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ALKBH2, a novel AlkB homologue, contributes to human bladder cancer progression by regulating MUC1 expression

Tomomi Fujii,[1] Keiji Shimada,[1] Satoshi Anai,[2] Kiyohide Fujimoto[2] and Noboru Konishi[1,3]

Departments of [1]Pathology, [2]Urology, Nara Medical University School of Medicine, Nara, Japan

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The ALKBH family of proteins are highly expressed in various types of human cancer where they are involved in tumor growth and progression. However, multiple isoforms of ALKBH exist and the effect of individual isoforms on the development of urinary bladder cancer is unknown, particularly the molecular mechanisms involved in the progression from a noninvasive to invasive phenotype. We examined the role and function of ALKBH2 in human bladder cancer development *in vitro* and provide the first report that suppression of ALKBH2 in a human urothelial carcinoma cell line, KU7, reduces the expression of the transmembrane mucin protein, MUC1, and induces G1 cell cycle arrest. Moreover, reduction of ALKBH2 suppressed epithelial to mesenchymal transition (EMT) via increasing E-cadherin and decreasing vimentin expression. Transfection of MUC1 siRNA inhibited cell proliferation and EMT to the same extent as ALKBH2 gene silencing *in vitro*. ALKBH2 knockdown significantly suppressed MUC1 expression and tumor volume of bladder cancers *in vivo* as assessed in an orthotopic mouse model using ALKBH2 shRNA transfected KU7 cells. Immunohistochemical examination showed high expression levels of ALKBH2 in human urothelial carcinoma samples, especially in high-grade, superficially and deeply invasive carcinomas (pT(1) and >pT(2)), and in carcinoma *in situ* but not in normal urothelium. This study demonstrates that ALKBH2 is an upstream molecule of the oncoprotein, MUC1, and regulates cell cycle and EMT, resulting in progression of urothelial carcinomas. (*Cancer Sci* 2013; 104: 321–327)

Urinary bladder cancer is a common cancer of the urogenital system. In 2006, it was estimated that 16 510 new patients were found with bladder cancer, with 6804 projected deaths from the disease in Japan.[1] Urinary bladder cancer is characterized by frequent recurrence and progression that invades the muscularis propria. The role of alterations in molecular pathways that control normal cellular homeostasis in the oncogenesis of bladder cancer is well established.[2] The most common histological type of bladder cancer is urothelial carcinoma. Approximately 80% of patients with urothelial carcinoma present with nonmuscular invasive urothelial carcinoma (from noninvasive papillary to superficially invasive phenotype). Superficially-invasive non-muscular-invasive urothelial carcinoma is notorious for its tendency to invade the muscular layer when it is left untreated, but rarely progress to muscular invasive life-threatening cancer when patients undergo medical treatment. In contrast, muscular invasive urothelial carcinoma and high-grade carcinoma *in situ* exhibit aggressive behavior with a tendency to develop distal metastatic disease and seem highly resistant to immunotherapy as an initial treatment for bladder cancer as well as surgical intervention and chemoradiation therapy for advanced phenotypes. These two different types of cancer harbor different genetic and molecular alterations.[2,3] Low-grade noninvasive urothelial carcinomas exhibit constitutively activated fibroblast growth factor receptor 3 and receptor tyrosine kinase-RAS pathways. In contrast, tumors that demonstrate high-grade or invasive phenotype show structural and functional defects in the p53 and retinoblastoma (RB) proteins. Loss of heterozygosity of chromosome 9 is frequently found in both types of urothelial carcinomas.

In *Escherichia coli*, the AlkB protein is involved in the repair of methylation-induced DNA after damage by oxidative demethylation.[4,5] To date, eight mammalian homologues, human AlkB homologues 1–8, have been identified by bioinformatic analysis.[5,6] Only two of the corresponding human proteins, hABH2 and hABH3, have been shown to possess a similar repair activity as AlkB from *E. coli*.[7] Accumulating molecular evidence suggests that ABH2 is the primary dioxygenase to repair methyl and εADNA damage.[8] We previously found that ALKBH8 contributes to human urothelial carcinoma development via NADPH oxidase 1-dependent generation of reactive oxygen species.[9] Furthermore, it has been demonstrated that overexpression of ALKBH3 is involved in the genesis and progression of prostate cancer, non-small cell lung cancer, pancreatic cancer and urinary bladder cancer.[9–12] Thus, ALKBH family members may be involved in development of various types of human malignancies.

The membrane-bound oncoprotein MUC1 is expressed at the apical plasma membranes of normal secretory epithelial cells.[13] MUC1 is aberrantly overexpressed in diverse human malignancies and inhibits stress-induced apoptosis,[14–16] enhances invasion and metastasis, and promotes epithelial to mesenchymal transition (EMT) via the upregulation of the expressions of transcription factors Snail and Slug, together with downregulation of E-cadherin expression.[16]

In the present study, we found that ALKBH2 is overexpressed in bladder cancer and that MUC1 functions as an important downstream signal. In addition, our results demonstrate that ALKBH2/MUC1 signals strongly affect proliferation in urothelial carcinogenesis.

Materials and Methods

Cancer cell line and transfection. The human urothelial carcinoma cell line, KU7, was cultured in RPMI1640 medium supplemented with 10% FBS and 50 units/mL penicillin/streptomycin. KU7 is derived from papillary bladder cancer.

Preparation of total RNA and reverse transcription PCR. We extracted total RNA using the RNeasy Mini kit (Qiagen, Venlo, the Netherlands). Fast-strand cDNA was synthesized from 1 μg of total RNA using the PrimeScript RT Master Mix

[3]To whom correspondence should be addressed.
E-mail: nkonishi@naramed-u.ac.jp

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(Perfect Real Time, TaKaRa, Shiga, Japan) and SYBR Premix Ex Taq II (TliRNaseH Plus, TaKaRa). PCR conditions were 95°C for 30 s followed by 55–63°C for 30 s, for a total of 35–45 cycles.

siRNA transfection of MUC1 and ALKBH2. For transfections, 10^4 KU7 cells/well were seeded in a 6-well dish and transfected with 100-ng/L siRNA against either MUC1 or ALKBH2 for 48 h. Transfections were carried out using Lipofectamine RNAiMAX (Life Technologies, Foster city, CA, USA) in accordance with the manufacturer's protocol. The MUC1 and ALKBH2 siRNA sequences were designed after selection of appropriate DNA target sequences and are as follows: 5′-GCAGCCTCTCTTACACAAA-3′ for MUC1 and 5′-CGGACGTGTTGCAGTGATA-3′ for ALKBH2.

Preparation and transfection of shRNA plasmids expressing MUC1 or ALKBH2. Synthetic sense and antisense oligonucleotides (TaKaRa), which constitute the template for generating RNA composed of two identical sequence motifs in an inverted orientation, were separated by a 15-bp spacer (5′-TAGT GCTCCTGGTTG) to form a double-strand hairpin of siRNA, annealed to generate double-stranded DNAs, and ligated into the linearized empty vector pBAsi hU6 Pur DNA (TaKaRa). A plasmid designed to encode a hairpin RNA containing the GAPDH sequence (sequence: 5′-CGGGAAGCTTGTCATCAAT-3′) was used as the negative control. Plasmid DNA transfection into KU7 cells was performed using FuGENE HD Transfection Reagent (Promega, Madison, WI, USA). Briefly, a total of 5×10^3 cells were seeded into each well of a 24-well tissue culture plate (BD Falcon, San Jose, CA, USA). When the cells reached 40–50% confluency, they were transfected using 0.5 μg of DNA mixed with 1.5 μL of FuGENE HD in a 0.5-mL medium containing 10% serum and incubated for 48 h. The transfected cells were then replated onto a 60-mm dish and cultured in selective medium containing 10% serum and 1 mg/mL puromycin (Sigma, St. Louis, MI, USA) for at least 2 weeks until all the nontransfected KU7 cells cultured in parallel had died. Each colony of stable transfectants was then picked, and 20 stable clones were cultured in a maintenance medium containing 10% serum and 0.5 mg /mL puromycin. Real-time PCR was used to analyze the ALKBH2 and MUC1 mRNA expression level of stable clones. We used the most efficiently inhibited clone in our experiment (ALKBH2, clone No. 9; MUC1, clone No. 2).

Preparation of cell lysates and western blotting analysis. We resolved the cell lysates in SDS-polyacrylamide gels and transferred them onto polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA), which were then blocked in 5% skim milk at room temperature for 1 h. The membranes were incubated with the indicated primary antibody for 1 h, and then incubated with horseradish peroxidase–conjugated antimouse or antigoat IgG (Amersham Pharmacia Biotech, Buckinghamshire, UK). We detected peroxidase activity on X-ray films using an enhanced chemiluminescence detection system.

Wound-healing assay. KU7 cells were plated in 6-well dishes at a density of 3×10^5 cells per well and grown overnight before transfection with 100-ng/L siRNA against either MUC1 or ALKBH2. Cells that had received no treatment and the siRNA-transfected cells were scratched using a 200-mL pipette tip. The wound was imaged at time 0. The cells were incubated, and the same areas were again imaged at 48 h. The percentage of wound closure was calculated by subtracting the 0-h wound size from the 24-h wound size.

Cell proliferation assay. We performed a cell proliferation assay using a methane thiosulfonate (MTS) reagent as previously described.[17] All the experiments were performed in triplicate.

Tissue samples and immunohistochemistry. We obtained cancer specimens from patients with various grades of urothelial carcinoma (pTa $n = 21$, pT1 $n = 23$, pT2 $n = 17$ and pTis $n = 19$) who were undergoing transurethral resection or radical cystectomy, without previous radiation or chemotherapy, at Nara Medical University Hospital. Normal urothelial tissue samples were obtained from autopsy cases ($n = 17$). Informed consent was obtained from all patients before the collection of specimens, and tumor stage and grade were noted at the time of diagnosis by two independent urological pathologists (KS and NK). We performed immunohistochemical analysis for ALKBH2 and MUC1, as previously described.[9,18] The sections were incubated with the appropriate primary antibodies (anti-MUC1 mAb: clone Ma695 (Novocastra, Newcastle Upon Tyne, UK); and anti-ALKBH2 [V-13]: goat polyclonal antibody [Santa Cruz Bio, Bath, UK]) diluted 1:100, for 16 h at 4°C. Reactions were visualized using a Histofine SAB-PO kit (Nichirei, Tokyo, Japan), with diaminobenzidine as the chromogen and light hematoxylin counterstaining. The number of ALKBH2-positive cells per 100 cells was designated as the percentage of positive cells with at least 1000 cells examined.

Cancer instillation into the mouse bladder. The animal experiments were approved by the institutional animal care and use committee at Nara Medical University. Eight-week-old female nude mice (BALB/c) were maintained on a daily 12-h light-dark cycle and fed the standard diet and water. Bladder cancer was established by inoculating KU7 cells (5×10^6 cells) or KU7/pBAsi-ALKBH2 cells (5×10^6 cells) into the mouse bladder using a transurethral catheter (five mice were injected with control KU7/pBAsi-control cells and another five mice KU7/pBAsi-ALKBH2 cells).[9] The mice were killed 2 weeks after the inoculations, and the urinary bladders were excised and fixed in 10% formalin for histological examination and immunohistochemistry.

Statistical analysis. Differences in the measure of continuous variables were analyzed using ANOVA or nonparametric tests (Mann–Whitney and Kruskal–Wallis tests). All the experiment results were analyzed using one-way ANOVA and Tukey's *post-hoc* test. A two-tail Student *t*-test was used to compare two data points. Results were considered significant if $P < 0.05$.

Results

ALKBH2 gene silencing induces an increase in E-cadherin with a concurrent decrease in vimentin. To clarify the effect of ALKBH2 on EMT-related molecules in urothelial carcinoma cells, we first determined the efficiency of a commercially validated siRNA to knock down the expression of ALKBH2 in our selected cell line, the urothelial carcinoma cell line KU7. We examined the mRNA expression of ALKBH2 and MUC-1 of some urothelial carcinoma cell lines: KU7, T24, UMUC2 and J82. KU7 was the most highly expressed ALKBH2 and MUC-1, so we used KU7 cell line in *in vitro* and *in vivo* experiments (Fig. 1A). Compared with the control cells (cells transfected with control RNA), ALKBH2 mRNA and protein expression levels were significantly reduced in cells transfected with ALKBH2 siRNA (Fig. 1B,E). To confirm the involvement of ALKBH2 in tumor progression and invasion, KU7 cells with stable knockdown of ALKBH2 were created by transfecting an ALKBH2 short-hairpin RNA (shRNA)-expressing pBAsi expression vector driven by a human U6 promoter (KU7 /pBAsi-ALKBH2). As shown in Figure 1(B), ALKBH2 mRNA levels were reduced by 60–70% in KU7/pBAsi-ALKBH2 cells relative to control cells. Figure 1(C) and d demonstrate that the E-cadherin mRNA expression level was increased, whereas the vimentin mRNA expression level tended to decrease in the KU7/pBAsi-ALKBH2 cells compared with the control cells. These results reveal that ALKBH2 might contribute to EMT in urothelial carcinoma cells.

ALKBH2 regulates MUC1 expression. The role of MUC-mediated and MUC1-mediated intracellular signaling in promoting EMT has been previously reported, with modifications in Slug,

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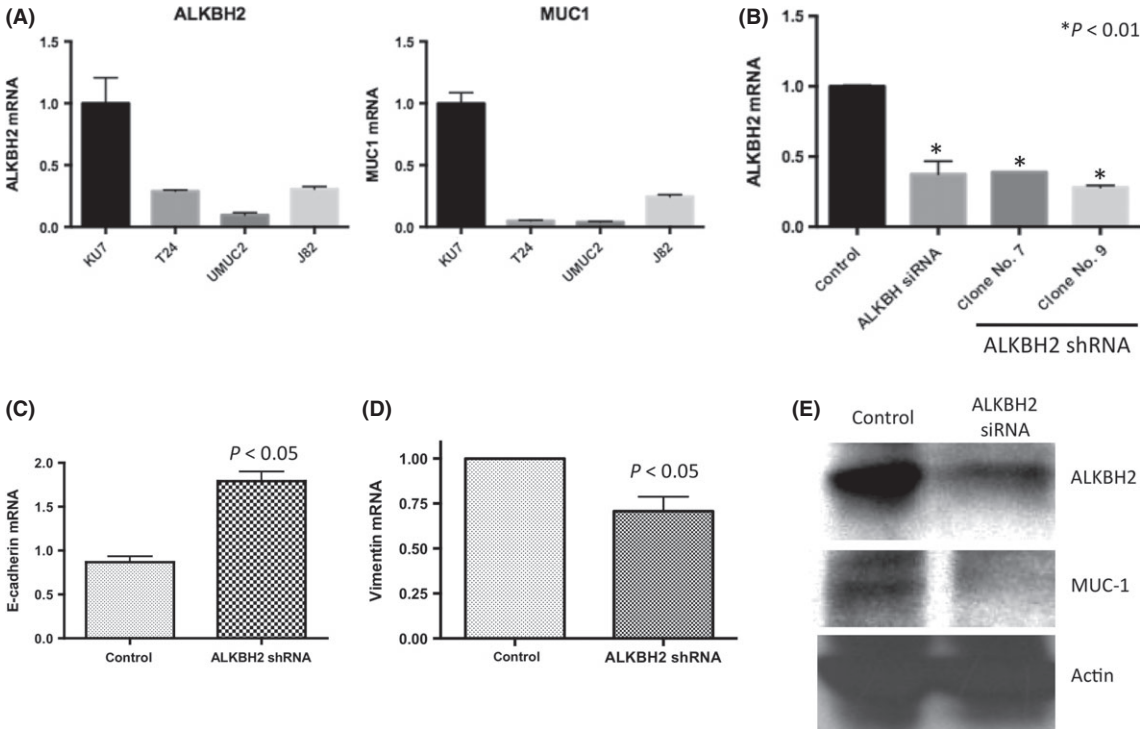

Fig. 1. ALKBH2 silenced KU7 cells are associated with epithelial to mesenchymal transition EMT. (A) ALKBH2 and MUC1 mRNA expression in utothelial carcinoma cell lines. (B) ALKBH2 mRNA expression of KU7 control cells, transiently transfected ALKBH2 siRNA and stable ALKBH2 knockdown. KU7 cells with stable ALKBH2 knockdown were created using a pBAsi expression vector with the human U6 promoter expressing ALKBH2 shRNA. ALKBH2 mRNA expression levels in the KU7 cells with transient and stable ALKBH2 knockdown were reduced by 60–70% compared with the control cells. The columns indicate mean ± SEM. (C, D) E-cadherin mRNA expression was significantly enhanced in the KU7/pBAsi-ALKBH2 cells. Decreased vimentin mRNA expression level was found in the KU7/pBAsi-ALKBH2 cells compared with the nontransfected cells, significantly. The columns indicate the mean ± SEM. (E) KU7 cells were transiently transfected with ALKBH2 siRNA. In the ALKBH2 silenced siRNA transfected cells, the ALKBH2 and MUC1 protein levels were significantly reduced compared with the control cells.

Snail, vimentin and E-cadherin playing a key role.[16,19–21] That is, previous reports suggest that the interaction between MUC1 and β-catenin lead to the repression of E-cadherin expression and activation of the EMT-associated transcription factors Snail and Slug. Furthermore, MUC1 signaling leads to the initiation of EMT. We examined whether ALKBH2 expression is linked to the expression of MUC1-related and EMT-related molecules in human urothelial carcinoma cells. We determined the efficiency of a commercially validated-siRNA to knock down the expression of MUC1 in KU7. Transient and stable knockdown of MUC1 in the KU7 cells reduced the MUC1 mRNA expression levels by more than 90% compared with the nontransfected cells (Fig. 2B). Interestingly, ALKBH2 gene silencing decreased the mRNA and protein expression levels of MUC1, as shown in Figures 1(E) and 2(A). In addition, several gene expressions that are associated with epithelial phenotypes, such as E-cadherin, desmoplakin and claudin-1, were increased in ALKBH2 and MUC1 siRNA-transfected cells (Fig. 2C). Conversely, the mRNA expression of genes associated with mesenchymal phenotype, such as vimentin and Snail, were decreased in ALKBH2 and MUC1 silenced cells (Fig. 2D). Furthermore, the KU7/pBAsi-MUC1 cells demonstrated significantly increased E-cadherin mRNA expression levels but decreased vimentin mRNA expression levels (Fig. 2E,F). We next performed a scratch assay to examine cell motility and the proliferation capacity of theKU7 cells *in vitro*. Control KU7 cells plated at approximately 80% confluency can close a constant diameter wound by approximately 83% in 48 h. The motility and proliferation was significantly reduced in MUC1 and ALKBH2 silenced cells (Fig. 2G). To confirm that ALKBH2 was involved in EMT, we used a Matrigel invasion assay. The number of cancer cells that pass through the matrigel was significantly decreased by ALKBH2 gene silencing in KU7 cells (data not shown). These results suggest that ALKBH2 is involved in EMT in urothelial carcinoma cells through the regulation of MUC1.

Suppression of MUC1 expression inhibited KU7 cell proliferation. As demonstrated in Figure 3(A) and (B), ALKBH2 and MUC1 gene silencing induced cell cycle arrest at the G1 phase, resulting in cell growth inhibition. Reduction of MUC1 and ALKBH2 inhibited cell proliferation, as was quantitatively assessed by the MTS assay (Fig. 3C). Our results reveal that the ALKBH2-MUC1 pathway positively regulates the proliferation of urothelial carcinoma cells through the G1 phase.

ALKBH2 knockdown suppressed tumor volume *in vivo*. To assess the effects of ALKBH2 knockdown on tumor growth, KU7/pBAsi-ALKBH2 cells were inoculated into the urinary bladder of nude mice using a transurethral catheter. We excised bladder tumors 14 days after instillation and measured the tumor volume. As demonstrated in Figure 4(A–C), the control mice showed large tumor volumes with papillary-proliferated cancer cells. In contrast, the total tumor volume was decreased as a result of ALKBH2 gene silencing *in vivo*. The immunohistochemical analyses of the tumor specimens revealed that the MUC1 and ALKBH2 protein expression levels were significantly decreased in the mice that received KU7/pBAsi-ALKBH2 cells. Taken together, these results suggest that ALKBH2 contributes to tumor growth in urothelial carcinoma cells via MUC1 signaling.

ALKBH2 and MUC1 were overexpressed in human urothelial carcinoma cells. Immunohistochemical analysis showed that the percentages for immunopositive cancer cells for ALKBH2 and

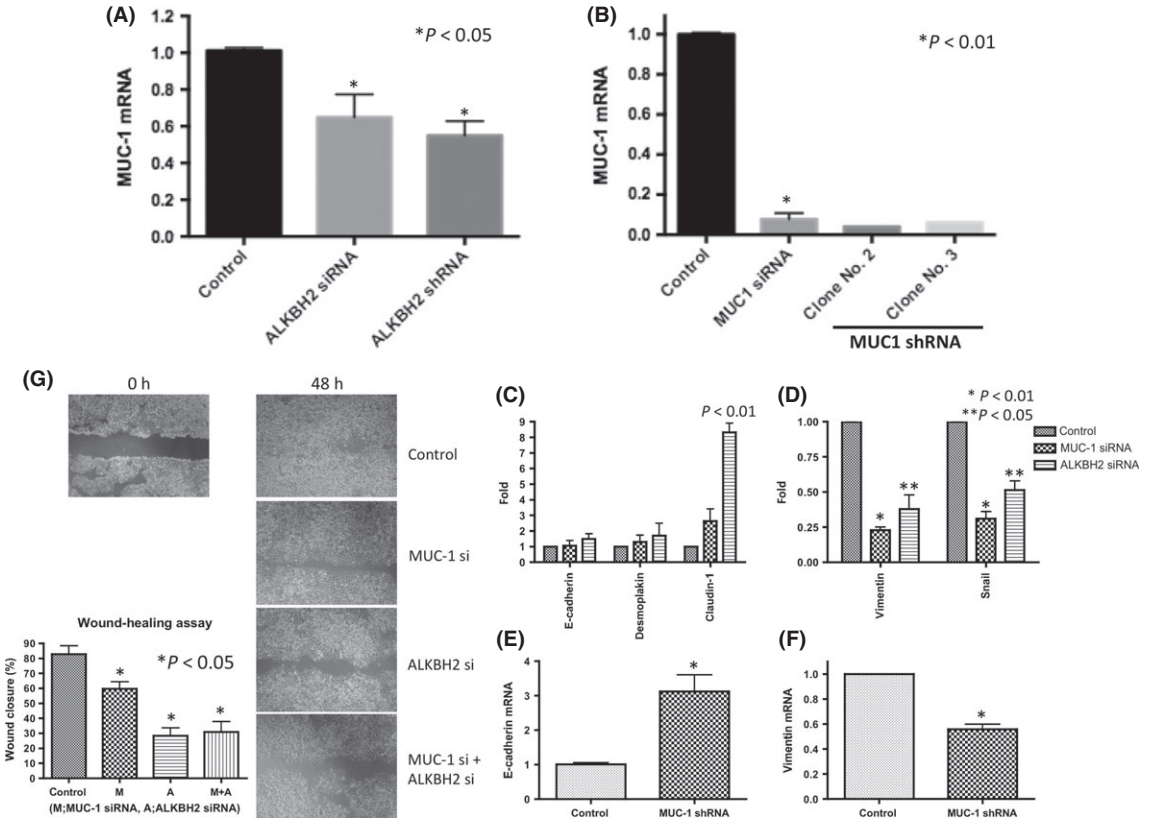

Fig. 2. ALKBH2 and MUC1 knockdown in the KU7 cells is associated with the epithelial to mesenchymal transition mechanism. (A) Transient transfection of ALKBH2 siRNA in the KU7 cells and stable ALKBH2 knockdown cells reduced the MUC1 mRNA expression levels significantly. The columns indicate mean ± SEM. (B) In the KU7 cells with stable MUC1 knockdown and transiently transfected MUC1 siRNA, the MUC1 mRNA expression levels were reduced by more than 90% compared with control cells. (C, D) RT-PCR analysis of the KU7 control cell, MUC1 knockdown cells and ALKBH2 knockdown siRNA cells. (C) The mRNA expression levels of genes generally associated with epithelial phenotypes, but E-cadherin and desmoplakin were not statistically significantly different. (D) The mRNA expression levels of genes generally associated with mesenchymal phenotype. (E, F) The E-cadherin mRNA expression was significantly enhanced, and the vimentin mRNA expression level was decreased in the KU7/pBAsi-MUC1 cells compared with the nontransfected cells. The columns indicate the mean ± SEM. *$P < 0.01$. (G) Representative images and the graphical display of wound closure (%) from the wound-healing assay using the KU7 control, MUC1 knockdown, ALKBH2 knockdown, and cells with simultaneous MUC1 siRNA and ALKBH2 knockdown.

MUC1 are tightly associated with both tumor grade and stage. As shown in Figure 5(A), a higher percentage of ALKBH2-positive cells were found in high-grade/invasive urothelial carcinomas and noninvasive/high-grade carcinoma *in situ* (CIS) than that measured in low-grade urothelial carcinoma (low grade 39.95, SE 3.01%; high grade 69.34, SE 2.12%). The normal urothelial epithelium is mostly negative for ALKBH2.

The expression pattern of MUC1 was similar to that of ALKBH2 in that MUC1 was highly expressed in high-grade and invasive cancer compared with the normal urothelium (Fig. 5B). It is interesting that MUC1 was predominantly expressed in the apical membrane of the cells of the surface layer of low-grade/noninvasive urothelial carcinomas, whereas it was expressed in the basolateral membrane and apical side of the whole cancer foci in high-grade/invasive cancer.

Discussion

We demonstrate for the first time that the human alkB homologue, ALKBH2, contributes to urothelial carcinoma development by regulating MUC1-dependent EMT and cell growth. Reduction in ALKBH2 levels dramatically upregulated the E-cadherin expression while downregulating vimentin expression (a reversal of what is observed at the EMT) and induced cell cycle arrest at G1 phase. MUC1, a transmembrane mucin glycoprotein, is a key modulator of several signaling pathways that affect oncogenesis, motility and metastasis.[22–25] In addition, its expression is closely associated with poor prognosis in patients with several types of malignancies.[22,26,27] Recent investigations have found that MUC1 regulates growth factors such as transforming growth factor and platelet-derived growth factor, both of which are well documented to accelerate cell cycle progression, the cadherin switch, stress kinase-mediated snail induction and extracellular matrix remodeling in cancer cells.[16,28,29] MUC1 is a transmembrane mucin expressed at the apical surface and affects cell polarity by cooperation with β-catenin at the apical–lateral membrane and epidermal growth factor receptor at the basolateral membrane.[30] Therefore, upregulation of MUC1 can lead to loss of E-cadherin/β-catenin complexes at the adherens junctions, and nuclear shuffling of β-catenin/MUC1-CT (cytoplasmic tail) complexes, resulting ultimately in the upregulation and nuclear localization of the Wnt nuclear effector, Lef-1.[30,31] The importance of signaling pathways in urinary bladder cancer development has yet to be clarified, but they may target ALKBH2-mediated cell growth and EMT.

The present study provides evidence that ALKBH2 tightly controls MUC1-dependent cell proliferation and EMT in urothelial carcinoma cells. The direct regulation of EMT by a DNA repair molecule seems biologically reasonable in cancer

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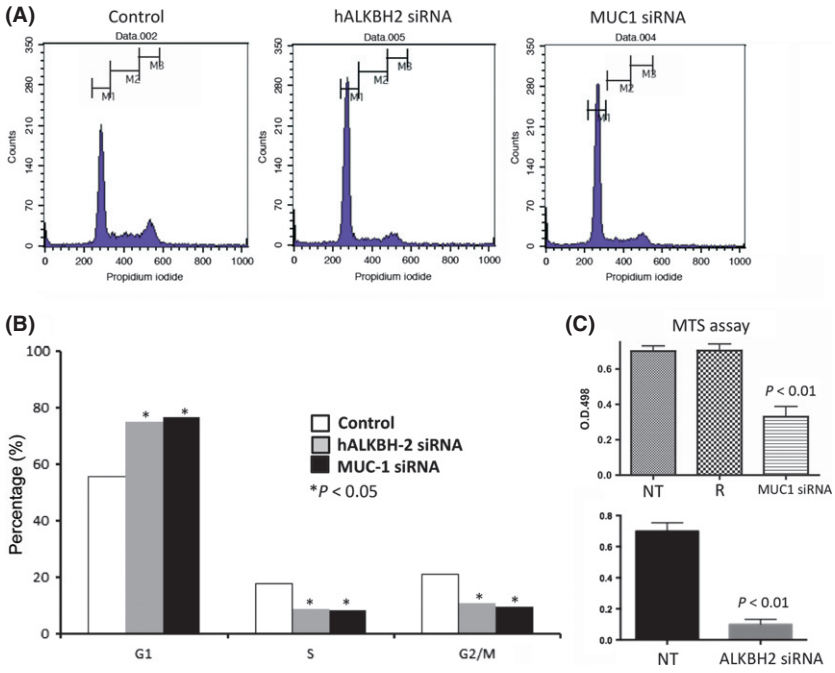

Fig. 3. Cell cycle arrest was induced in KU7 cells with either ALKBH2 or MUC1 knockdown. (A, B) The cell cycle analysis performed by flow cytometry using propidium iodide, as described in the Materials and Methods section. (C) Reduction of MUC1 and ALKBH2 inhibited cell proliferation as was quantitatively assessed by the MTS assay. The columns indicate the mean ± SEM. NT, no treatement; R, reagent only.

cells. Growing data demonstrates that cells with an EMT phenotype contain an abundant source of cancer stem cells that harbor cytotoxic damage-induced repair signals, suggesting a biological link between EMT and the DNA repair system: EMT will provide the most appropriate microenvironment for DNA repair molecules to perform their functions.

To date, the biological function of MUC1 on tumor progression has been well studied, particularly in ductal adenocarcinoma of the pancreas. Such studies have led to increasing recognition of MUC1 as a potential diagnostic marker and therapeutic target in pancreatic carcinomas.[16] However, immune neutralization against MUC1 alone is not sufficient for clinical application. The present results suggest a more successful therapeutic strategy by targeting MUC1 and its upstream molecule, ALKBH2, in urinary bladder cancer.

It is of great interest that ALKBH2 is overexpressed not only in invasive but also in noninvasive papillary urothelial carcinoma cells and that the expression is much higher in invasive phenotypes. Immunohistochemical analysis of MUC1 indicated the similar correlation with tumor grade in agreement with *in vitro/in vivo* data (data not shown). No histological evidence of EMT was found, including loss of E-cadherin or increased expression of vimentin in non-invasive urothelial carcinoma cells. We postulate that when the expression of MUC1, tightly parallel to that of ALKBH2, increases above a certain level, EMT is activated, resulting in

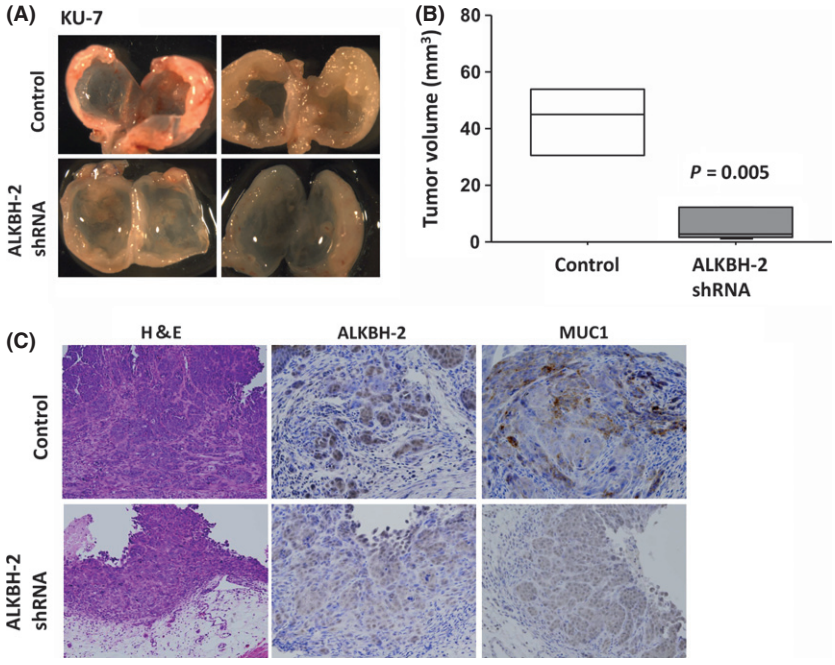

Fig. 4. Intravesical injection of KU7/pBAsi-ALKBH2 inhibits tumor growth *in vivo* in a mouse orthotopic bladder cancer implant model. (A) Macroscopic examination of the urinary bladder dissected 14 days after instillation of the KU7 cells transfected with either the control RNA or ALKBH2 shRNA, as described in the Materials and Methods section. After the excision of the urinary bladder, the tumor volume was measured (B) and the specimen was stained by H&E, together with immunohistochemical analysis of ALKBH2 or MUC1.

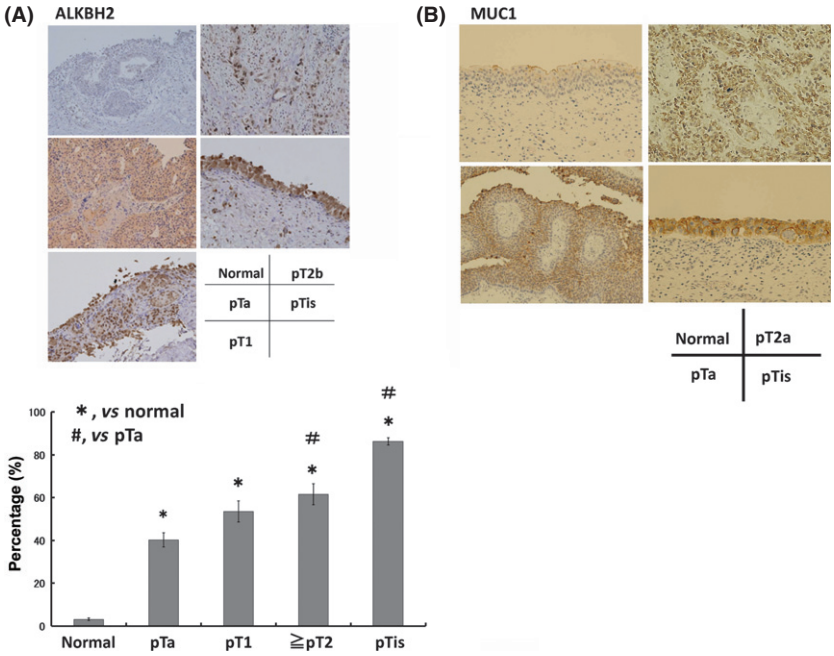

Fig. 5. The results of immunohistochemical analyses of ALKBH2 and MUC1 in human urothelial carcinomas of the urinary bladder indicate a correlation with grade. Immunohistochemistry for expression of ALKBH2 (A) and MUC1 (B) was performed on representative samples from different grades of human urothelial carcinoma. The percentage of immunopositive cells was calculated per 1000 cells per high-power field (A, lower panel). There appears to be a positive correlation between the percentages of ALKBH2 immunopositive cells and tumor grade. The columns indicate mean ± SEM.

acquisition of an invasive phenotype. Moreover, low-grade, noninvasive urothelial carcinomas predominantly showed apical and superficial MUC1 expression, whereas basal cells with cytoplasmic and/or circumferential membrane positivity were frequently observed in high-grade invasive phenotypes. ALKBH2 might affect the localization of MUC1 at the apical membrane, basolateral membrane or cytoplasm, thereby modulating the E-cadherin–β-catenin interaction, resulting in the gain of EMT and advanced phenotype in urothelial carcinoma cells. This issue should be further evaluated to address whether any other unidentified molecules affect MUC1-dependent EMT and cell cycle progression after ALKBH2 activation.

It is well known that CIS frequently progresses to invasive urothelial carcinoma; however, the key mechanisms directing this process remain unclear. The present study showed that ALKBH2 was overexpressed in CIS; therefore, the positive regulation of MUC1-dependent enhancement of EMT by ALKBH2 provides a molecular mechanism to explain why CIS cancer cells are more likely to progress to invasion.

In summary, a member of the AlkB family, ALKBH2 plays an important role in cell survival and cancer development by regulating MUC1-dependent signaling in human urothelial carcinoma. Amplification of ALKBH2/MUC1 expression is closely associated with transformation from a noninvasive to an invasive phenotype. Molecular therapy focusing on ALKBH2 may represent an attractive tool to achieve successful therapy in the treatment of human bladder carcinoma.

Disclosure Statement

The authors have no conflict of interest to declare.

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