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# The epigenetic factor KDM2B regulates cell adhesion, small rho GTPases, actin cytoskeleton and migration in prostate cancer cells

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ABSTRACT

The histone demethylase KDM2B is an epigenetic factor with oncogenic properties that is regulated by the basic fibroblasts growth factor (FGF-2). It has recently been shown that KDM2B *co*-operates with Polycomb Group proteins to promote cell migration and angiogenesis in tumors. In the present study we addressed the role of KDM2B in regulating actin cytoskeleton signaling, cell-cell adhesion and migration of prostate tumor cells. We report here that KDM2B is functionally expressed in DU-145 prostate cancer cells, activated by FGF-2 and regulates EZH2. KDM2B knockdown induced potent up-regulation of gene transcription and protein expression of the epithelial markers E-cadherin and ZO-1, while KDM2B overexpression down-regulated the levels of both markers, suggesting control of cell adhesion by KDM2B. RhoA and RhoB protein expression and activity were diminished upon KDM2B-knockdown and upregulated in KDM2B-overexpressing cell clones. In accordance, actin reorganization with formation of stress fibers became evident in KDM2B-overexpressing cells and abolished in the presence of the Rho inhibitor C3 transferase. DU-145 cell migration was significantly enhanced in KDM2B overexpressing cells and abolished in C3-pretreated cells. Conversely, the retardation of cell migration observed in KDM2B knockdown cells was enhanced in C3-pretreated cells. These results establish a clear functional link between the epigenetic factor KDM2B and the regulation of cell adhesion and Rho-GTPases signaling that controls actin reorganization and cell migration.

## 1. Introduction

The histone demethylase KDM2B is an epigenetic factor that was isolated in a screen for novel oncogenes by using retroviral insertion mutagenesis in rodents [1–4]. This gene codes for a lysine-specific protein that demethylates H3K4me3 and H3K36me2 leading to transcriptional repression [ 5–7]. The function of KDM2B depends on its CxxC-ZF domain, which mediates its genome-wide binding to unmethylated CpG islands [7–11]. Ectopic expression of KDM2B enables MEFs to undergo immortalization and bypasses replicative senescence, by *co*-operating with Polycomb Repressor Complex-2 (PRC-2) [7]. PRC-2-complexes include EZH2, which is over expressed in solid tumors [ 1,7]. In human cancers KDM2B is regulated by the basic fibroblast growth factor (bFGF) that promotes tumor cell proliferation, migration, and invasiveness, as well as angiogenesis and the cycling of cancer stem cells [ 10,12]. In addition, regulation of FGF transcription involves actin cytoskeleton reorganization [13]. Previous studies established an important role of KDM2B in cell migration and angiogenesis. Indeed, KDM2B is essential for FGF-2-induced angiogenesis, as its knockdown abrogates in vitro tube formation in HUVECs [12]. These findings suggested that growth factor signals may drive the upregulation of KDM2B, which, in *co*-operation with Polycomb Group proteins, promotes motility and invasion in tumor cell lines and primary tumors.

Several lines of evidence suggest that epigenetic mechanisms may regulate actin cytoskeleton [ 14,15], which plays a central role in conferring cancer cells with migratory and invasive characteristics [ 16–22]. In this process, epithelial cells lose their polarity and cell-cell adhesion, and gain migratory and invasive properties, in a process termed epithelial-mesenchymal transition (EMT). EMT involves a dramatic reorganization of the cytoskeleton accompanied by the formation of membrane protrusions required for invasive growth [ 23–27]. Alterations in chromatin regulators have been recently linked to deregulated cytoskeletal functions [12,28] however, the detailed mechanisms behind these processes are only beginning to emerge. In the

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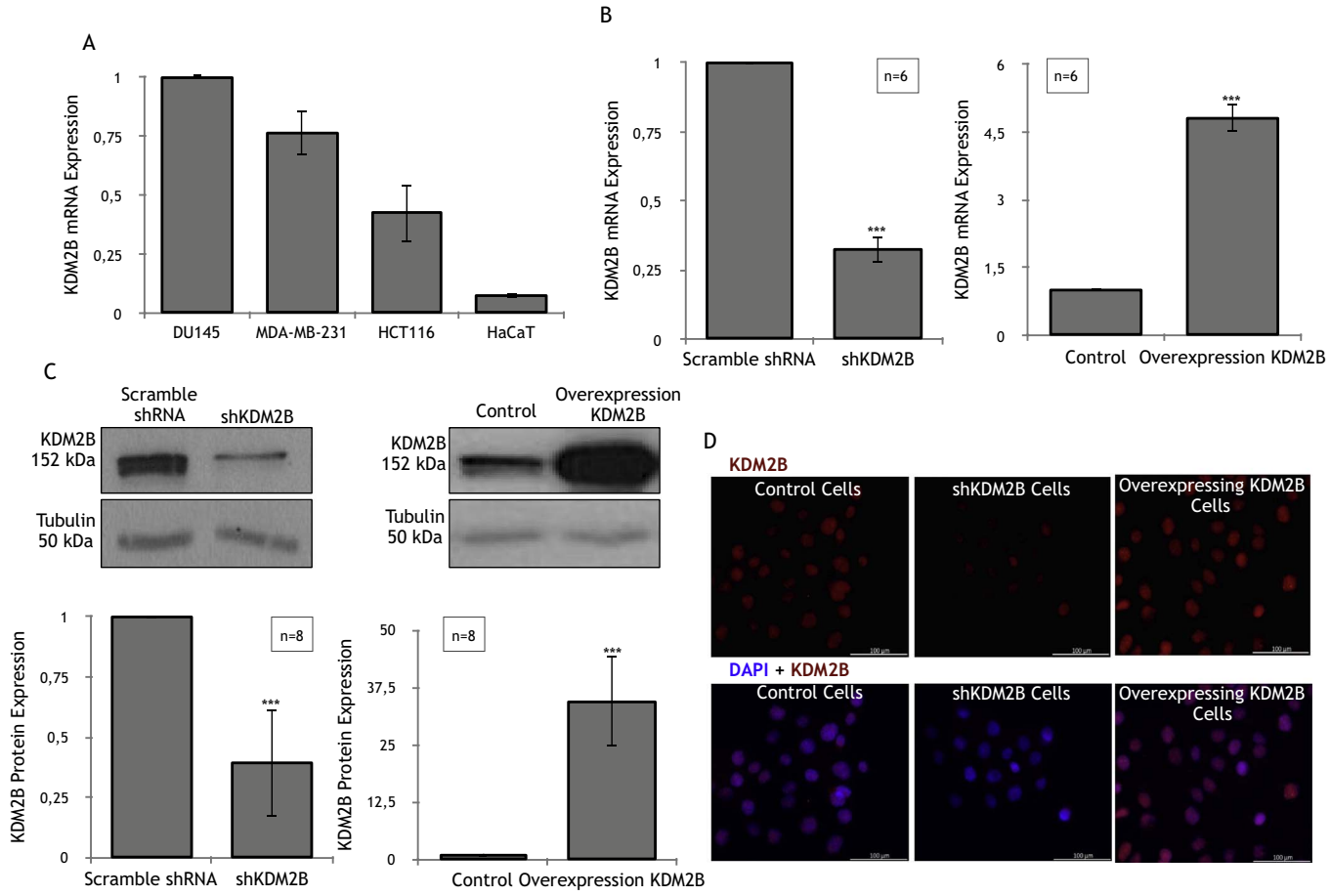


**Figure 1.** Expression of KDM2B in tumor cells.

(A) Transcriptional expression of KDM2B in various tumor cells. RT-PCR analysis established differential basic KDM2B expression levels in various tumor cells.

(B) Transcriptional expression of KDM2B in DU-145 cells upon knockdown and overexpression of the gene. KDM2B mRNA levels were measured by quantitative real time RT-PCR, with b-actin as the internal control in scramble shRNA, shKDM2B (left panel), and control overexpression, overexpression of KDM2B (right panel) DU-145 cell lysates. The data are presented as mean ± standard deviation (SD) from $n = 6$ independent experiments, while y axis represents the ratio between KDM2B and actin genes; $^{***}p < 0.001$ indicates statistical significance.

(C) Protein expression of KDM2B in DU-145 cells upon knockdown and overexpression of the gene. Original Western blot and quantification of the expression of KDM2B and tubulin (control) protein in scramble shRNA, shKDM2B (left panel) and control overexpression, overexpression of KDM2B (right panel) DU-145 cell lysates. Graph shows mean expression of KDM2B relative to control from $n = 8$ independent experiments; $^{***}p < 0.001$ indicates statistical significance.

(D) Immunofluorescence of KDM2B (red) staining and nuclei (blue) in control (left), shKDM2B (middle) and overexpression of KDM2B (right) DU-145 cells. Scale bar represents 100 μm.

present study we addressed whether epigenetic alterations result in the deregulation of the normal cytoskeleton organization and function that in turn promotes motility and invasiveness and we studied the underlying mechanism. To this end, we focused on KDM2B and analyzed its role in regulating Rho small GTPases, cell adhesion and migration potential in prostate cancer cells. Our findings provide strong evidence that in tumor cells, epigenetic mechanisms control the expression and function of cytoskeletal genes, invasiveness and migration.

## 2. Material and methods

RPMI 1640 medium, penicillin/streptomycin for cell culture, Trypsin, puromycin, SuperScript II Reverse Transcriptase, Trizol reagent for RNA extraction, dNTPs, ProLong Gold Antifade reagent with Dapi, Rhodamin-Phalloidin, Anti-Zo1 (339100) and primers rt-PCR for *E*-Cadherin, RhoA, RhoB and Zo-1 were purchased from Invitrogen/Life Technologies (Carlsbad, CA, USA). Fetal Bovine Serum (FBS) was purchased from Bioline (Bioline Reagents Limited, London, UK). Primers rt-PCR for KDM2B was purchased from Eurofins MWG Operon (Ebersberg, Germany). FITC-IgG antibody were from Molecular probes Inc. (Eugene OR USA). Fibroblast growth factor 2 (FGF-2), Anti-Ezh2 (D2C9), anti-Bmi1 (D20B7), anti-pFAK (#3283) and anti-AKT (#9272) antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Anti-RhoA (sc-418), anti-RhoB (sc-480), FAK (sc-557) and PI-3 K (sc-423) were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). KAPA SYBR Green/ROX qPCR Master Mix was purchased from Fermentas (Maryland, USA). Anti-KDM2B antibody, Goat anti-rabbit IgG Peroxidase Conjugated, Goat anti-mouse IgG Peroxidase Conjugated and ECL Immobilon western-Chemiluminescent HRP Substrate were purchased from Millipore Corporation (Billerica, MA, USA). Dc Protein Assay was purchased from BioRad Laboratories (California, USA). Albumin Fraction V (BSA) was purchased from PanReac Applichem, ITW Reagents (Gatersleben, Germany). All other chemicals were obtained from usual commercial sources at the purest grade available.

### 2.1. Cell lines and culture conditions

The cell lines DU-145 and HCT116 were cultured in RPMI 1640, supplemented with 1% penicillin/streptomycin and 10% heat-inactivated fetal bovine serum in a humidified atmosphere containing 5% $CO_2$ at 37 °C.

### 2.2. Lentiviral and retroviral packaging and transduction

HEK293T cells were transiently co-transfected with retroviral