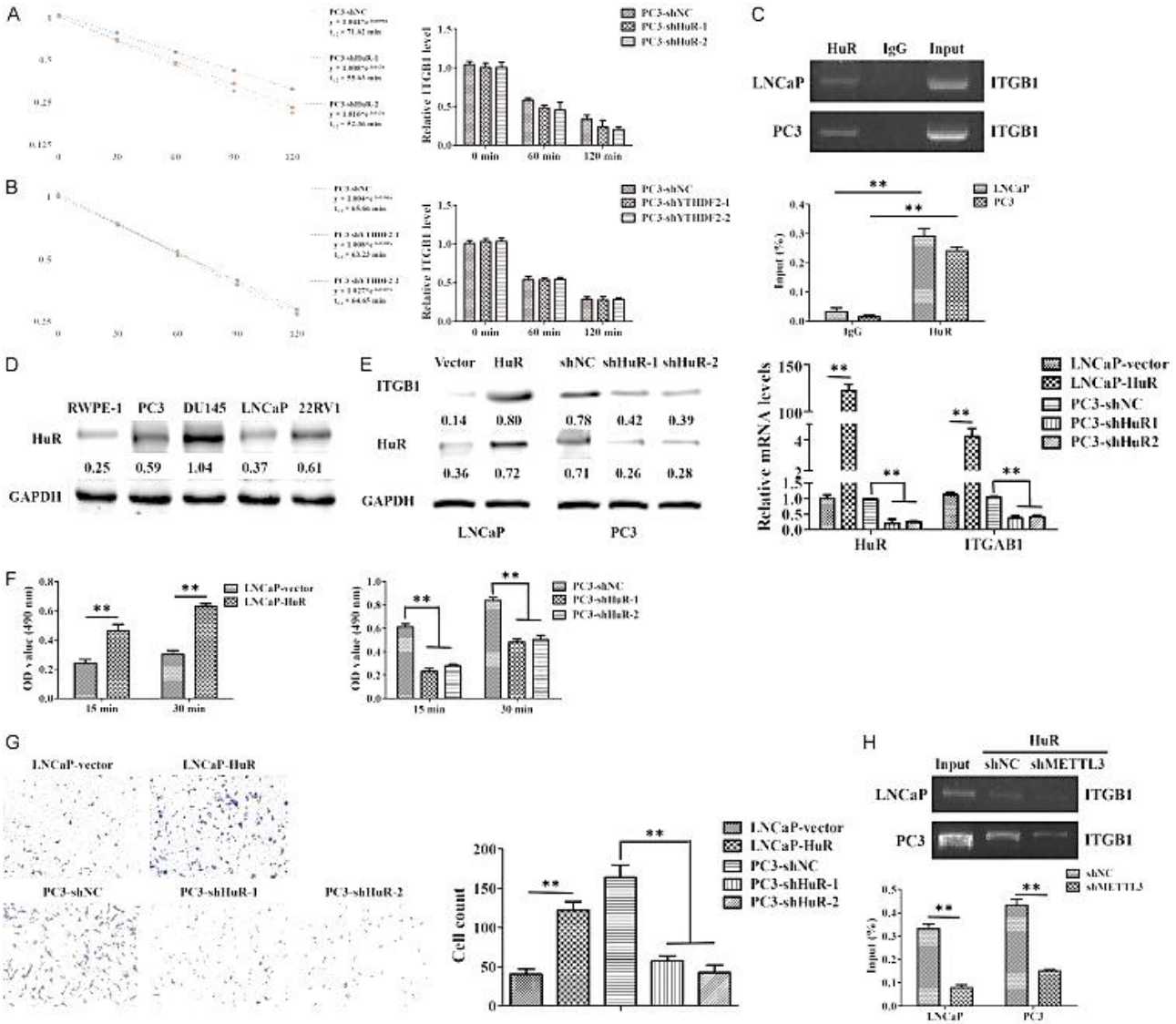

Figure 5. HuR promotes ITGB1 expression and stability, thereby mediating METTL3-dependent adhesion and migration. (A-B) ITGB1 mRNA stability assays showing reduced transcript stability following HuR knockdown. (C) RIP-qPCR confirming the interaction of HuR with ITGB1 mRNA in LNCaP and PC3 cells. (D-E) Western blot analysis of HuR and ITGB1 expression and their modulation by HuR overexpression or knockdown. (F-G) Adhesion and Transwell migration assays demonstrating that HuR enhances cell adhesion and migration, whereas HuR depletion suppresses these effects. (H) RIP analysis showing reduced HuR-ITGB1 mRNA interaction following METTL3 knockdown. **P < 0.01.

3.6 METTL3 Knockdown Represses Bone Metastasis of Osteosarcoma Cells *in Vivo*

In order to evaluate METTL3's function in osteosarcoma bone metastasis, PC3-shMETTL3-1/2, LNCaP-METTL3, or control cells were injected intratibially into mice models, and the results were examined eight weeks later. X-ray imaging confirmed histological examination, which showed bone resorption and degradation brought on by tumour cells. In contrast to 0-1 of 4 animals in the METTL3-silenced PC3-shMETTL3-1/2 group, tumour development was seen in 4 of 4 mice in the METTL3-positive PC3-shNC group. Two out of four LNCaP-METTL3 mice developed bone tumours, but no tumours appeared in the LNCaP-vector group. These findings show that, in comparison to METTL3-silenced cells, METTL3-positive osteosarcoma cells clearly prefer bone colonisation *in vivo*.

The in vivo tibial injection model was used as a preliminary approach to explore the potential role of METTL3 in osteosarcoma bone colonization. After eight weeks of observation, tumor formation was detected in 4 of 4 mice injected with PC3-shNC cells, whereas tumor incidence appeared reduced in mice receiving METTL3-silenced PC3-shMETTL3-1/2 cells. In contrast, tumors were observed in 2 of 4 mice injected with LNCaP-METTL3 cells, while no tumors were detected in the LNCaP-vector control group. Histological analysis and X-ray imaging supported these observations.

To evaluate the role of METTL3 *in vivo*, osteosarcoma cells with altered METTL3 expression were implanted into a mouse tibial model. METTL3 overexpression promoted tumor growth and bone colonization, whereas METTL3 knockdown significantly reduced tumor formation and osteolytic lesions. Histological examination and X-ray imaging

supported these findings, suggesting that METTL3 facilitates osteosarcoma bone metastasis and contributes to disease progression *in vivo* (Figure 6).

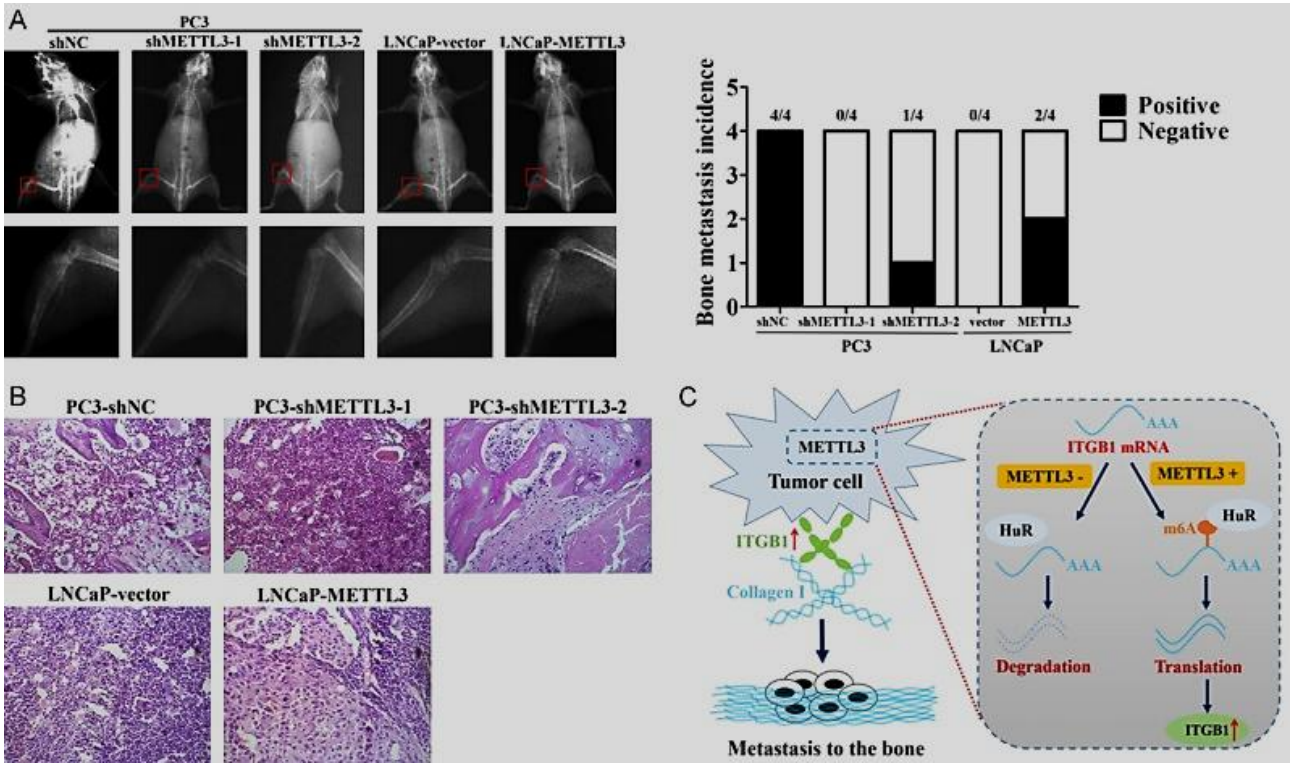

Figure 6. *In vivo*, METTL3 promotes prostate cancer bone metastasis through the ITGB1-HuR axis. (A) Representative X-ray images and quantification of bone metastasis in mice injected with METTL3-modified PC3 and LNCaP cells. (B) Representative H&E staining of metastatic bone tissues showing reduced metastasis following METTL3 knockdown and enhanced metastasis following METTL3 overexpression. (C) Schematic model illustrating METTL3-mediated m6A modification of ITGB1 mRNA, which enhances HuR-dependent mRNA stability and ITGB1 expression, thereby promoting tumor cell adhesion and bone metastasis.

4. Discussion

RNA m6A modification is increasingly recognized as one of the most influential epitranscriptomic marks, capable of exerting a wide spectrum of post-transcriptional regulatory effects. These include modulation of transcriptional output, alteration of alternative splicing patterns, control of nuclear export, translational efficiency, and even the determination of cellular identity [8]. Dysregulation of the enzymes that deposit, remove, or recognize m6A collectively referred to as "writers," "erasers," and "readers" has been implicated in the initiation and progression of various malignancies. However, despite the clear relevance of m6A pathways in many cancers, their specific function in osteosarcoma has remained insufficiently characterized [20].

Beyond its role in promoting osteosarcoma cell adhesion and bone colonization, the METTL3-HuR-ITGB1 regulatory axis may also have important implications for the tumor immune microenvironment. The extracellular matrix is increasingly recognized as a critical regulator of immune cell behavior within tumors. Changes in matrix composition and integrin-mediated adhesion can influence the infiltration and spatial organization of immune cells such as cytotoxic T lymphocytes, macrophages, and dendritic cells. By enhancing ITGB1 expression and strengthening tumor cell interactions with collagen I, METTL3 may contribute to extracellular matrix remodeling that alters immune accessibility within the bone tumor niche. Moreover, epitranscriptomic regulation through m6A modification has been shown to modulate immune-related gene expression and responsiveness to immunotherapy in several malignancies. Consequently, Our findings suggest that the METTL3-HuR-ITGB1 axis represents a mechanistically relevant pathway in osteosarcoma progression and may serve as a potential target for future therapeutic investigation by suppressing metastatic progression while also reshaping the tumor immune microenvironment to enhance immune-mediated tumor control.

Although the present study primarily investigated the role of METTL3 in regulating osteosarcoma cell adhesion and bone metastatic behavior, our findings may also have broader implications for the tumor microenvironment. ITGB1-mediated interactions with collagen I are known to influence extracellular matrix architecture, which can indirectly affect immune-cell trafficking and localization within tumors. In addition, previous studies have demonstrated that m6A RNA methylation regulates multiple immune-related signalling pathways and responses to immunotherapy in several malignancies. However, the current study did not directly evaluate immune-cell infiltration, cytokine production, or antitumor immune responses. Therefore, the potential immunological significance of the METTL3-HuR-ITGB1 axis

should be regarded as a biologically plausible hypothesis that warrants future experimental investigation rather than a conclusion established by the present work [21]. Although previous literature has yielded inconsistent conclusions regarding whether METTL3 acts as an oncogene or tumor suppressor in Osteosarcoma, the present findings support a predominantly oncogenic role in promoting Osteosarcoma progression. These observations reinforce the concept that the biological consequences of m6A modifications are highly context-dependent, shaped by the identity of specific methylated transcripts and the repertoire of m6A-binding proteins present within a given tumor [22].

m6A RNA methylation is regulated by a network of methyltransferases, demethylases, and RNA-binding proteins that collectively influence RNA metabolism. Among these regulators, METTL3 functions as a key catalytic component of the m6A methyltransferase complex. In the present study, we specifically investigated the role of METTL3-mediated m6A modification in regulating osteosarcoma progression, with particular focus on its interaction with the RNA-binding protein HuR and its downstream effect on ITGB1 mRNA stability.

Tumor metastasis requires cancer cells to dramatically remodel their adhesion and signaling properties. Alterations in integrin expression and downstream pathways are particularly important for enabling tumor cells to detach, invade, migrate, and ultimately colonize distant tissues. In the unique microenvironment of bone which is rich in extracellular matrix proteins collagen type I serves as the principal structural protein and a powerful attractant for Osteosarcoma cells [23]. Binding of Osteosarcoma cells to collagen I is largely mediated through the α2β1 integrin receptor, which activates signaling cascades that promote cell anchoring, survival, and metastatic outgrowth in bone. Emerging evidence has also suggested that m6A methylation may influence integrin-related signaling events, though the mechanistic basis remained unclear [24].

In this context, the current study shows that METTL3 enhances the ability of Osteosarcoma cells to home to and adhere to collagen I by upregulating ITGB1 in an m6A-dependent fashion. Interestingly, METTL3 manipulation did not affect other integrin subunits such as α2, αV, or β3, nor did it alter adhesion to other extracellular matrix proteins including fibronectin, laminin, or vitronectin. This selective enhancement of the collagen I-ITGB1 axis provides a compelling mechanistic explanation for the osteotropism often observed in Osteosarcoma metastasis. To further delineate the molecular pathways involved, the authors analyzed GEO microarray dataset GSE40272, identifying integrin signaling as one of the major pathways linked to bone metastasis [25,26]. RIP assays and luciferase reporter analyses confirmed that METTL3 directly regulates ITGB1 by depositing m6A marks within its 3′UTR and coding sequence, thereby facilitating its post-transcriptional activation. METTL3 knockdown, as well as direct ITGB1 silencing in LNCaP-METTL3 cells, impaired the enhanced adhesion phenotype. Moreover, functional blockade of ITGB1 with neutralizing antibodies selectively suppressed METTL3-driven adhesion and migration on collagen I substrates. Collectively, these findings position METTL3 as a central m6A-dependent driver of Osteosarcoma cell recruitment to collagen I through ITGB1 upregulation, revealing a crucial mechanism that may underlie bone-specific metastatic behavior.

A further layer of regulation involves m6A reader proteins, which determine the fate of methylated transcripts. Among them, HuR (ELAVL1) is widely expressed and known to stabilize numerous oncogenic mRNAs, thereby influencing proliferation, invasion, and treatment resistance. HuR has already been identified as an important regulator of Osteosarcoma growth and chemoresistance. Recent work suggests that HuR preferentially interacts with m6A-modified transcripts, enhancing their stability, although the literature describes context-dependent variability in this relationship [27].

In the present study, HuR expression was found to be elevated in both Osteosarcoma tissues and cultured Osteosarcoma cells. METTL3-mediated m6A modification increased HuR binding to ITGB1 mRNA, resulting in greater transcript stability and enhanced downstream protein expression. This aligns with previous reports showing that HuR supports malignant phenotypes in multiple cancers, particularly in metastatic settings [28]. However, some studies have observed the opposite effect namely, that m6A deposition can actually obstruct HuR binding at specific sites, destabilizing certain transcripts, and that METTL3/METTL14 depletion may enhance HuR-mRNA interactions by altering microRNA accessibility [29,30]. These seemingly contradictory findings underscore the context-dependent, transcript-specific, and cell-type-specific nature of HuR function as an m6A reader, highlighting the complexity of epitranscriptomic regulation in cancer.

Beyond its direct role in metastasis, the METTL3-HuR-ITGB1 axis may also have important immunological implications. ITGB1-mediated adhesion and extracellular matrix interactions are increasingly recognized as key modulators of the tumor immune microenvironment, influencing immune cell infiltration, immune synapse formation, and tumor immune evasion [31]. Aberrant m6A signaling has been shown to regulate immune-related gene expression, antigen presentation, and responsiveness to immune checkpoint blockade in several malignancies. Therefore, targeting METTL3 or disrupting HuR-dependent stabilization of ITGB1 may not only impair osteosarcoma cell adhesion and bone colonization but also reprogram the tumor microenvironment to enhance antitumor immune responses [32]. Such interventions could potentially synergize with immunotherapeutic approaches, including immune checkpoint inhibitors or immune-modulating agents, to improve therapeutic efficacy in osteosarcoma. These findings position the METTL3-HuR-ITGB1 axis as a promising epitranscriptomic-immunologic target, warranting further investigation for the development of combination strategies aimed at preventing or treating osteosarcoma bone metastasis.

5. Study Limitations and Future Directions

Despite providing mechanistic insights into the role of the METTL3-HuR-ITGB1 axis in osteosarcoma progression, several limitations of the present study should be acknowledged. First, although our findings demonstrate that METTL3 promotes osteosarcoma cell adhesion, migration, invasion, and metastatic behavior through m6A-dependent stabilization of ITGB1 mRNA, the precise upstream regulatory mechanisms controlling METTL3 activation in osteosarcoma remain incompletely understood. Future studies should investigate the genomic, epigenetic, and signaling pathways responsible for METTL3 dysregulation, including potential interactions with oncogenic pathways, transcription factors, and additional RNA-binding proteins that may cooperate with METTL3 during osteosarcoma progression. While our study highlights potential implications of the METTL3-HuR-ITGB1 axis for the tumor microenvironment, direct immunological mechanisms were not experimentally evaluated. Specifically, immune-cell infiltration, immune-cell activation, cytokine and chemokine profiles, immune checkpoint expression, and functional antitumor immune responses were not assessed in the current study. Therefore, any potential influence of METTL3-mediated ITGB1 regulation on tumor-immune interactions remains hypothetical and should be interpreted as a future research direction rather than an experimentally established conclusion. Future investigations using immunocompetent osteosarcoma models, patient-derived immune profiling, single-cell RNA sequencing, spatial transcriptomics, and multiplex immunohistochemical approaches will be necessary to determine whether METTL3-driven extracellular matrix remodeling affects immune-cell recruitment, immune surveillance, or responses to immunotherapy.

Although the *in vivo* experiments provide supportive evidence that METTL3 contributes to osteosarcoma growth and bone metastatic progression, the relatively small number of animals used in each experimental group ($n = 4$) limits the statistical power and generalizability of these findings. Therefore, the animal results should be considered preliminary and require validation in larger independent cohorts and additional metastatic models. Future studies incorporating larger sample sizes, orthotopic implantation models, spontaneous metastasis models, and patient-derived xenograft or organoid systems will provide stronger evidence regarding the biological significance of the METTL3-HuR-ITGB1 pathway in osteosarcoma metastasis. Although our study identifies METTL3 as an important molecular regulator of osteosarcoma progression, we did not directly evaluate the therapeutic potential of targeting METTL3. Pharmacological inhibition of METTL3, genetic depletion strategies, or combination approaches with existing osteosarcoma therapies were not investigated. Therefore, the clinical applicability of METTL3 targeting remains to be established. Future studies should evaluate selective METTL3 inhibitors, optimize delivery strategies, and determine whether METTL3 inhibition can effectively suppress tumor progression while maintaining acceptable toxicity profiles. Furthermore, investigations combining METTL3 targeting with chemotherapy, targeted therapy, or immunotherapeutic approaches may provide valuable insights into potential therapeutic combinations.

Our clinical tissue analyses support the relevance of METTL3 and ITGB1 expression in osteosarcoma progression, the relatively limited patient cohort size and retrospective nature of the study represent additional limitations. Larger multicenter clinical cohorts with comprehensive clinical annotation and long-term follow-up will be required to validate the prognostic significance of the METTL3-HuR-ITGB1 axis. Future studies should also evaluate whether expression levels of METTL3, HuR, or ITGB1 correlate with treatment response, metastatic recurrence, and patient survival outcomes. While our study establishes the importance of m6A-mediated regulation of ITGB1 in osteosarcoma biology, m6A modification is a complex and dynamic regulatory process involving multiple writers, erasers, and readers. The present study primarily focused on METTL3-mediated regulation of ITGB1; however, additional m6A-regulated transcripts and regulatory networks may contribute to osteosarcoma progression. Future transcriptome-wide m6A sequencing (MeRIP-seq/m6A-seq), RNA interactome analysis, and integrated multi-omics approaches will be valuable for identifying additional METTL3-dependent pathways involved in osteosarcoma metastasis.

The present study establishes the METTL3-HuR-ITGB1 axis as an important molecular mechanism regulating osteosarcoma adhesion and metastatic progression. While our findings provide a foundation for understanding the contribution of m6A-mediated regulation in osteosarcoma biology, further studies incorporating larger clinical cohorts, advanced *in vivo* models, therapeutic validation, and comprehensive immune profiling are required to determine the translational potential of targeting this pathway.

6. Conclusion

The present study demonstrates that METTL3 promotes osteosarcoma progression by enhancing cell adhesion, migration, invasion, and bone metastatic potential through m6A-dependent stabilization of ITGB1 mRNA mediated by the RNA-binding protein HuR. Analyses of clinical specimens, cellular models, and complementary molecular assays consistently support the role of the METTL3-HuR-ITGB1 regulatory axis in osteosarcoma progression. The *in vivo* findings provide preliminary evidence that is consistent with these mechanistic observations, although confirmation in larger animal cohorts is warranted. These results identify the METTL3-HuR-ITGB1 pathway as an important molecular mechanism underlying osteosarcoma metastasis and provide a foundation for future studies investigating its potential as a therapeutic target. Furthermore, the possible involvement of this pathway in regulating the tumor microenvironment

or influencing antitumor immune responses remains hypothetical and should be evaluated in dedicated mechanistic and immunological studies.

Acknowledgements

We are Grateful to Aga Khan University and Abdul Wali Khan University for providing the best environment for research.

Funding

Not received.

Ethics Statement

The study protocol was reviewed and approved by the Institutional Review Board (IRB), Aga Khan University, Karachi, Pakistan (Approval No. AWKUM/2025/4877). All procedures involving human participants were conducted in accordance with the Declaration of Helsinki, and written informed consent was obtained from all participants prior to tissue collection.

Data Availability Statement

All the data are available in the manuscript and its supplementary file.

Author Contributions

Awais Ali and Syed Luqman Ali conceptualized and designed the research project, performed all analyses, and contributed to the writing, revision, and critical review of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest related to this work.

Generative AI statement

AI-assisted tools were used solely for manuscript language editing, grammar correction, and improving clarity and readability. The authors reviewed and approved all AI-assisted edits and take full responsibility for the final manuscript content.

References

[1] Ritter J, Bielack SS. Osteosarcoma. Annals of Oncology, 2010, 21(7), vii320-vii325. DOI: 10.1093/annonc/mdq276
[2] Liu JF, Shanmugavadivel D, Ball-Gamble A, Walker D. Clinical presentation of bone tumours in children and young people: A systematic review and meta-analysis. Archives of Disease in Childhood, 2025, 110(8), 622-629. DOI: 10.1136/archdischild-2024-327879
[3] Hosseini H, Heydari S, Hushmandi K, Daneshi S, Raesi R. Bone tumors: A systematic review of prevalence, risk determinants, and survival patterns. BMC Cancer, 2025, 25(1), 321. DOI: 10.1186/s12885-025-13720-0
[4] Iacobellis G, Leggio A, Salzillo C, Lucà S, Ortega-Ruiz R, Marzullo A. Analysis and historical evolution of paediatric bone tumours: The importance of early diagnosis in the detection of childhood skeletal malignancies. Cancers, 2025, 17(3), 451. DOI: 10.3390/cancers17030451
[5] Biermann JS, Hirbe A, Ahlawat S, Bernthal NM, Binitie O, Boles S. Bone cancer, version 2.2025, NCCN clinical practice guidelines in oncology. Journal of The National Comprehensive Cancer Network, 2025, 23(4), 1-21. DOI: 10.6004/jnccn.2025.0017
[6] Vignati G, Zantonelli G, Albano D, Gitto S, Sconfienza LM. Introduction to malignant bone tumors: Clinical presentation and diagnostic imaging. Malignant Bone Lesions and Beyond, 2025, 1-16. DOI: 10.1007/978-3-032-00018-7_1
[7] Zheng BX, Sun XC, Zhang L, Qu GJ, Ren CM, Yan P. Inhibition of anlotinib-induced autophagy attenuates invasion and migration by regulating epithelial-mesenchymal transition and cytoskeletal rearrangement through ATG5 in human osteosarcoma cells. Brazilian Journal of Medical and Biological Research, 2024, 57, e13152. DOI: 10.1590/1414-431X2023e13152
[8] Lindsey BA, Markel JE, Kleinerman ES. Osteosarcoma overview. Rheumatology and Therapy, 2017, 4, 25-43. DOI: 10.1007/s40744-016-0050-2
[9] Maden BEH. The numerous modified nucleotides in eukaryotic ribosomal RNA. Progress in Nucleic Acid Research and Molecular Biology, 1990, 39, 241-303. DOI: 10.1016/S0079-6603(08)60629-7
[10] Wang X, Zhao BS, Roundtree IA, Lu Z, Han D, Ma HH, et al. N6-methyladenosine modulates messenger RNA translation

efficiency. Cell, 2015, 161(6), 1388-1399. DOI: 10.1016/j.cell.2015.05.014
[11] Wang X, Lu Z, Gomez A, Hong GC, Yue Y, Han D, et al. N6-methyladenosine-dependent regulation of messenger RNA stability. Nature, 2013, 505, 117-120. DOI: 10.1038/nature12730
[12] Geuens T, Bouhy D, Timmerman V. The hnRNP family: Insights into their role in health and disease. Human Genetics, 2016, 135, 851-867. DOI: 10.1007/s00439-016-1683-5
[13] Xiao YL, Chen J, Zhou H, Zeng XD, Ruan ZP, Pu ZY, et al. Combining p53 mRNA nanotherapy with immune checkpoint blockade reprograms the immune microenvironment for effective cancer therapy. Nature Communications, 2022, 13, 758. DOI: 10.1038/s41467-022-28279-8
[14] Wang CB, He Y, Zheng J, Wang X, Chen SH. Dissecting order amidst chaos of programmed cell deaths: Construction of a diagnostic model for KIRC using transcriptomic information in blood-derived exosomes and single-cell multi-omics data in tumor microenvironment. Frontiers in Immunology, 2023, 14, 1130513. DOI: 10.3389/fimmu.2023.1130513
[15] Agris PF, Vendeix FAP, Graham WD. tRNA's wobble decoding of the genome: 40 years of modification. Journal of Molecular Biology, 2007, 366(1), 1-13. DOI: 10.1016/j.jmb.2006.11.046
[16] Dai DJ, Wang HY, Zhu LY, et al. N6-methyladenosine links RNA metabolism to cancer progression. Cell Death & Disease, 2018, 9, 124. DOI: 10.1038/s41419-017-0129-x
[17] Manzoor U, Ali A, Ali SL, Abdelkarem O, Kanwal S, Alotaibi SS, et al. Mutational screening of GDAP1 in dysphonia associated with charcot-marie-tooth disease: Clinical insights and phenotypic effects. Journal of Genetic Engineering and Biotechnology, 2023, 21(1), 119. DOI: 10.1186/s43141-023-00568-9
[18] Aiman S, Ahmad A, Khan AA, Alanazi AM, Samad A, Ali SL, et al. Vaccinomics-based next-generation multi-epitope chimeric vaccine models prediction against Leishmania tropica-A hierarchical subtractive proteomics and immunoinformatics approach. Frontiers in Immunology, 2023, 14, 1259612. DOI: 10.3389/fimmu.2023.1259612
[19] Yi L, Zheng C. Dual-luciferase reporter assay to investigate virus-host interactions. Molecular Virology, 2025, 337-341. DOI: 10.1007/978-1-0716-4615-1_29
[20] Yao N, Zhou J, Jiang Y, Jin QM, Zhu H, Zhang J, et al. Rhizoma Paridis saponins suppresses vasculogenic mimicry formation and metastasis in osteosarcoma through regulating miR-520d-3p/MIG-7 axis. Journal of Pharmacological Sciences, 2022, 150(3), 180-190. DOI: 10.1016/j.jphs.2022.08.005
[21] Yao N, Zhou J, Jiang Y, Jin QM, Zhu H, Zhang J, et al. Rhizoma Paridis saponins suppresses vasculogenic mimicry formation and metastasis in osteosarcoma through regulating miR-520d-3p/MIG-7 axis. Journal of Pharmacological Sciences, 2022, 150(3), 180-190. DOI: 10.1016/j.jphs.2022.08.005
[22] Wang H, Zuo HN, Liu J, Wen F, Gao YW, Zhu XD, et al. Loss of YTHDF2-mediated m6A-dependent mRNA clearance facilitates hematopoietic stem cell regeneration. Cell Research, 2018, 28, 1035-1038. DOI: 10.1038/s41422-018-0082-y
[23] Hall CL, Dai JL, van Golen KL, Keller ET, Long MW. Type I collagen receptor (α2β1) signaling promotes the growth of human prostate cancer cells within the bone. Cancer Research, 2006, 66(17), 8648-8654. DOI: 10.1158/0008-5472.CAN-06-1544
[24] Zhu JJ, Ye ZJ, Zhang Z, Liu JY, Ali SL. AI-integrated 3D imaging and modelling for hip morphology assessment in athletes. Computer Methods in Biomechanics and Biomedical Engineering, 2025, 1-11. DOI: 10.1080/10255842.2025.2502828
[25] Li H, Guan KF, Liu DD, Liu M. Identification of mitochondria-related hub genes in sarcopenia and functional regulation of MFG-E8 on ROS-mediated mitochondrial dysfunction and cell cycle arrest. Food Funct, 2022, 13(2), 624-638. DOI: 10.1039/d1fo02610k
[26] Getachew N, Meten M. Weights of evidence modeling for landslide susceptibility mapping of Kabi-Gebro locality, Gundomeskel area, Central Ethiopia. Geoenvironmental Disasters, 2021, 8, 6. DOI: 10.1186/s40677-021-00177-z
[27] Ali SL, Ali A, Khan A. Identification and assessment of ferroptosis-related genes and their implication as therapeutic agents for pancreatic ductal adenocarcinoma. Annals of Pancreatic Cancer, 2025, 8. DOI: 10.21037/apc-25-2
[28] Li E, Wei B, Wang XL, Kang R. METTL3 enhances cell adhesion through stabilizing integrin β1 mRNA via an m6A-HuR-dependent mechanism in prostatic carcinoma. American Journal of Cancer Research, 2020, 10(3), 1012-1025.
[29] Chen XR, Luo Y, Zhu Q, Zhang JZ, Huang H, Kan YS, et al. Small extracellular vesicles from young plasma reverse age-related functional declines by improving mitochondrial energy metabolism. Nature Aging, 2024, 4, 814-838. DOI: 10.1038/s43587-024-00612-4
[30] Zhuang DX, Wang SS, Liu GY, Liu PP, Deng HT, Sun JF, et al. Phenformin suppresses angiogenesis through the regulation of exosomal microRNA-1246 and microRNA-205 levels derived from oral squamous cell carcinoma cells. Frontiers in Oncology, 2022, 12, 943477. DOI: 10.3389/fonc.2022.943477
[31] Lawrence P, Escudero-Pérez B. Henipavirus immune evasion and pathogenesis mechanisms: lessons learnt from natural infection and animal models. Viruses, 2022, 14(5), 936. DOI: 10.3390/v14050936
[32] Ali A, Ali SL, Ullah W, Khan A. Gene expression profiling identifies CAV1, CD44, and TFRC as potential diagnostic markers and therapeutic targets for multiple myeloma. Cell Biochemistry and Biophysics, 2025, 83, 3633-3650. DOI: 10.1007/s12013-025-01743-0

Supplementary

Table S1. Description of the shRNA sequences.

PRIMER	SEQUENCE
SIMETTL3-1	5’-CTGCAAGTATGTTCACTATGA-3’
SIMETTL3-2	5’-UCAUAGUGAACAUACUUGCAG-3’
SIHUR-1	5’-GCAGCAUUGGUGAAGUUGAAUCU-3’
SIHUR-2	5’- GCCCAUCACAGUGAAGUUUGCA-3’
SIYTHDF2-1	5’-TTGGCTATTGGGAACGTCCTT-3’
SIYTHDF-2	5’-AAGGACGTTCCCAATAGCCAA-3’
SIITGB1	5’-AUGGGACACGGGUGAAAAUTT-3’
NONSPECIFIC OLIGOS	5’-GCUUCGCGCCGUAGUCUUATT-3’

Table S2A. Description of the primer sequences for PCR.

PRIMER	SEQUENCE
METTL3	
FORWARD	5’-TTGCGGCCGCAAGGTGTCCGCGTGAGAATTGG-3’
REVERSE	5’-GCTCTAGAGCCTTAGCTCTGTAAGGAAGTGCTTC-3’
HUR	
FORWARD	5’-TTGCGGCCGCAACTTCCCGCCCGTGTTCAGAT-3’
REVERSE	5’-CCCTCGAGGGCGTGAGCGAGTTATTTGTGGG-3’

Table S2B. Description of the primer sequences for qRT-PCR.

PRIMER	SEQUENCE
METTL3	
FORWARD	5’-TTGTCTCCAACCTTCCGTAGT-3’
REVERSE	5’-CCAGATCAGAGAGGTGGTGTAG-3’
ITGB1	
FORWARD	5’-CACGGCTGCTGGTGTTTTCC-3’
REVERSE	5’-CCTTCTATTGCTCACCTTGTCC-3’
HUR	
FORWARD	5’-GGGTGACATCGGGAGAACG-3’
REVERSE	5’-CTGAACAGGCTTCGTAACTCAT-3’
ITGA2	
FORWARD	5’-CCTACAATGTTGGTCTCCCAGA-3’
REVERSE	5’-AGTAACCAGTTGCCTTTTGGATT-3’
ITGAV	
FORWARD	5’-ATCTGTGAGGTCGAAACAGGA-3’
REVERSE	5’-TGGAGCATACTCAACAGTCTTTG-3’
ITGB3	
FORWARD	5’-GTGACCTGAAGGAGAATCTGC-3’
REVERSE	5’-CCGGAGTGCAATCCTCTGG-3’
GAPDH	
FORWARD	5’-CGCTGAGTACGTCGTGGAGTC-3’
REVERSE	5’-GCTGATGATCTTGAGGCTGTTGTC-3’