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Article

KDM6B regulates the epigenetic mechanism of epithelial-mesenchymal transition in differentiated thyroid cancer in response to extracellular matrix stiffness

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Abstract: Objective: This study aims to investigate the epigenetic mechanisms by which KDM6B regulates epithelial-mesenchymal transition (EMT) in differentiated thyroid cancer cells in response to changes in extracellular matrix (ECM) stiffness, and to elucidate its role in tumor invasion and metastasis. **Methods:** The differentiated thyroid cancer cell line K1 was used to prepare soft matrices (1 kPa), moderately stiff matrices (10 kPa), and stiff matrices (30 kPa). KDM6B was knocked down using siRNA and overexpressed using pcDNA3.1-KDM6B. The expression and activity of EMT markers (E-cadherin, N-cadherin, vimentin) and KDM6B were detected using real-time quantitative polymerase chain reaction (qRT-PCR), Western blot, Chromatin Immunoprecipitation-quantitative Polymerase Chain Reaction (ChIP-qPCR), and RNA sequencing (RNA-seq). Additionally, Transwell migration and invasion assays were performed to assess the migratory and invasive capabilities of the cells. GO and KEGG enrichment analyses were conducted to explore the key genes and signaling pathways regulated by KDM6B. **Results:** Under different ECM stiffness conditions, the mRNA and protein expression levels and enzymatic activity of KDM6B significantly increased ($p < 0.001$). Increased matrix stiffness led to a significant decrease in E-cadherin and a significant increase in N-cadherin and vimentin ($p < 0.001$). Following KDM6B knockdown, E-cadherin significantly decreased, N-cadherin and vimentin significantly increased, and cell migration and invasion capabilities were enhanced ($p < 0.01$). Conversely, overexpression of KDM6B significantly upregulated E-cadherin, significantly downregulated N-cadherin and vimentin, and inhibited cell migration and invasion capabilities ($p < 0.01$). ChIP-qPCR analysis indicated that KDM6B binding to the promoters of EMT genes such as Snail and Twist was significantly enhanced in high-stiffness matrices and regulated their transcriptional activity through demethylation of H3K27me3 ($p < 0.05$). RNA-seq and enrichment analyses revealed that KDM6B regulates key genes and multiple signaling pathways, including PI3K/Akt and MAPK, which are involved in biological processes such as cell proliferation and apoptosis. **Conclusion:** KDM6B regulates the EMT process in differentiated thyroid cancer cells in response to changes in ECM stiffness, primarily by affecting the expression of EMT-related genes through epigenetic modifications, thereby regulating cell migration and invasion capabilities. KDM6B provides a new theoretical basis as a potential therapeutic target for differentiated thyroid cancer.

Keywords: KDM6B; extracellular matrix stiffness; epithelial-mesenchymal transition; epigenetic modification

1. Introduction

Differentiated thyroid carcinoma is the most common type of thyroid cancer, accounting for more than 90% of thyroid cancer cases. With changes in global lifestyle and environmental factors, the incidence of DTC has shown a year-by-year upward trend [1]. Although most DTC patients have a good prognosis, some patients have poor prognosis due to tumor invasion and metastasis, making clinical treatment challenging [2,3]. At present, the pathogenesis of DTC is not yet fully understood, and in-depth research on its molecular biological characteristics is of great significance for developing new therapeutic strategies. Epithelial-mesenchymal transition (EMT), as an important process of cellular phenotype transformation, plays a key role in tumor invasion and metastasis [4,5]. During EMT, the downregulation of epithelial markers such as E-cadherin and the upregulation of mesenchymal markers such as N-cadherin and vimentin endow tumor cells with stronger migratory and invasive capabilities [6]. In recent years, the stiffness of the extracellular matrix (ECM), as an important physical property of the microenvironment, has received extensive attention for its role in regulating the EMT process through mechanical signal transduction pathways. However, how ECM stiffness regulates key factors in the EMT process through epigenetic mechanisms, especially the specific mechanism of KDM6B, has not been fully investigated [7,8]. This study aims to explore the epigenetic mechanism by which KDM6B regulates the EMT process of DTC under different ECM stiffness conditions. By knocking down and overexpressing KDM6B, combined with real-time quantitative polymerase chain reaction (qRT-PCR), Western blot, Chromatin Immunoprecipitation-quantitative Polymerase Chain Reaction (ChIP-qPCR), and RNA-seq, we systematically analyzed the role of KDM6B in regulating the expression of EMT markers as well as cell migration and invasion. The results revealed that KDM6B, as an epigenetic regulatory factor, exhibits a bidirectional role in inhibiting or promoting the EMT process in response to changes in ECM stiffness. This provides new molecular insights into the mechanisms of DTC invasion and metastasis and offers a theoretical foundation for developing therapeutic strategies targeting KDM6B.

2. Materials and methods

2.1. Cell culture and treatment

This study used the differentiated thyroid carcinoma cell line K1 (Catalog No. CCTCC-T4062), purchased from the Cell Bank of the Chinese Academy of Sciences. The cells were maintained in RPMI-1640 medium containing 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin-streptomycin (100 U/mL, Gibco, USA) at 37 ℃ with 5% CO_2, until the cells reached 70%–80% confluence for experiments.

Before the start of the experiments, all cells were cultured under drug-free conditions for 48 h so that they were in the logarithmic growth phase. Then, the cells were grouped and seeded on substrates of different stiffness, including soft substrate (1 kPa), medium-stiff substrate (10 kPa), and stiff substrate (30 kPa), to simulate different extracellular matrix (ECM) stiffness environments. Each stiffness group was set up with three independent replicates.

2.2. Preparation and verification of extracellular matrix (ECM) stiffness

According to the established formulation, polyacrylamide (Sigma-Aldrich, USA) was used to prepare substrates of different stiffness: (1) Soft substrate (1 kPa) by preparing 10% polyacrylamide solution (volume ratio 10:0.05, acrylamide: crosslinker N,N′-methylenebisacrylamide (Sigma-Aldrich, USA)); (2) medium-stiff substrate (10 kPa) by preparing 7.5% polyacrylamide solution (volume ratio 7.5:0.0375, acrylamide:crosslinker) [9]; (3) stiff substrate (30 kPa) by preparing 5% polyacrylamide solution (volume ratio 5:0.025, acrylamide:crosslinker). The polyacrylamide solution and crosslinker were mixed at the above ratios and then quickly applied to pre-cleaned and treated silicone slides (Thermo Fisher Scientific, USA). The slides were placed in a humid chamber at room temperature for 1 h to complete polymerization [10]. After polymerization, a 0.1 M NaOH solution was used to neutralize any unreacted acrylamide on the substrate surface, followed by incubation with 1% collagen (Collagen I, Sigma-Aldrich, USA) at 4 °C overnight to promote cell adhesion.

A nanoindentation instrument (Nanoindentation, Bruker, USA) was used to measure the stiffness of each prepared substrate. The specific procedures were as follows: (1) Fix the prepared substrate slides on the sample stage of the nanoindentation instrument; (2) select an appropriate indenter (such as a Berkovich tip), set the indentation speed to 1 μm/s, and set the maximum indentation depth to 200 nm; (3) perform five indentation tests at different locations on each type of substrate, record and calculate the average stiffness value (Young's modulus) to ensure that the actual stiffness of each substrate meets the expected value.

2.3. KDM6B expression and activity detection

2.3.1. qRT-PCR detection of KDM6B mRNA

Use TRIzol reagent (Invitrogen, USA) to extract total RNA from the cells. Then, use the PrimeScript™ RT reagent kit (Takara, Japan) to reverse-transcribe 2 μg of total RNA into cDNA, followed by real-time quantitative PCR (qRT-PCR) using SYBR® Green Master Mix (Applied Biosystems, USA). The amplification program is 95 ℃ for 10 min, then 95 ℃ for 15 s and 60 ℃ for 1 min, for a total of 40 cycles. Primers (**Table 1**) were designed for KDM6B and the internal reference gene GAPDH, and the results were relatively quantified using the $2^{(-\Delta\Delta Ct)}$ method.

Primer and siRNA Sequences (**Table 2**).

Table 1. qRT-PCR and ChIP-qPCR primers.

Gene	Primer	Sequence (5′→3′)	Expected Size (bp)
KDM6B	Forward	ACGAGAGCGAACAGTTCCTG	~180
	Reverse	GGTTGCTGAACTTTCTGGGA	
E-cadherin	Forward	TGCCCAGAAAATGAAAAAGG	~200
	Reverse	GTGTATGTGGCAATGCGTTC	
N-cadherin	Forward	AGGCCTTCACTGACTGAGCAA	~220
	Reverse	GGCCTTTCTGCCAAACTTCT	

Table 1. (*Continued*).

Gene	Primer	Sequence (5′→3′)	Expected Size (bp)
Vimentin	Forward	GACGCCATCAACACCGAGTT	~220
	Reverse	CTTTGTCGTTGGTTAGCTGGT	
Snail (mRNA)	Forward	TTCTTCTGCGCTACTGCTGCG	~190
	Reverse	GGGCTGCTGGAAGGTAAACTC	
Twist (mRNA)	Forward	GAGCAAGATTCAGACCCTCA	~210
	Reverse	TTAGCTTGCCATCTTGGAGT	
GAPDH	Forward	ACAACTTTGGTATCGTGGAAGG	~150
	Reverse	GCCATCACGCCACAGTTTC	
Snail (ChIP)	Forward	CAAACCCAGGCTACACGTAAC	~180
	Reverse	TTGGGATTGAGGAGGCTGTT	
Twist (ChIP)	Forward	ACTGGCAGCATCTTGGATTT	~200
	Reverse	GGAGACATCTCGAAGTGTTGG	

Table 2. siRNA sequences.

Name	Sequence (5′→3′)	Purpose
siKDM6B (Sense)	GCUUCUACUUCAGAUACAUdTdT	Knockdown KDM6B (sense)
siKDM6B (Antisense)	AUGUAUCUGAAGUAGAAGCdTdT	Knockdown KDM6B (antisense)
siNC (Sense)	UUCUCCGAACGUGUCACGUdTdT	Negative control (sense)
siNC (Antisense)	ACGUGACACGUUCGGAGAAdTdT	Negative control (antisense)

2.3.2. Western blot detection of KDM6B protein

Use RIPA buffer (Beyotime, Shanghai, China) to lyse the cells, then centrifuge at 4 ℃, 12,000 rpm for 10 min, and collect the supernatant. The protein concentration is determined using a BCA kit (Thermo Fisher, Waltham, MA, USA). Then, take an equal amount of protein (30 μg) for 10% SDS-PAGE electrophoresis and transfer to a PVDF membrane (Millipore, USA). After blocking with 5% skim milk at room temperature for 1 h, incubate overnight at 4 ℃ with primary antibodies KDM6B (1:1000, Cell Signaling Technology, Danvers, MA, USA) and β-actin (1:5000, Cell Signaling Technology, USA) [11]. The next day, incubate with HRP-labeled secondary antibody (1:5000, Jackson ImmunoResearch, West Grove, PA, USA) at room temperature for 1 h, develop the signal with an ECL detection reagent, and collect the results using a ChemiDoc XRS+ imaging system (Bio-Rad, Hercules, CA, USA).

2.3.3. KDM6B enzymatic activity measurement

Use a KDM6B Activity Assay Kit (Abcam, Waltham, MA, USA). Prepare the reaction system according to the kit instructions and add 50 μg of protein lysate to the reaction system. After incubation at 37 ℃ for 1 h, terminate the reaction and then perform color development. Finally, measure the absorbance at 450 nm. Each group is set up in triplicate, and the results are represented by the average value.

2.4. Detection of epithelial-mesenchymal transition (EMT) markers

2.4.1. qRT-PCR detection of EMT-related genes

The method for detecting mRNA levels of EMT markers E-cadherin (epithelial marker), N-cadherin, and vimentin (mesenchymal markers) is the same as in 2.3.1, only replacing the corresponding gene-specific primers for amplification.

2.4.2. Western blot detection of EMT-related proteins

Protein extraction, SDS-PAGE, electrophoretic transfer, and blocking steps are the same as in Section 2.3.2. The only difference lies in the primary antibodies used, which are specific antibodies against E-cadherin, N-cadherin, and vimentin (all 1:1000, Cell Signaling Technology, USA). Development and analysis were performed as previously described.

2.4.3. Immunofluorescence staining

First, fix the cells with 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA) for 10 min, then permeabilize with 0.1% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) at room temperature for 5 min, followed by blocking with 5% BSA (Sigma-Aldrich, St. Louis, MO, USA) at room temperature for 1 h. Next, incubate the cells overnight at 4 ℃ with the primary antibodies E-cadherin (1:200, Abcam, Waltham, MA, USA) and vimentin (1:200, Abcam, Waltham, MA, USA). On the following day, incubate with FITC-labeled secondary antibody (1:500, Jackson ImmunoResearch, West Grove, PA, USA) at room temperature for 1 h. Finally, stain the cell nuclei with DAPI (Sigma-Aldrich, St. Louis, MO, USA) and observe using a fluorescence microscope (Leica, Wetzlar, Germany).

2.5. Role of KDM6B in the EMT process induced by ECM stiffness

2.5.1. KDM6B knockdown and overexpression

Knockdown experiment: (1) Design and synthesize specific siRNA (siKDM6B) and non-specific control siRNA (siNC); (2) use Lipofectamine RNAiMAX transfection reagent (Invitrogen, USA) to perform the transfection according to the manufacturer's instructions. The specific steps include: plate 4×10^4 cells in a 6-well plate, wait until the cells adhere and reach 70%–80% confluence, prepare the transfection mixture by mixing 20 pmol siRNA with 2 μL Lipofectamine RNAiMAX per well, incubate at room temperature for 20 min, and then add the transfection mixture to the cell culture medium with gentle shaking to mix evenly [12]; (3) after 48 h of transfection, proceed with subsequent experiments.

Overexpression experiment: (1) Construct the KDM6B overexpression vector pcDNA3.1-KDM6B (GeneCopoeia, Rockville, MD, USA) and the empty vector pcDNA3.1; (2) use Lipofectamine 3000 transfection reagent (Invitrogen, Carlsbad, CA, USA) to perform the transfection according to the manufacturer's instructions. Prepare the transfection mixture by mixing 2 μg pcDNA3.1-KDM6B or pcDNA3.1 with 5 μL Lipofectamine 3000 per well, incubate at room temperature for 10 min, and then add the transfection mixture to the cell culture medium with gentle shaking to mix evenly; (3) after 48 h of transfection, proceed with subsequent experiments.

2.5.2. Analysis of EMT markers and cellular behavior

Under different substrate stiffness and KDM6B regulatory conditions, the expression changes of E-cadherin, N-cadherin, and vimentin were detected by qRT-PCR, Western Blot, and immunofluorescence methods, as described in 2.3.1, 2.3.2, and 2.4.3, respectively.

2.6. Cell Migration and invasion assays

2.6.1. Transwell migration assay

A Corning Transwell chamber (Corning, NY, USA) was used for the migration assay. The specific steps include: (1) Suspend 1×10^5 treated cells in the upper chamber containing RPMI-1640 medium with 1% FBS; (2) add RPMI-1640 medium with 10% FBS to the lower chamber as a chemoattractant; (3) after incubating at 37 ℃ for 24 h, remove the non-migrated cells in the upper chamber, fix with methanol, and stain with crystal violet; (4) use a microscope (Olympus, Tokyo, Japan) to count the number of cells that migrated to the lower chamber in each well and take the average value.

2.6.2. Matrigel invasion assay

A Corning Matrigel invasion chamber (Corning, NY, USA) was used for the invasion assay. The specific steps include: (1) Pre-coat an appropriate thickness of Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) on the upper layer of the Transwell chamber and allow it to solidify; (2) suspend 1×10^5 treated cells in the upper chamber containing RPMI-1640 medium with 1% FBS; (3) add RPMI-1640 medium with 10% FBS to the lower chamber as a chemoattractant; (4) after incubating at 37 ℃ for 48 h, remove the non-invaded cells in the upper chamber, fix with methanol, and stain with crystal violet; (5) use a microscope to count the number of cells that invaded to the lower chamber in each well and take the average value.

2.7. Epigenetic modification mechanism mediated by KDM6B

2.7.1. Chromatin immunoprecipitation (ChIP-qPCR)

When performing chromatin immunoprecipitation using a Millipore ChIP kit (Millipore, USA), first crosslink the cells with 1% formaldehyde for 10 min and neutralize with glycine. Then, lyse the cells and sonicate the chromatin into 200–500 bp fragments. Subsequently, add antibodies against KDM6B (Cell Signaling Technology, USA), H3K27me3 (Abcam, Waltham, MA, USA), or an IgG control antibody for immunoprecipitation, and purify the obtained DNA after reverse crosslinking. Next, use promoter region-specific primers for EMT-related genes (Snail, Twist, etc.) for qPCR (amplification program same as 2.3.1), while using the IgG control as the internal reference to detect the enrichment of KDM6B and H3K27me3 in the promoter regions of target genes.

2.7.2. H3K27me3 modification level detection

In the ChIP-qPCR experiment, the H3K27me3 antibody is used to detect the H3K27me3 modification level in the promoter regions of EMT-related genes. The specific steps are the same as in ChIP-qPCR. The results are quantified using the same

qPCR method to analyze changes in H3K27me3 under different substrate stiffness and KDM6B regulatory conditions.

2.8. RNA sequencing (RNA-seq)

Collect cell samples under different substrate stiffness conditions (1 kPa, 10 kPa, 30 kPa) and KDM6B knockdown/overexpression conditions. Extract total RNA using TRIzol reagent, and after evaluating RNA quality (OD260/280 > 1.8, RIN > 7), construct the sequencing library using the TruSeq RNA Library Prep Kit (Illumina, USA). Then, perform PE150 high-throughput sequencing on the Illumina NovaSeq 6000 platform to obtain high-quality raw sequence data. After mapping the raw reads to the reference genome using HISAT2 software, use StringTie software for transcript assembly and quantification, and then perform differential gene expression analysis with DESeq2 to identify key EMT-related genes regulated by KDM6B. Further, use the ClusterProfiler R package to conduct GO and KEGG pathway enrichment analyses on differentially expressed genes to screen significantly enriched biological processes and signaling pathways, thereby elucidating the mechanism of KDM6B in the EMT process.

2.9. Statistical analysis

All experimental data are expressed as mean ± standard error. One-way ANOVA is used for comparisons among groups, and paired *t*-test is used for comparisons within groups. The significance level is set at $p < 0.05$. Statistical analysis is performed using SPSS 25.0 software and GraphPad Prism 8 software for data processing and graphing.

2.10. Ethical statement

This study did not involve animal experiments; all cell experiments were conducted under sterile conditions. Relevant ethical requirements were strictly followed during the research process to ensure scientific rigor and compliance.

3. Results

3.1. Preparation and verification of extracellular matrix (ECM) stiffness

To verify the stiffness of the prepared substrates, this study conducted five measurements of the soft, medium, and hard substrates. The results are shown in **Table 3**.

Table 3. Verification of ECM stiffness.

Substrate Type	Measurement 1 (kPa)	Measurement 2 (kPa)	Measurement 3 (kPa)	Measurement 4 (kPa)	Measurement 5 (kPa)	Average (kPa)	SEM (kPa)
Soft Substrate (1 kPa)	0.93	1.07	0.99	1.02	1.05	1.01	0.05
Medium-Stiff Substrate (10 kPa)	9.78	10.21	10.05	9.95	10.18	10.03	0.11
Stiff Substrate (30 kPa)	29.67	30.23	30.11	29.89	30.35	30.05	0.13

3.2. Expression and activity changes of KDM6B under different ECM stiffness

Under different ECM stiffness conditions, one-way ANOVA showed a significant increase ($p < 0.001$) in both the mRNA and protein expression levels of KDM6B (**Figure 1A**), and a significant increase ($p < 0.001$) in the enzymatic activity of KDM6B (**Figure 1B**).

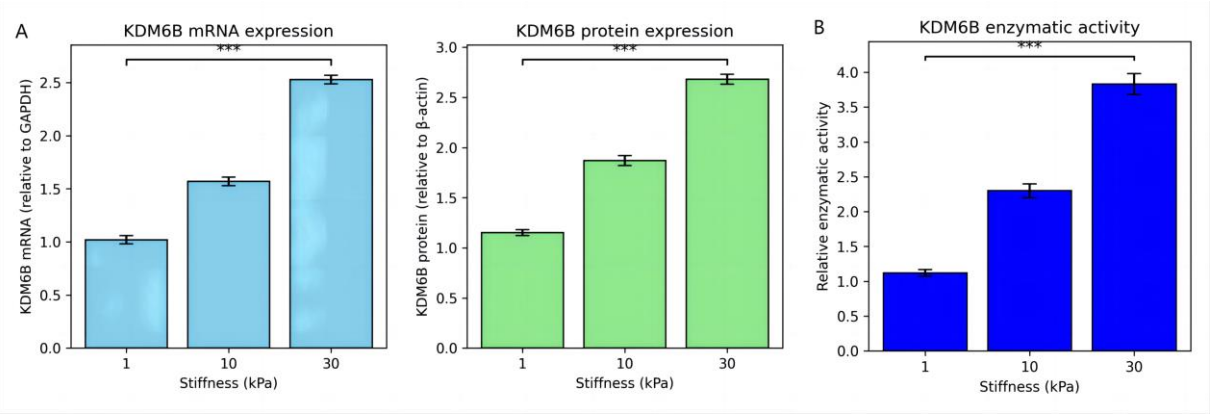

Figure 1. Effect of ECM stiffness on KDM6B expression and enzymatic activity. **(A)** mRNA and protein expression levels of KDM6B under different ECM stiffness; **(B)** Enzymatic activity of KDM6B under different ECM stiffness; one-way ANOVA.

***$P < 0.001$. Data are presented as mean ± SEM from three independent experiments.

3.3. Effects of ECM stiffness on epithelial-mesenchymal transition (EMT) markers

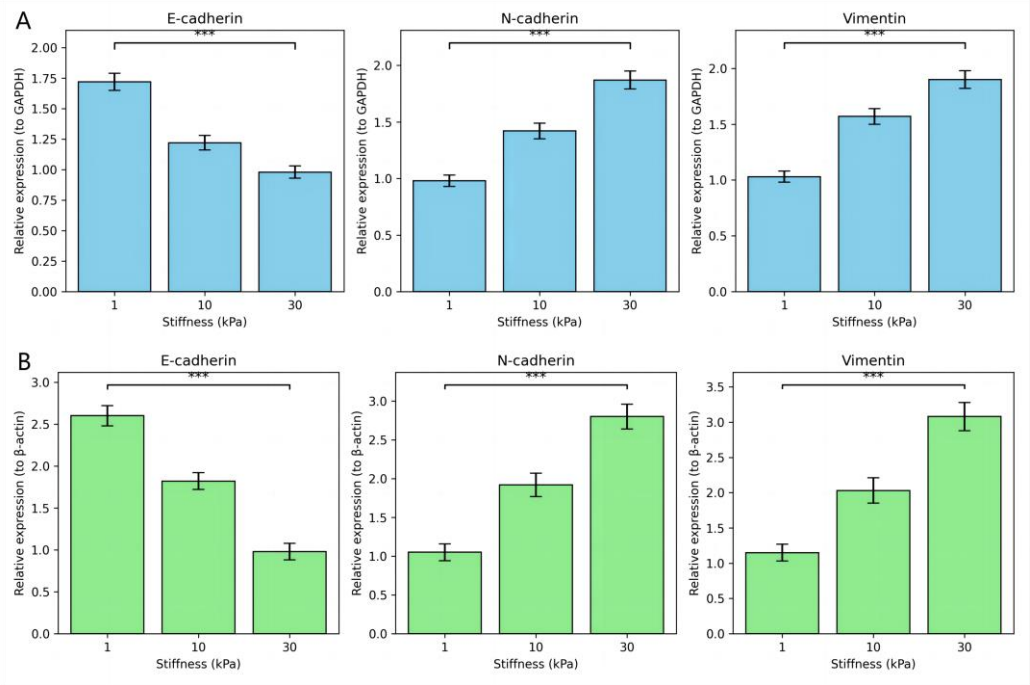

Figure 2. Effect of ECM stiffness on EMT marker expression. **(A)** mRNA expression levels of EMT markers under different ECM stiffness; **(B)** protein expression levels of EMT markers under different ECM stiffness, one-way ANOVA.

***$P < 0.001$. Data are presented as mean ± SEM from three independent experiments.

Under different ECM stiffness conditions, the expression levels of EMT markers showed significant changes. qRT-PCR analysis indicated that as substrate stiffness increased, the mRNA expression of E-cadherin significantly decreased, while the mRNA expression of N-cadherin and vimentin significantly increased ($p < 0.001$) (**Figure 2A**). Western blot results further confirmed these findings, showing a significant reduction in E-cadherin protein expression and a significant increase in N-cadherin and vimentin protein expression ($p < 0.001$) **(Figure 2B**).

3.4. Regulatory role of KDM6B in the EMT process induced by ECM stiffness

After KDM6B knockdown, the mRNA and protein expression levels of the EMT marker E-cadherin decreased significantly, while those of N-cadherin and vimentin increased significantly. Both qRT-PCR and Western blot analyses showed that these changes in marker expression were highly significant ($p < 0.01$) (**Figure 3A**,**B**). These results suggest that KDM6B knockdown promotes the EMT process and enhances cell migration and invasion. In contrast, after KDM6B overexpression, the mRNA and protein expression levels of E-cadherin increased significantly, whereas those of N-cadherin and vimentin decreased significantly. A paired *t*-test revealed that all changes were highly significant ($p < 0.01$) (**Figure 3C**,**D**). These results indicate that KDM6B overexpression inhibits the EMT process and reduces cell migration and invasion.

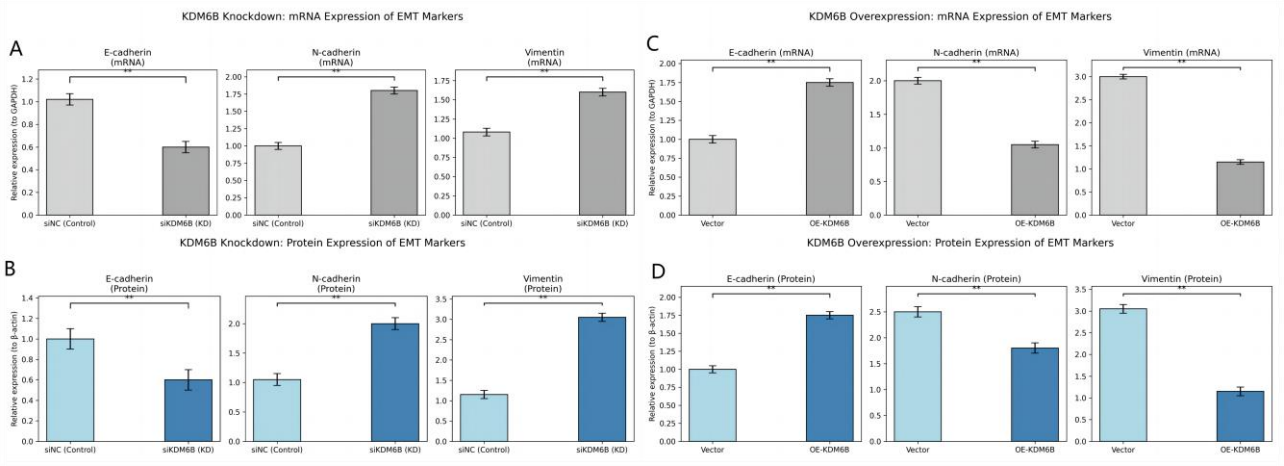

Figure 3. Effects of KDM6B knockdown and overexpression on EMT marker expression. **(A)** Effect of KDM6B knockdown on the mRNA expression of EMT markers; **(B)** effect of KDM6B knockdown on the protein expression of EMT markers; **(C)** effect of KDM6B overexpression on the mRNA expression of EMT markers; **(D)** effect of KDM6B overexpression on the protein expression of EMT markers, paired *t*-test.

***P* < 0.01. Data are presented as mean ± SEM from three independent experiments.

3.5. Effects of KDM6B regulation on cell migration and invasion

In the Transwell migration and Matrigel invasion assays, the results of the paired *t*-test showed that the number of migrating and invading cells in the siKDM6B group was significantly higher than in the siNC group ($p < 0.01$). These findings suggest that KDM6B knockdown markedly enhances cell migration and invasion, indicating its inhibitory role in the metastasis of differentiated thyroid carcinoma cells (**Figure 4**).

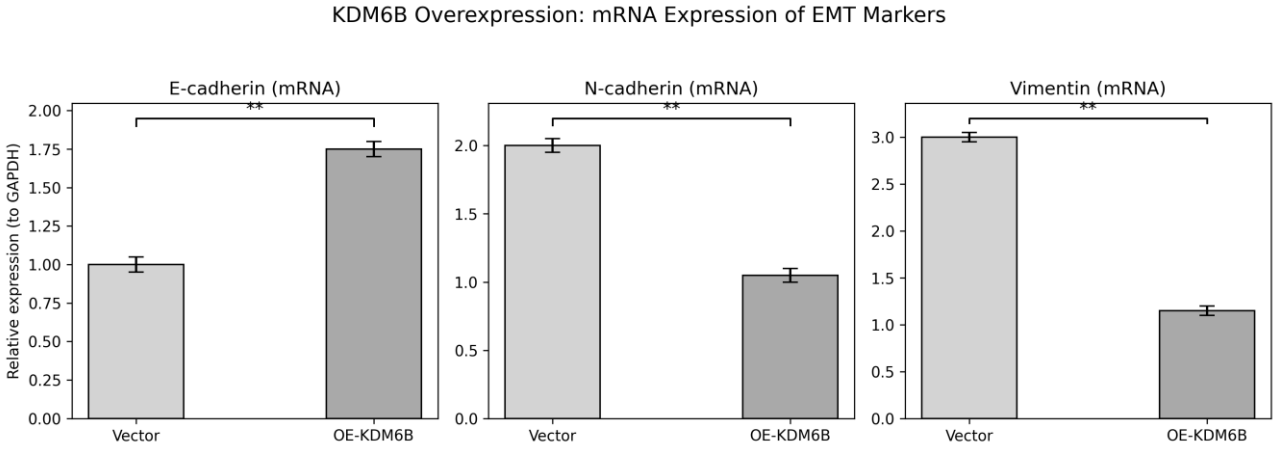

Figure 4. Effects of KDM6B regulation on cell migration and invasion, paired *t*-test.
***P* < 0.01. Data are presented as mean ± SEM from three independent experiments.

3.6. Epigenetic modification mechanism mediated by KDM6B

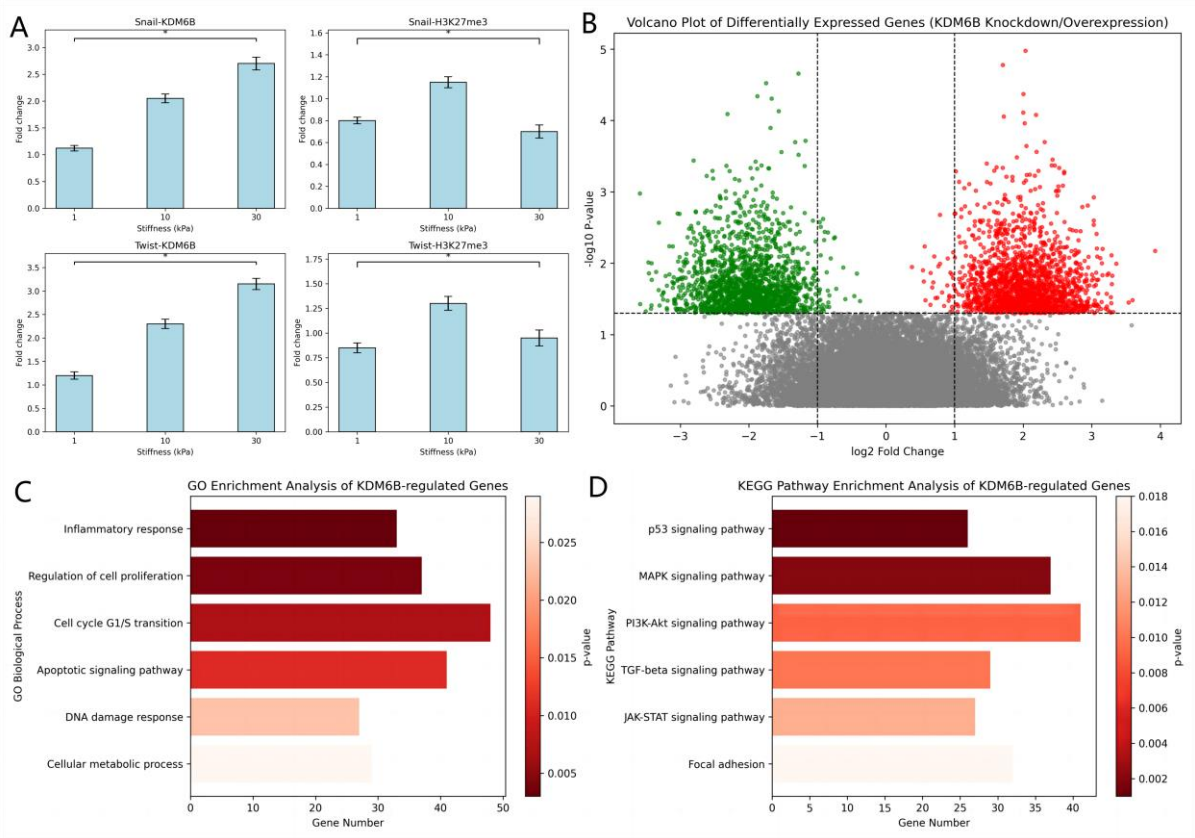

Figure 5. Comprehensive ChIP-qPCR and transcriptomic analysis of KDM6B-mediated EMT regulation. **(A)** Binding of KDM6B and H3K27me3 to EMT gene promoters (ChIP-qPCR), one-way ANOVA; **(B)** volcano plot of RNA-seq differential gene expression; **(C)** GO enrichment analysis results; **(D)** KEGG pathway analysis results.
**P* < 0.05. Data are presented as mean ± SEM from three independent experiments.

In the ChIP-qPCR analysis, one-way ANOVA results showed that under higher stiffness conditions, the binding of KDM6B to the promoters of Snail and Twist was enhanced ($p < 0.05$), whereas H3K27me3 modification was most active at medium stiffness (10 kPa) ($p < 0.05$), suggesting a non-linear relationship between ECM

stiffness and the epigenetic modification status (**Figure 5A**). The volcano plot of RNA-seq differential gene expression revealed that most genes showed no significant change, with only a few genes on both sides being significantly upregulated or downregulated, suggesting that KDM6B knockdown/overexpression affects the transcription of only certain target genes (**Figure 5B**). GO and KEGG enrichment analyses indicated that most differentially expressed genes were significantly enriched in biological processes such as cell proliferation and apoptosis, as well as related signaling pathways. The depth of color indicates the level of significance, suggesting that KDM6B influences the progression of differentiated thyroid carcinoma through multiple signaling pathways (**Figure 5C**,**D**).

4. Discussion

This study found that increased extracellular matrix (ECM) stiffness significantly upregulated the mRNA and protein expression levels of KDM6B in differentiated thyroid carcinoma cells and enhanced its enzymatic activity. This result indicates that KDM6B, as a demethylase, is activated in a high-stiffness environment and may promote the expression of related genes by removing the H3K27me3 modification in specific gene promoter regions [13,14]. ECM stiffness regulates the expression of KDM6B through mechanical signal transduction pathways such as the YAP/TAZ pathway. A high-stiffness substrate can activate YAP/TAZ, thereby upregulating KDM6B expression and enhancing its demethylation activity, which in turn affects the transcriptional activity of downstream genes [15]. This mechanism reveals how cells sense and respond to the physical properties of the microenvironment, regulating key epigenetic factors and thus influencing cell behavior and fate. With the increase in substrate stiffness, the expression levels of EMT markers changed significantly. E-cadherin, as a marker of epithelial cells, was significantly decreased, whereas N-cadherin and vimentin, as markers of mesenchymal cells, were significantly increased [16]. This change is consistent with classic EMT characteristics, indicating that cells transition from an epithelial phenotype to a mesenchymal phenotype, thereby enhancing their migration and invasion capabilities. ECM stiffness further promotes the EMT process by regulating KDM6B. KDM6B knockdown experiments showed that reduced KDM6B expression exacerbated the decrease in E-cadherin and the increase in N-cadherin and vimentin, promoting EMT progression and enhancing cell migration and invasion. In contrast, KDM6B overexpression inhibited the changes in EMT markers, maintained epithelial characteristics, and reduced cell migration and invasion. This suggests that KDM6B exerts an inhibitory effect on tumor invasion and metastasis in a high-stiffness environment by suppressing the EMT process [17]. KDM6B regulates the expression of EMT-related genes through demethylation, further influencing the phenotypic transformation of cells. High-stiffness substrates upregulate KDM6B via mechanical signal transduction pathways, suppressing its demethylation activity, leading to upregulation of EMT-related genes and promoting cellular invasiveness and metastatic potential. This finding not only reveals the profound impact of ECM stiffness on tumor cell behavior but also provides a new perspective on understanding the role of epigenetic regulation in tumor progression.

KDM6B exhibits bidirectional regulatory effects in differentiated thyroid carcinoma cells, underscoring its critical role in the epithelial-mesenchymal transition (EMT) process. Experimental results indicate that knocking down KDM6B significantly promotes the reduction of the EMT marker E-cadherin and the increase of N-cadherin and vimentin, thereby enhancing cell migration and invasion. This finding suggests that under normal conditions, KDM6B exerts tumor-suppressive functions by inhibiting the EMT process. As a demethylase, KDM6B may inhibit the expression of EMT-related genes by removing H3K27me3 modifications, thus maintaining the phenotypic stability of epithelial cells [18]. When KDM6B is knocked down, the level of H3K27me3 modifications increases, leading to downregulation of EMT-inhibitory genes, promoting the transition of cells toward a mesenchymal phenotype and enhancing their invasiveness. Conversely, overexpression of KDM6B inhibits the expression of the EMT markers N-cadherin and vimentin while upregulating E-cadherin, significantly reducing cell migration and invasion. This indicates that KDM6B, by increasing H3K27me3 modifications, suppresses the transcriptional activity of EMT-related genes, thereby inhibiting the EMT process. Overexpression of KDM6B not only restores the expression of epithelial markers but also effectively suppresses the upregulation of mesenchymal markers, maintaining epithelial characteristics of the cells and reducing their migratory and invasive capacities [19]. This mechanism further supports the role of KDM6B as an EMT suppressor, regulating phenotypic transitions through epigenetic modifications. By modulating H3K27me3 modifications, KDM6B influences the expression of EMT-related genes, highlighting its importance in epigenetic regulation. High-stiffness ECM environments upregulate KDM6B expression and activity via mechanical signal transduction pathways, which in turn inhibit the EMT process and reduce tumor cell invasion and metastasis [20]. This finding not only elucidates the mechanism by which substrate stiffness affects tumor cell behavior but also provides a potential therapeutic strategy targeting KDM6B. By regulating its epigenetic modification function, it may be possible to inhibit tumor progression and metastasis.

KDM6B significantly regulates the expression of EMT-related genes in a high-stiffness environment through epigenetic modifications. Studies have shown that KDM6B enhances its binding to the promoters of EMT genes such as Snail and Twist in high-stiffness substrates, accompanied by marked changes in H3K27me3 modifications. This indicates that KDM6B inhibits the expression of these key transcription factors by demethylating H3K27me3, thereby suppressing the EMT process. As an inhibitory epigenetic mark, H3K27me3 can activate EMT-inhibitory genes when demethylated, maintaining the epithelial characteristics of cells [21]. Through mechanical signal transduction pathways, such as the YAP/TAZ pathway, high-stiffness ECM environments regulate KDM6B expression and activity, enabling it to exert stronger demethylation functions in gene promoter regions and inhibit the transcriptional activity of EMT-related genes [22]. RNA-seq and subsequent GO and KEGG enrichment analyses further revealed the key genes and signaling pathways regulated by KDM6B. Knockdown or overexpression of KDM6B significantly affected the expression of certain target genes, which mainly participate in biological processes such as cell proliferation, apoptosis, and migration. The enrichment analysis results showed that differentially expressed genes were significantly enriched in key

signaling pathways such as PI3K/Akt and MAPK, which play important roles in regulating cell growth, survival, and migration. By regulating these pathways, KDM6B further influences cell behavior during the EMT process. For example, activation of the PI3K/Akt pathway can promote cell survival and migration, whereas the MAPK pathway plays a key role in cellular stress responses and differentiation. KDM6B, through its demethylation function, suppresses abnormal activation of these pathways, thereby inhibiting the EMT process and reducing cell invasion and metastasis [23]. The role of KDM6B in regulating epigenetic modifications is not limited to the demethylation of H3K27me3 but may also involve interactions with other epigenetic marks and transcription factors. These multilayered regulatory mechanisms enable KDM6B to flexibly respond to different ECM stiffness environments, accurately regulating the EMT process and maintaining cellular homeostasis and function. In this way, KDM6B plays a key role in the adaptation of cells to microenvironmental changes, regulating tumor cell phenotypic transformation and invasiveness.

This study mainly used the differentiated thyroid carcinoma cell line K1 for experiments, which did not encompass other thyroid cancer cell lines and may limit the generalizability of the results. Different cell lines exhibit variations in gene expression and phenotypic characteristics; therefore, future research should be extended to multiple thyroid cancer cell models to validate the regulatory role of KDM6B under different backgrounds. In addition, differences in transfection efficiency and the uniformity of substrate stiffness during the experiments may introduce experimental errors, affecting the accuracy of the results. Transfection conditions should be optimized, and more precise substrate preparation methods should be employed to reduce potential biases. Future investigations should delve deeper into the synergistic effects of KDM6B with other epigenetic modification enzymes, revealing the complex mechanisms by which it regulates the EMT process. Functional validation using in vivo tumor models will further confirm the role of KDM6B in tumor progression and provide a basis for clinical application. Combined with clinical sample analysis, assessing the expression level of KDM6B in thyroid cancer patients and its correlation with prognosis will help establish its value as a potential therapeutic target. Through multi-level and multi-perspective research, a comprehensive analysis of how KDM6B regulates EMT in response to extracellular matrix stiffness will offer new theoretical foundations and practical avenues for treatment strategies against differentiated thyroid carcinoma.

5. Conclusion

This study demonstrates that extracellular matrix stiffness upregulates KDM6B expression and activity in differentiated thyroid carcinoma cells, thereby suppressing EMT through H3K27me3 demethylation and reducing migration and invasion. KDM6B knockdown enhances EMT, whereas its overexpression reverses this effect. These findings underscore KDM6B’s crucial role in thyroid cancer progression and highlight its potential as a therapeutic target.

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