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METTL14-mediated m6A modification upregulates HOXB13 expression to activate NF-κB and exacerbate cervical cancer progression

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ABSTRACT

Cervical cancer (CC) is one of the common malignant tumors in women, and the incidence rate is located in the second place of female tumors. As a major RNA N6-methyladenosine (m6A) methyltransferase, methyltransferase-like 14 (METTL14) is involved in tumor progression by catalyzing methylation modifications in mRNAs. However, the molecular mechanism of METTL14-mediated m6A modification in CC remains not fully revealed. The expression of METTL14 was detected by RT-qPCR and western blot. Cell function was assayed by cell counting kit-8 (CCK-8) assay and flow cytometry analysis. Methylated RNA immunoprecipitation (MeRIP) was used to confirm the relationship between METTL14 and homeobox B13 (HOXB13). In our study, we found that the level of METTL14 was elevated in CC tissues and cells compared with their controls. The inhibition of METTL14 significantly impaired cell proliferation and the epithelial-mesenchymal transition (EMT) process, while also induced apoptosis in HeLa and C33A cells. Furthermore, our findings indicated that homeobox B13 (HOXB13) was a target of METTL14, which positively regulated the expression of HOXB13 in an m6A-dependent manner. Rescue experiments indicated that overexpression of HOXB13 effectively reversed the tumor suppression induced by METTL14 knockdown. Finally, we confirmed that METTL14-modified HOXB13 exerted an oncogenic effect through activation of the nuclear factor kappa B (NF-κB) pathway. In conclusion, our data demonstrated that the m6A modification of HOXB13, mediated by METTL14, facilitated the advancement of CC through targeting the NF-κB pathway, which may be a potential molecular target for the treatment of CC.

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Cervical cancer; N6-methyladenosine; METTL14; HOXB13; NF-κB

1. Introduction

Cervical cancer (CC) is a common malignancy in gynecology, and infection with human papillomavirus (HPV) is identified as a major risk factor for CC.[1] Vaccine-driven prevention combined with HPV screening are currently best strategy for prevention and control of CC.[2,3] Great progression has made in the treatment of CC, such as the discovery of many novel biomarkers and therapeutic targets, including exosomes,[4,5] miRNAs,[6] small molecule inhibitors, and monoclonal antibodies of cancer-associated proteins.[7] Currently, surgery, chemotherapy,[8] radiotherapy,[9] and immunotherapy[10] including nanomaterials[11–13] are the mainly therapies for advanced CC, but the clinical outcomes of CC are still unsatisfied.[14] Therefore, it is necessary to investigate the underlying molecular mechanisms of CC pathogenesis and identify novel targets to improve diagnosis, prognosis of this disease.

N6 methyladenosine (m6A) modification is one of the most prevalent post-transcriptional modifications in mammalian RNA.[15] Multiple studies have revealed that the progression of many diseases is associated with abnormal genetic and epigenetic changes, including CC.[16,17] The functional regulation of the eukaryotic transcriptome is influenced by m6A RNA modifications, which in turn impact several processes, including mRNA export, splicing, translation, localization, and stability.[18] The m6A modification is introduced by m6A methyltransferases, which include methyltransferase-like 3 (METTL3), methyltransferase-like 14 (METTL14), and the Wilms' tumor-associated protein (WTAP), collectively known as "writers." Conversely, this modification is reversed by demethylases such as fat mass and obesity-associated protein (FTO) and alkylation repair homolog protein 5 (ALKBH5), referred to as "erasers." Additionally, m6A modification is recognized by m6A binding proteins, which comprise the YTH domain-containing family proteins YTHDC1/2, YTHDF1/2/3, and insulin-like growth factor 2 mRNA binding proteins IGF2BP1/2/3, commonly referred to as "readers".[19] Previous research has shown that the abnormal expression of m6A-related genes, such as ALKBH5, METTL3, METTL14, and FTO, is linked to the development of cervical cancer.[14,20–22] Moreover, METTL14-mediated mRNA m6A modification has been found in colorectal cancer,[23] pancreatic cancer,[24] CC,[25] and other tumors. However, the potential regulatory mechanisms of METTL14-mediated m6A modification in the progression of CC are remains to be further investigated.

Homeobox (HOX) genes are a group of transcription factors that contain homology domains, and it can be

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categorized into 4 gene clusters (i.e., A to D).[26] Specially, HOXB13, as a member of the HOXB cluster, is associated with cancer development, such as prostate cancer,[27] bladder cancer,[28] breast cancer,[29] hepatocellular carcinoma,[30] gastric cancer,[31] and CC.[32] Furthermore, HOXB13 acts as a pro-cancer or tumor suppressor in cancers by regulating multiple cellular functions. For instance, HOXB13 accelerates the tumorigenesis of hepatocellular carcinoma by promoting cell proliferation and invasion while also activating the AKT/mTOR pathway.[30] And inhibition of HOXB13 suppresses cell proliferation, migration and invasion in glioblastoma.[33] Specially, the upregulation of HOXB13 is related with the malignant progression of CC.[32] Besides, it has reported that demethylates FTO-mediated m6A modification regulates HOXB13 expression, thereby contributing to the advancement of endometrial cancer[34] and gastric cancer.[35] However, the role of HOXB13 and the potential influence of m6A modification in cervical cancer (CC) continue to be unclear.

In our study, we explored the role of m6A modification in CC development. Our findings indicate that METTL14 plays a crucial role in promoting CC tumorigenesis. Inhibition of METTL14 resulted in reduced cell viability, impaired epithelial-mesenchymal transition (EMT), and triggered apoptosis in CC cells. Furthermore, METTL14-mediated m6A modification upregulated the expression of HOXB13, thereby activating the nuclear factor kappa B (NF-κB) pathway in CC cells. In conclusion, we identified the METTL14/m6A/HOXB13/NF-κB axis as a novel mechanism of RNA epigenetic regulation in CC.

2. Materials and methods

2.1. Patient samples

CC specimens and normal tissues were obtained from CC patients who underwent surgery at the 926th Hospital of the Joint Logistics Support Force of the Chinese People's Liberation Army. None of the GC patients received radiotherapy, chemotherapy, targeted therapy, or immunotherapy before surgery. Ethics consent was approved by the Ethics Committee of the 926th Hospital of the Joint Logistics Support Force of the Chinese People's Liberation Army, and informed consent was obtained from each patient before sample collection.

2.2. Cells culture and treatment

Human normal cervical epithelial cells (Ect1/E6E7) were obtained from Shanghai Emzyme-linked Biotechnology Co., Ltd, and CC cell lines (HeLa and C33A) were purchased from Procell Life Science & Technology Co., Ltd. All cells were cultured in minimum essential medium (MEM) with non-essential amino acids (NEAA) (Pricella) supplemented with 10% fetal bovine serum (FBS, Pricella) and 1% penicillin-streptomycin (Pricella).

The full length of HOXB13 cDNA was cloned into the pcDNA3.1 vector to construct the HOXB13 overexpression (oe-HOXB13) plasmid. The shRNA of METTL14 (kd-METTL14) and the negative control (kd-NC) were obtained from GenePharma, and the sequences of shRNAs were shown in Table 1. Cell transfection was performed using Lipofectamine 3000 (Invitrogen). The NF-κB inhibitor BAY11–7082 was purchased from Selleck.

Table 1. The sequence of shRnas.

Name	Sequence
sh-NC	ACTCAAAAGGAAGTGACAAGA
sh-METTL14	GCTAAAGGATGAGTTAAT

2.3. RT-qPCR

Trizol reagent (Invitrogen) was used to extract total RNA from CC tissues and cells, and cDNA was synthesized by PrimeScript™ RT reagent Kit (TaKaRa). The expression levels of METTL14 and HOXB13 were detected by the SYBR Premix Ex Taq II kit (TaKaRa). The primers used in our study were listed in Table 2. The data were analyzed by the $2^{-\Delta\Delta Ct}$ method and normalized to GAPDH.

2.4. Western blot

CC tissues and cells were collected and lysed by RIPA buffer. Protein concentrations were quantified using BCA protein assay kit (Beyotime). Equal amounts of protein (20 μg) from each sample were separated and transferred onto PVDF membranes (Millipore). The membranes were hybridized with proper primary antibodies overnight at 4°C, and then incubated with HRP-labeled secondary antibody for 2 h at room temperature. The protein bands were visualized by enhanced chemiluminescence (Beyotime), and quantified by Image J software.

2.5. Cell counting kit-8 (CCK-8) assay

Cells (3×10^3) were cultured in a 96-well plate after transfection. Cell viability was measured every 24 h. Then, 10 μl CCK-8 reagent (MedChemExpress) was added to each well and incubated for another 2 h. The absorbance value of each well at 450 nm was recorded.

2.6. Flow cytometry analysis

Cell apoptosis was performed using the Annexin V-FITC/PI Apoptosis Detection kit (Vazyme). Cells were collected after transfection for 48 h, and washed twice with PBS. Cells were resuspended in 100 μl of binding buffer and then mixed with 5 μl of Annexin V and 5 μl of PI and left in the dark at room temperature for 20 min, and the apoptosis level was detected using a flow cytometer (BD Biosciences).

2.7. Methylated RNA immunoprecipitation (MeRIP)

Cells transfected with knockdown of METTL14 were subjected to MeRIP assay by the Magna MeRIP™ m6A Kit (Millipore).

Table 2. Sequences of primers for rt-qPCR.

Name	Forward (5′-3′)	Reverse (5′-3′)
METTL14	GAACACAGAGCTTAAATCCCCA	TGTCAGCTAAACCTACATCCCTG
HOXB13	GTGTTGCCAGGGAGAACAGA	TTGTTAGCCGCATACTCCCG
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG

Briefly, RNA is digested into 100 nt fragments and then magnetically immunoprecipitated with anti-m6A or anti-IgG antibodies. After immunoprecipitation, the isolated RNA fragments were subjected to RT-qPCR.

2.8. Statistical analysis

All data were expressed as the mean ± SD of three or more biological replicates, and statistical analyses were performed by GraphPad Prism 7.0 software (GraphPad, Inc.,). Differences between groups were estimated using a student's *t*-test (two groups) or One-way ANOVA (multiple groups). Pearson's chi-square test was used to analyze the correlation between METTL14 and HOXB13 expression. $p < .05$ was statistically significant.

3. Results

3.1. METTL14 expression is elevated in cervical cancer tissues and cell lines

To investigate the role of METTL14 in CC, we firstly collected CC tumor tissues and normal tissues, and the levels of METTL14 were measured by RT-qPCR. As shown in Figure 1a, the mRNA expression of METTL14 was notably increased in CC tissues compared with the normal tissues. Simultaneously, western blot analysis showed that METTL14 level was increased in CC tissues compared with normal tissues (Figure 1b–c). Next, we detected the expression of METTL14 *in vitro*. The results of RT-qPCR indicated that METTL14 mRNA expression was also greatly improved in CC cell lines (HeLa and C33A) compared with the normal Ect1/E6E7 cells (Figure 1d). Likewise, we also noted an increase in the protein level of METTL14 in HeLa and C33A cells (Figure 1e–f). In addition, an analysis of the clinical stage and prognostic significance of METTL14, utilizing existing the existed datasets, and we found that the expression of METTL14 was not associated with different clinical stage (Figure 1g, $p = .421$) or the prognosis of cervical squamous cell carcinoma (CESC) (Figure 1h, $p = .72$). Besides, the associations between METTL14 expression and the clinicopathological characteristics in patients with CC were determined. As detailed in Supplementary file: Table S1, there was no observed correlation between age, tumor size, and stage; however, the expression of METTL14 was linked to

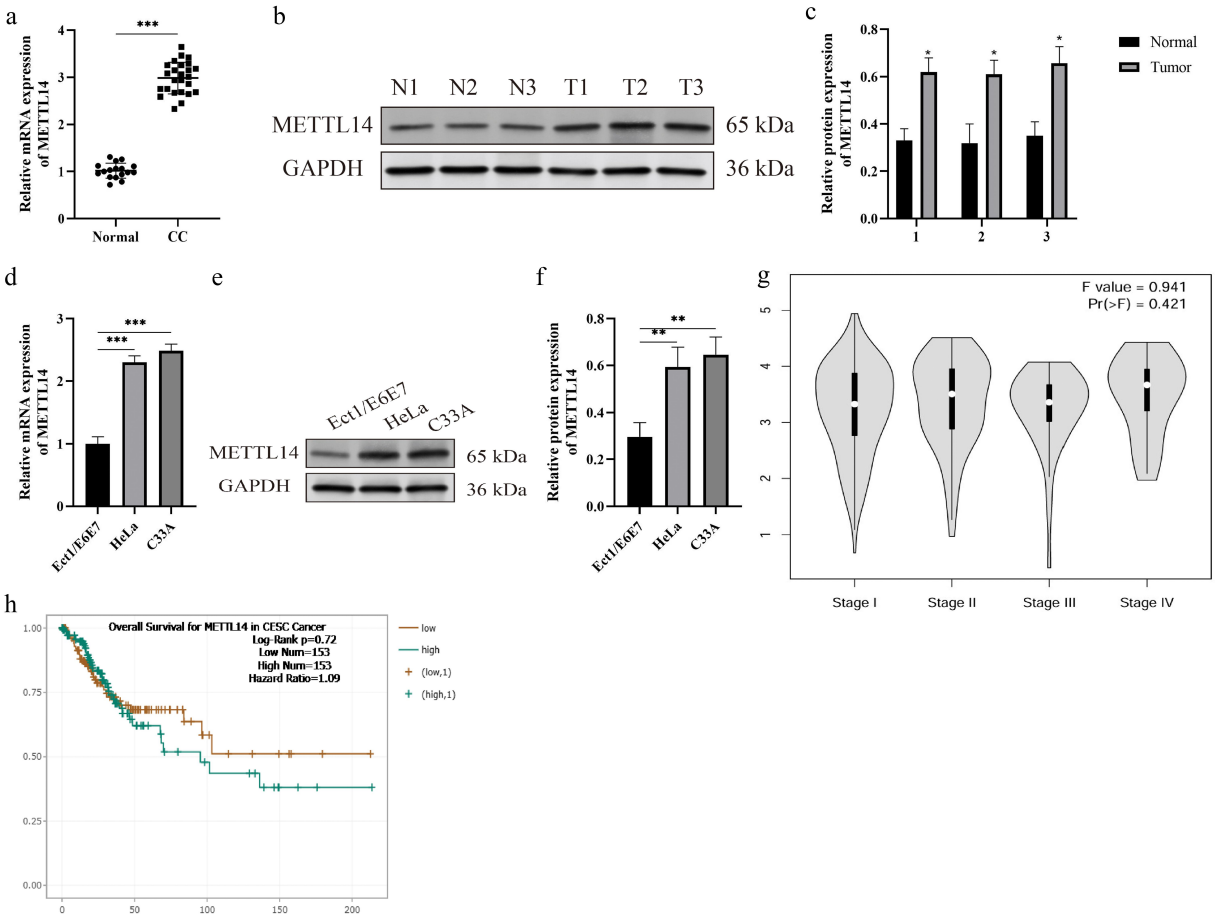

Figure 1. METTL14 expression is elevated in cervical cancer tissues and cell lines. A: METTL14 mRNA expression in CC tissues ($n = 25$) and normal tissues ($n = 17$) was detected by rt-qPCR. B-C: METTL14 protein expression in CC tissues and normal tissues was measured by western blot. D: METTL14 mRNA expression was assessed by rt-qPCR in human normal cervical epithelial cells (Ect1/E6E7) and cervical cancer cell lines (HeLa and C33A). E-F: METTL14 protein expression in Ect1/E6E7, HeLa and C33A cells was examined by western blot. G: METTL14 expression was evaluated in cervical squamous cell carcinoma (CESC) patients with different tumor stage using the GEPIA2 database. H: survival analyses categorized by METTL14 expression levels (METTL14 low expression = 153, METTL14 high expression = 153) in patients with CESC utilizing the ENCORI database. *$p < .05$, **$p < 0.01$, ***$p < .001$.

HPV infection. These data suggested that METTL14 was increased in tissues and cells of CC.

3.2. Inhibition of METTL14 reduces cell viability, EMT and induces apoptosis of cervical cancer cells

To investigate the role of METTL14 in the tumorigenesis of cervical cancer (CC), we conducted a depletion of METTL14 in HeLa and C33A cell lines, followed by a series of functional experiments. First of all, the knockdown efficiency of METTL14 was detected by RT-qPCR, and we found that METTL14 level was visibly decreased in HeLa and C33A cells transfected with METTL14-silencing vectors (Figure 2a). Next, the results of CCK-8 assay showed that the knockdown of METTL14 suppressed cell proliferation in both HeLa and C33A cells (Figure 2b–c). Furthermore, flow cytometry analysis disclosed that cell apoptosis was increased in METTL14-knockdown CC cells compared with the control kd-NC group (Figure 2d–e). In addition, western blot analysis revealed that the level of epithelial marker E-cadherin was increased and mesenchymal markers N-cadherin and vimentin was decreased in HeLa and C33A cells transfected with the METTL14 knockdown vectors, suggesting that inhibition of METTL14 declined the process of epithelial-to-mesenchymal transition (EMT) in CC cells (Figure 2f–g). Taken together, our data highlighted that METTL14 acted as an oncogene that promoted cell viability and EMT process and inhibited apoptosis of CC cells.

3.3. METTL14 promotes HOXB13 expression

It has been reported that METTL14 regulates stabilized mRNAs through m6A modification, thereby affecting cellular biological functions.[36] The m6A modification has been reported to regulate HOXB13 expression, including in endometrial cancer and gastric cancer.[34,35] And HOXB13 has been proven to be upregulated in cervical cancer.[32] However it remains unknown whether m6A modifications regulate

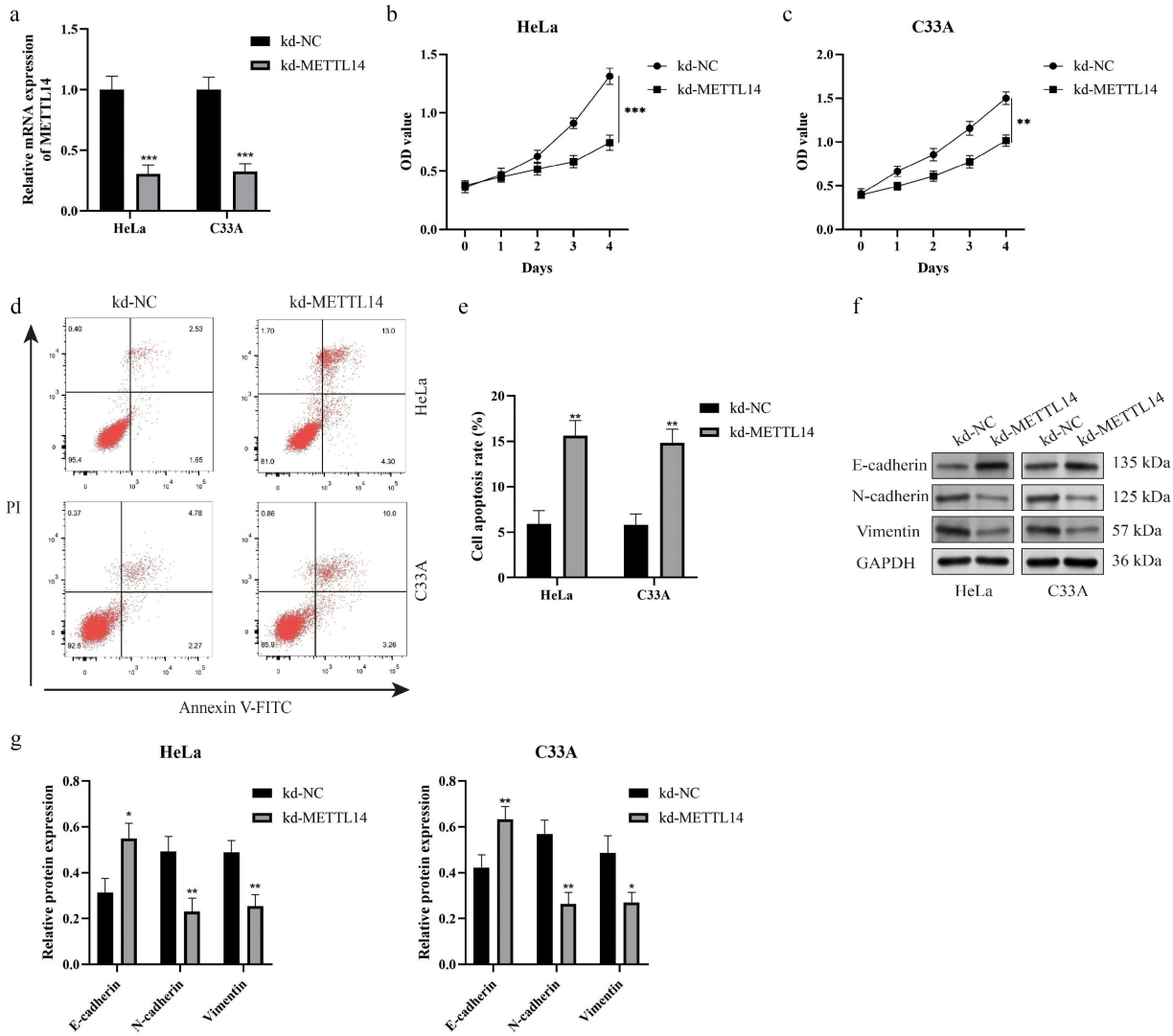

Figure 2. Inhibition of METTL14 reduces cell viability, EMT and induces apoptosis of cervical cancer cells. A: the expression of METTL14 in METTL14-depleted HeLa and C33A cells was detected by rt-qPCR. B-C: cell viability in METTL14 knockdown HeLa and C33A cells was examined by CCK-8 assay. D-E: cell apoptosis of HeLa and C33A cells after inhibition of METTL14 was assessed by flow cytometry analysis. F-G: emt-related markers (E-cadherin, N-cadherin and Vimentin) were measured by western blot. *$p < .05$, **$p < 0.01$, ***$p < .001$.

HOXB13 in CC. Base on the SRAMP database, we found that HOXB13 has m6A modification sites (Figure 3a). MeRIP-PCR results indicated that METTL14 knockdown decreased the m6A level of HOXB13 mRNA, suggesting that METTL14 may be involved in the m6A modification of HOXB13 (Figure 3b). Besides, RT-qPCR confirmed that the expression of HOXB13 was markedly suppressed in METTL14-depleting CC cells (Figure 3c). Western blot analysis revealed that HOXB13 protein level was also declined in HeLa and C33A cells after inhibition of METTL14 (Figure 3d). Moreover, we observed that HOXB13 expression in CC tissues was higher than that of the normal tissues (Figure 3e). And it was positively correlated with the mRNA expression of HOXB13 and METTL14 (Figure 3f). Furthermore, an examination of the clinical stage and prognostic relevance of HOXB13 by the GEPIA2 database and the ENCORI database, revealed that the expression levels of HOXB13 did not correlated with clinical stages (Figure 3g, $p = .605$) or the prognosis of CESC (Figure 3h, $p = .39$). Additionally, the relationships between HOXB13 expression and the clinicopathological features in

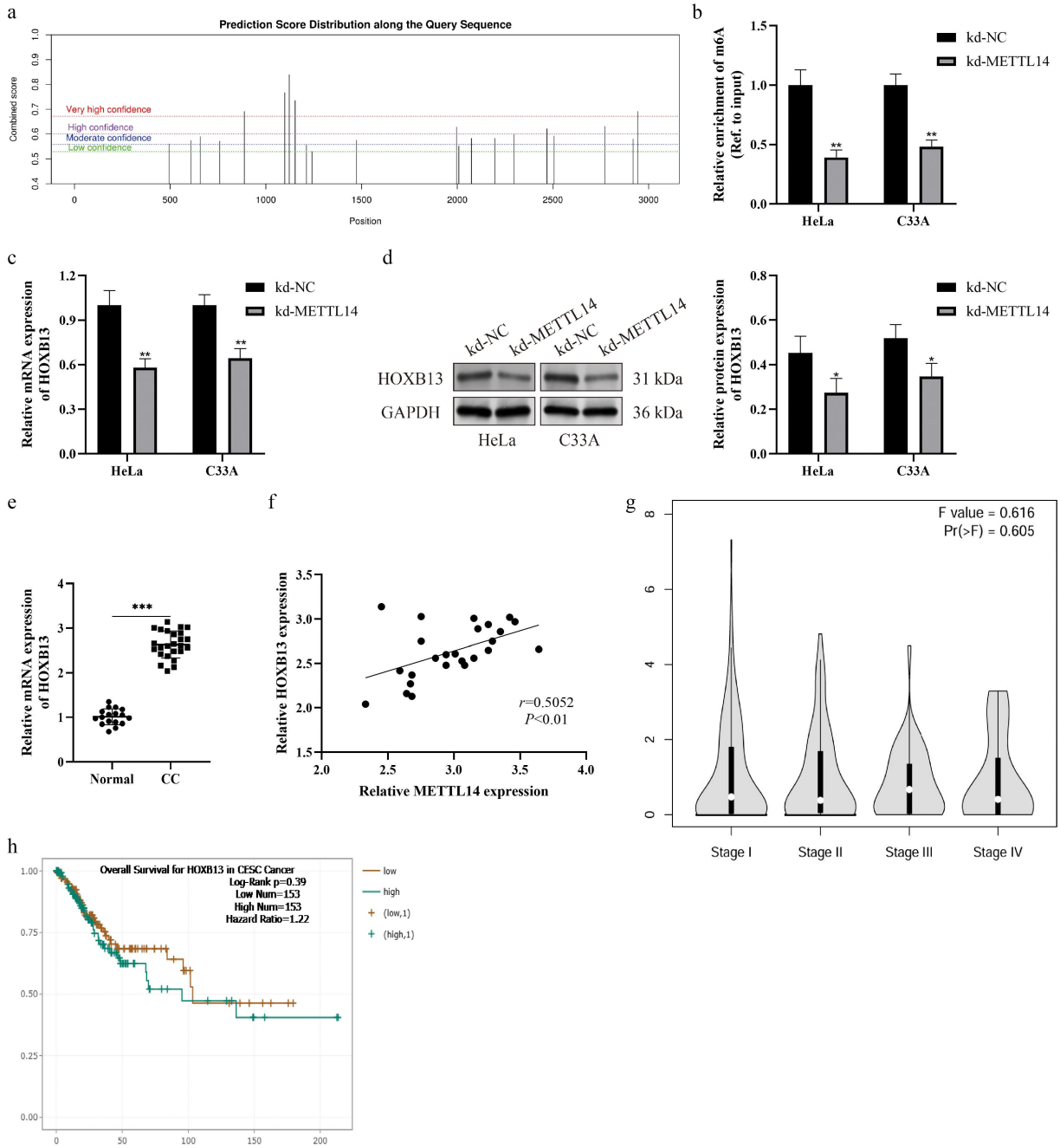

Figure 3. METTL14 promotes HOXB13 expression. A: the m6A modification site of HOXB13 was predicted by bioinformatics website SRAMP (https://www.Cuilab.cn/sramp). B: MeRIP-qPCR analysis was employed to demonstrate METTL14-mediated HOXB13 m6A modifications. The m6A modification of HOXB13 was decreased when METTL14 was knocked out. C: rt-qPCR analysis confirmed the expression of HOXB13 after METTL14 knockdown in HeLa and C33A cells. D: Western blot analysis was used to detect the expression of HOXB13 in METTL14-silencing CC cells. E: HOXB13 mRNA expression in CC tissues and normal tissues was detected by rt-qPCR. F: analysis of the correlation between HOXB13 and METTL14 expression in CC tissues. G: HOXB13 expression was evaluated in CESC patients with different tumor stage using the GEPIA2 database. H: survival analyses categorized by HOXB13 expression levels (HOXB13 low expression = 153, METTL14 high expression = 153) in CESC patients utilizing the ENCORI database.*$p < .05$, **$p < 0.01$, ***$p < .001$.

patients with CC were assessed. As shown in Supplementary file: Table S2, no significant correlation was identified between age and clinical stage; however, the expression of METTL14 was found to be associated with tumor size and HPV infection. In conclusion, METTL14 positively regulated the m6A methylation and expression level of HOXB13.

3.4. Upregulation of HOXB13 alleviates the inhibitory effect of silencing METTL14 on cervical cancer tumorigenesis

To further elucidate the role of the METTL14/HOXB13 axis in CC progression, we analyzed the functional changes in cells after overexpression of HOXB13. RT-qPCR results demonstrated that the downregulation of HOXB13 in METTL14-silencing CC cells was effectively recovered after overexpression of HOXB13 (Figure 4a). CCK-8 assay pointed out that upregulation of HOXB13 reversed the inhibitory effect of knockdown of METTL14 on cell viability (Figure 4b–c). Flow cytometry analysis manifested that overexpression of HOXB13 contributed to the alleviation of apoptosis induced by repression of METTL14 (Figure 4d–e). Furthermore, the knockdown of METTL14, which led to the upregulation of E-cadherin and the downregulation of N-cadherin and Vimentin, was counteracted by the overexpression of HOXB13 (Figure 4f–h). These results suggested that HOXB13 promoted cell proliferation, EMT and inhibited apoptosis, which was regulated by METTL14-mediated m6A medication.

3.5. HOXB13 activates NF-κB signaling pathway

It is increasingly recognized that the abnormal expression of the NF-κB pathway plays a crucial role in the development and progression of cancer.[37] We next investigated whether the NF-κB signaling pathway was activated by HOXB13 in HeLa and C33A cells. The protein expression of p-NF-κB (p-p65), p-IKBα, NF-κB (p65) and IKBα total protein were measured by western blot analysis. In HOXB13 overexpressing CC cells, protein expression of p-p65 and p-IKBα was visibly upregulated, while the levels of total p65 and IKBα remained relatively unchanged (Figure 5a). Quantitative analysis of protein expression showed that p-p65/p65 and p-IKBα/IKBα were significantly increased in the HOXB13 upregulated CC cells (Figure 5b–c). These data demonstrated that HOXB13 induced the activation of NF-κB signaling pathway in CC cells.

3.6. METTL14-regulated HOXB13 promotes cervical cancer progression through NF-κB signaling pathway

To further explore the role of NF-κB signaling pathway in the progression of CC, NF-κB inhibitor BAY11–7082 was used in the following experiments. CCK-8 assay showed that upregulation of HOXB13 increased cell viability and this promotion was reversed by inhibiting METTL14, whereas treatment with BAY11–7082 in CC cells of the kd-METTL14+oe-HOXB13 group significantly inhibited cell viability (Figure 6a–b). In addition, upregulation of HOXB13 did not affect the level of cell apoptosis, whereas simultaneous silencing of METTL14 promoted apoptosis, which was exacerbated by co-treatment with BAY11–7082 (Figure 6c–d). Finally, the expression level of EMT-related biomarkers was detected by western blot, and we found that overexpression of HOXB13 inhibited E-cadherin level and promoted N-cadherin and Vimentin expression, where as silencing of METTL14 abolished this EMT-promoting

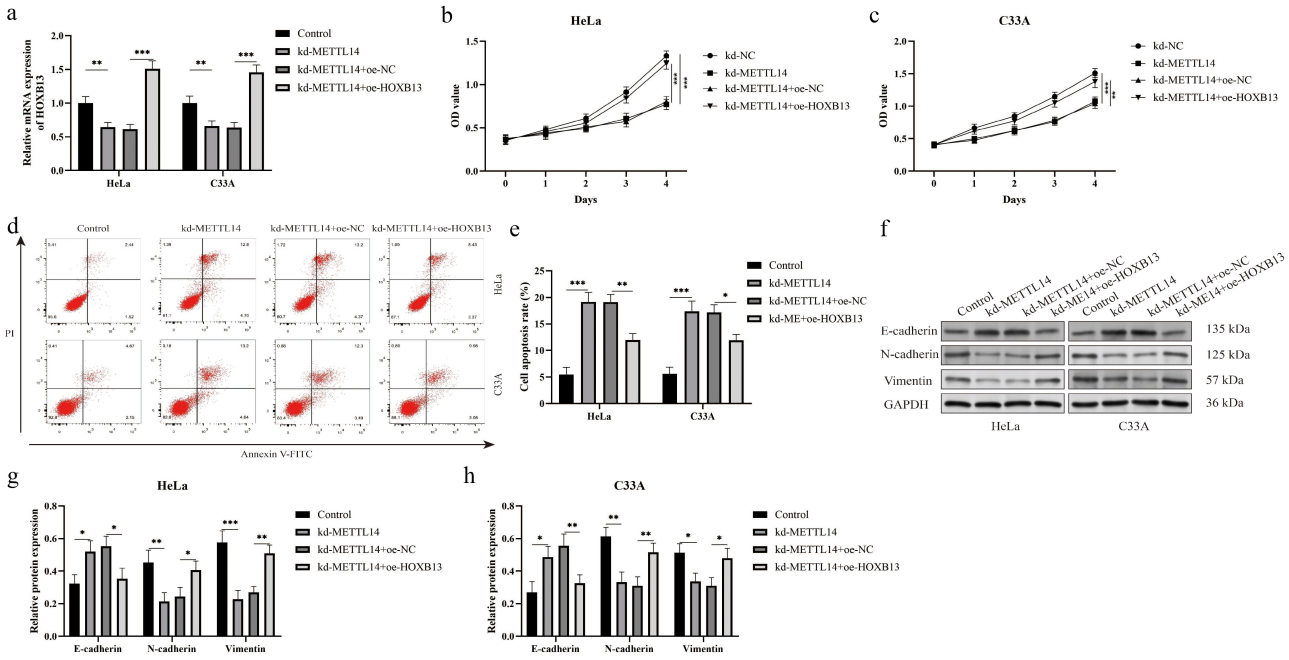

Figure 4. Upregulation of HOXB13 alleviates the inhibitory effect of silencing METTL14 on cervical cancer tumorigenesis. A: the expression of HOXB13 in HeLa and C33A cells of control, kd-METTL14, kd-METTL14+oe-nc and kd-METTL14+oe-HOXB13 group was assessed by rt-qPCR. B-C: cell viability in HeLa and C33A cells of four groups was examined by CCK-8 assay. D-E: cell apoptosis of HeLa and C33A cells in four groups was assessed by flow cytometry analysis. F-H: the protein expression of E-cadherin, N-cadherin and Vimentin was measured by western blot. *$p < .05$, **$p < 0.01$, ***$p < .001$.

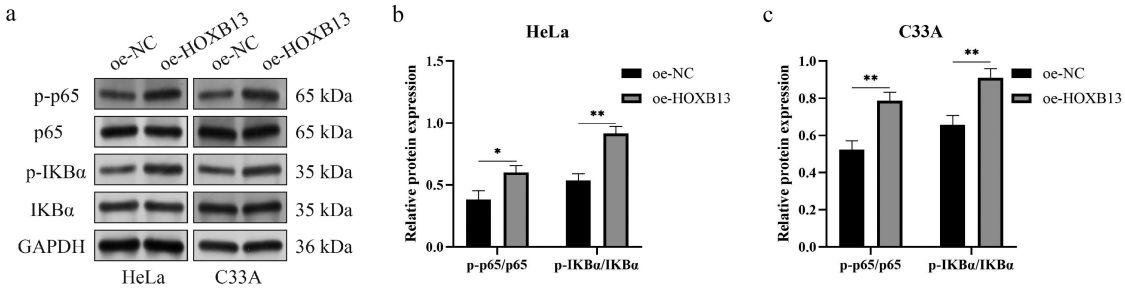

Figure 5. HOXB13 activates nf-κB signaling pathway. A: the expression of p-p65, p65, p-IKBα, IKBα and GAPDH was detected by western blot in CC cells transfected with HOXB13 overexpression vectors. B-C: quantitative analysis of p-p65/p65 and p-IKBα/IKBα in HeLa and C33A cells. *$p < .05$, **$p < 0.01$, ***$p < .001$.

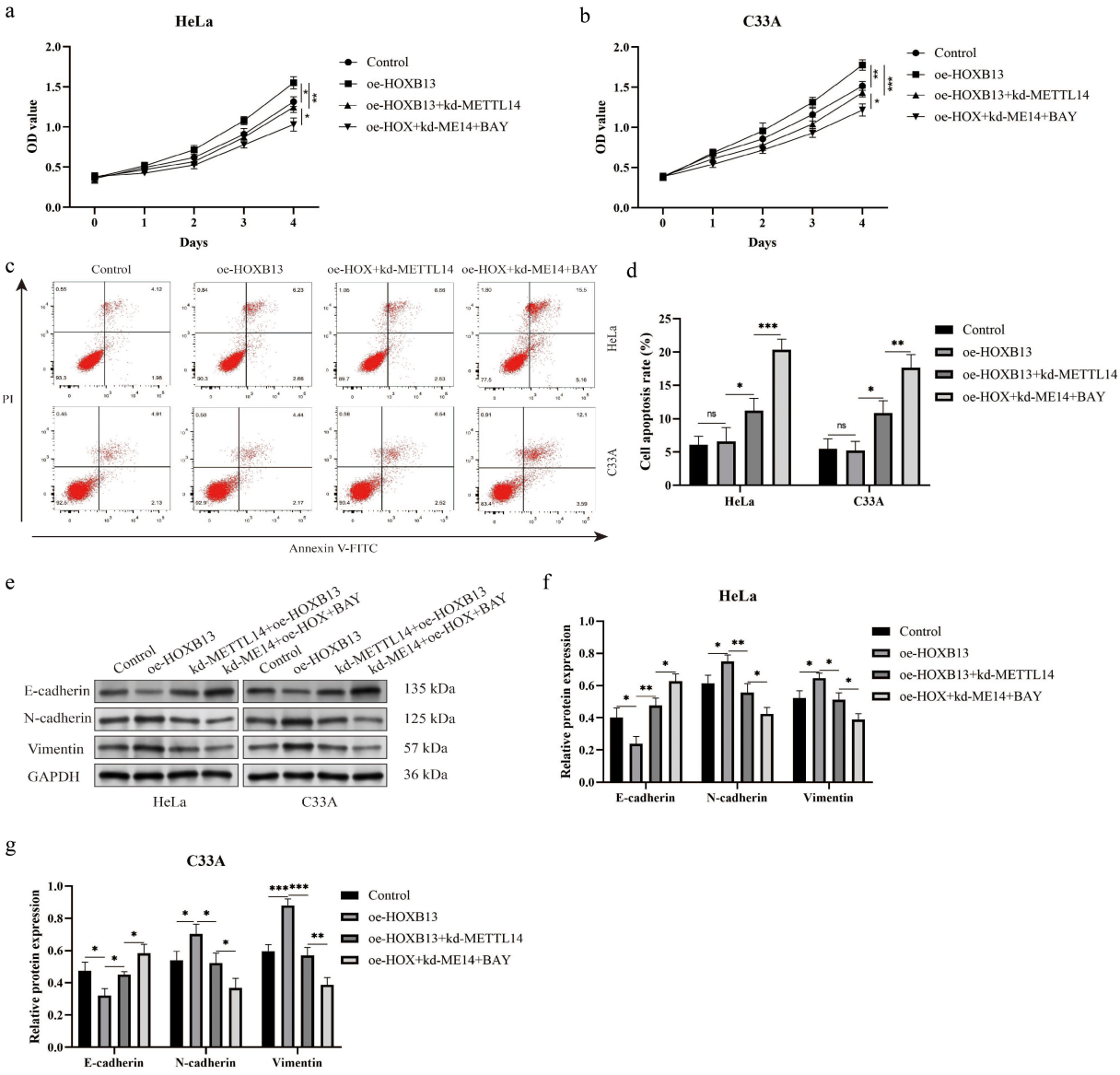

Figure 6. METTL14-regulated HOXB13 promotes cervical cancer progression through nf-κB signaling pathway. A-B: cell viability in HeLa and C33A cells of control, oe-HOXB13, kd-METTL14+oe-HOXB13 and kd-METTL14 +oe-HOXB13 +BAY11-7082 group was examined by CCK-8 assay. C-D: cell apoptosis of HeLa and C33A cells in four groups was assessed by flow cytometry analysis. E-G: the expression of E-cadherin, N-cadherin and Vimentin in four groups of HeLa and C33A cells was measured by western blot. *$p < .05$, **$p < 0.01$, ***$p < .001$.

effect, and BAY11–7082 co-treatment exacerbated the EMT-inhibitory effect of silencing METTL14 (Figure 6e–g). In summary, METTL14-modified HOXB13 exacerbated CC progression by activating the NF-κB pathway.

4. Discussion

As the fourth most common cancer among women globally, CC has a high incidence and mortality rate, especially in

developing countries.[38] Despite notable advancements in tumor treatment, the current treatment of advanced CC is not satisfactory.[39] Hence, there is an immediate need to unravel the underlying causes of CC and pinpoint novel targets for diagnosis and treatment. Numerous studies have demonstrated that the abnormal functioning of m6A regulators plays a crucial role in the progression of CC.[40–43] Notably, METTL14 plays a significant role as either an oncogene or a tumor suppressor gene, regulating the progression of various cancer types.[44] In our data, we observed that METTL14 level was increased in CC tissues and cells, and knockdown of METTL14 reduced cell proliferation, EMT and promoted apoptosis. Taken together, METTL14 could serve as a promising prognostic indicator and a potential target for therapeutic strategies in CC.

M6A methylation-associated enzymes regulate gene expression by impacting various facets of mRNA metabolism, encompassing pre-mRNA processing, nuclear export, degradation, and translation.[45] A growing number of researches have shown that mRNAs regulated by METTL14 through the m6A mechanism are widespread in a variety of diseases. For example, Li et al. demonstrated that METTL14 plays a crucial role in regulating the m6A methylation modification of MN1, inhibits the degradation of MN1 facilitated by IGF2BP2, and subsequently contributes to the advancement of osteosarcoma as well as resistance to all-trans-retinoic acid.[46] Zhang et al. suggested that overexpression of METTL14 increases PTEN expression, which is enriched through m6A modification mediated by YTHDF1, thereby inhibiting cell proliferation and migration in clear-cell renal-cell carcinoma.[47] In our research, we discovered that METTL14 plays a crucial role in regulating the expression of HOXB13, promoting its expression in an m6A-dependent manner, which subsequently expedited the development of CC. These findings suggested that both METTL14 and the m6A methylation of HOXB13 significantly influence the progression of CC.

Over the past few decades, the transcription factor NF-κB has attracted increasing attention in the field of cancer research.[48] The aberrant activation of the NF-κB pathway is frequently present in various malignant tumors and is associated with processes such as tumor cell proliferation, survival, angiogenesis, invasion, metastasis, and drug resistance.[49] For example, Hu et al. revealed that the expression of NF-κB and IKKβ was enhanced in epithelial ovarian cancer.[50] Specially, inhibition of NF-κB signaling pathway by baicalein has been shown to represses cell proliferation and induces apoptosis in CC.[51] Besides, HOXB13 has been identified as a factor that activates the NF-κB pathway in several cancers, including prostate cancer[52] and esophageal squamous cell carcinoma (ESCC).[53] Li et al. indicated that HOXB13-mediated activation of the NF-κB/p65 pathway was observed in ESCC,[53] Kim et al. suggested that HOXB13 reduces intracellular zinc concentrations, thereby activating NF-κB signaling pathways, which subsequently enhances the invasion and metastasis of prostate cancer,[52] and Taminiau et al. found that HOXA1 interacts with RBCK1 and TRAF2 and activate the NF-κB signaling pathway.[54] In our study, we found that HOXB13 activated the NF-κB pathway, and inhibition this pathway effectively decreased the tumorigenesis of CC. Overall, our findings revealed that the m6A modification of HOXB13, mediated by METTL14, increased its expression and promoted the NF-κB pathway in CC. However, whether HOXB13 regulated the NF-κB pathway by affecting molecules in this study still required further study.

In conclusion, we demonstrated that METTL14 regulated cell growth, apoptosis and EMT in CC cells through m6A modification of HOXB13 and activating NF-κB pathway, which may provide biomarkers and potential therapeutic targets for the diagnosis and treatment of CC.

Disclosure statement

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Author contributions

Qian Li, Na Zhao and Jufen Zhao designed the study, performed the data analysis. Xuejing Ding carried out the experiments. Xuejing Ding, Na Zhao and Qian Li drafted the paper. Jufen Zhao revised the article. All authors contributed to the article and approved the final manuscript.

Data availability statement

The data of this study are available upon a reasonable request to the corresponding author.

Ethical approval

Ethics consent was approved by the Ethics Committee of the 926th Hospital of the Joint Logistics Support Force of the Chinese People's Liberation Army, and informed consent was obtained from each patient before sample collection.

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