Research article

Loss of MEG3 and upregulation of miR-145 play an important role in the invasion and migration of Cr(VI)-transformed cells

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ABSTRACT

Chronic exposure of human bronchial epithelial BEAS-2B cells to hexavalent chromium (Cr(VI)) causes malignant cell transformation. These transformed cells exhibit increases in migration and invasion. Neuronal precursor of developmentally downregulated protein 9 (NEDD9) is upregulated in Cr(VI)-transformed cells compared to that of passage-matched normal BEAS-2B cells. Knockdown of NEDD9 by its shRNA reduced invasion and migration of Cr(VI)-transformed cells. Maternally expressed gene 3 (MEG3), a long noncoding RNA, was lost and microRNA 145 (miR-145) was upregulated in Cr(VI)-transformed cells. MEG3 was bound to miR-145 and this binding reduced its activity. Overexpression of MEG3 or inhibition of miR-145 decreased invasion and migration of Cr(VI)-transformed cells. Overexpression of MEG3 was able to decrease miR-145 level and NEDD9 protein level in Cr(VI)-transformed cells. Ectopic expression of MEG3 was also shown to reduce β-catenin activation. Inhibition of miR-145 in Cr(VI)-transformed cells decreased Slug, an important transcription factor that regulates epithelial-to-mesenchymal transition (EMT). Inhibition of miR-145 was found to increase MEG3 in Cr(VI)-transformed cells. Further studies showed that mutation of MEG3 at the binding site for miR-145 did not change NEDD9 and failed to decrease invasion and migration. The present study demonstrated that loss of MEG3 and upregulation of miR-145 elevated NEDD9, resulting in activation of β-catenin and further upregulation of EMT, leading to increased invasion and migration of Cr(VI)-transformed cells.

1. Introduction

Hexavalent chromium (Cr(VI))-containing compounds are carcinogenic [1,2,3,4,5,6,7,8,9,10,11]. Environmental exposure to Cr(VI) is associated with lung carcinogenesis [10]. The International Agency for Research on Cancer (IARC) classifies Cr(VI) compounds as an established human carcinogen [11]. It has been reported that chronic exposure of normal cells to Cr(VI) at low doses can cause malignant cell transformation [12]. These transformed cells exhibit phenotypes of cancer cells, such as resistance to apoptosis [13,14], rapid cell proliferation/growth [15], and tumor growth in vivo [13,14].

Neural precursor cell expressed developmentally down-regulated protein 9 (NEDD9), the CRK-associated substrate (CAS) family of adaptor proteins [16,17], is a multidomain scaffolding protein. NEDD9 upregulation has been linked to the progression of various cancers including lung [18], breast [19], melanoma [20], liver [21], pancreatic [22], prostate [23], and colon [24]. Cancer cells acquire epithelial-mesenchymal transition (EMT), resulting in the acquisition of metastatic ability [25]. During an EMT, epithelial cells express the

mesenchymal markers and increase motility and invasiveness [26]. It has been reported that NEDD9 promotes EMT [27] and mesenchymal protease-dependent migration [28].

Maternally expressed gene 3 (MEG3), a noncoding lncRNA, is expressed in normal tissues but is downregulated or lost in various human tumor tissues. MEG3 is associated with cancer initiation, progression, and metastasis [29]. Studies have shown that MEG3 regulates p53, retinoblastoma protein (RB), Myc, and transforming growth factor (TGF-β), leading to inhibition of proliferation and invasion of cancer cells [30, 31, 32]. MEG3 gene expression is turned off by DNA methyltransferases (DNMTs) which methylates its promoter. It has been reported that MEG3 regulates cell proliferation, apoptosis, and angiogenesis by sequestering miRNAs [33]. In turn, these miRNAs suppress the translation of mRNAs.

lncRNA, a competing endogenous RNA (ceRNA), is associated with the initiation and progression of various diseases, including cancers [34, 35]. MEG3 acts as a ceRNA of the PH domain and leucine-rich repeat protein phosphatase 2 (PHLPP2) in competing with miR-27a, thus promoting PHLPP2 protein translation, resulting in inhibition of invasion of

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bladder cancer cells and lung metastasis [36]. The mechanism of invasion or migration of Cr(VI)-transformed cells remains largely unknown. The present study investigated the mechanisms of NEDD9 as a key mediator linking MEG3/miR-145 signaling in the invasion and migration of Cr(VI)-transformed cells.

2. Materials and methods

2.1. Reagents

Sodium dichromate dihydrate ($Na_2Cr_2O_7$) was from Sigma (St Louis, MO, USA). Dulbecco's Modified Eagle Medium (DMEM) and F12/K were purchased from Fisher Sci (Waltham, MA). Transfection reagent PolyJet was purchased from SignaGen Laboratories (Rockville, MD). Primary antibodies against NEDD9, Slug, non-p-β-catenin, and β-catenin, and secondary antibodies against mouse and rabbit were purchased from Cell Signaling Inc (Beverly, MA, USA). GAPDH antibody was purchased from Gentex Corporation (Irvine, CA, USA). SuperScript first-strand synthesis kit was purchased from Invitrogen (Waltham, MA, USA). miRScript PCR kit was purchased from Qiagen (Hilden, Germany). PowerUP SYBR green master mix was purchased from Applied Biosystems (Waltham, MA). A Dual-Luciferase Assay kit was purchased from Promega (WI, USA). ECF substrate for Western blot was purchased from GE Healthcare (Pittsburgh, PA, USA).

2.2. Plasmids

The full-length human MEG3 sequences (NR_002766) were synthesized and subcloned into the pEGFP-C1 vector (Clontech, Palo Alto, CA, USA) as described previously [37]. The miR-145 binding site mutation of the MEG3 expressing vector (MEG3/miR-145 mt) was constructed using a site-directed mutagenesis kit (Agilent Tech, TX, USA) following manufactory instruction. Primers for the construct are followed. MEG3-F: GGCATGGACGAGCTGTACAAG, MEG3-R: GAGCGAGTCAGGAAGCAGT; MEG3 mt-S: TCACCTGCTAGCAAACTCCAGTGTTTCCCTCCCCAA, MEG3 mt-A: TTGGGGAGGGAAACACTGGAGTTTGCTAGCAGGTGA. MEG3 shRNA and its control vector were kindly provided by Dr. Shau-Ping Lin (Institute of Biotechnology, National Taiwan University, Taipei, Taiwan). NEDD9 shRNA plasmids and control vector were purchased from Addgene (Cambridge, MA, USA). miR-145 inhibitor plasmid and its control vector were purchased from GeneCopoeia (Rockville, MD). Human miR-145 promoter (from −1548 to −30) was cloned into the pGL3-basic luciferase reporter as previously reported [38].

2.3. Cell culture, stably expressing cells, and Cr(VI)-transformed cells

Both human bronchial epithelial cells (BEAS-2B) and human adenocarcinoma alveolar basal epithelial cells (A549) were from ATCC. BEAS-2B cells were cultured in DMEM medium with 10% FBS and A549 cells were cultured in F12/K medium with 10% FBS. The cells were split when reaching 90% confluent. For the establishment of stable expression cells, the cells were transfected with 2 μg plasmid DNA in each well of a six-well plate followed by antibiotics G418 or puromycin selection for at least one month. The verification of gene expression was carried out by immunoblotting or real-time qPCR analysis.

BEAS-2B cells were exposed to a low dose (0.1 μM) of $Na_2Cr_2O_7$ for 6 months. The cells were split twice a week and fresh culture media was added. After 6 months, a Soft Agar assay was performed. Single colonies from soft agar were isolated and expanded in tissue culture. These cells were considered Cr(VI)-transformed cells. Passage-matched BEAS-2B cells without Cr(VI) exposure were used as control.
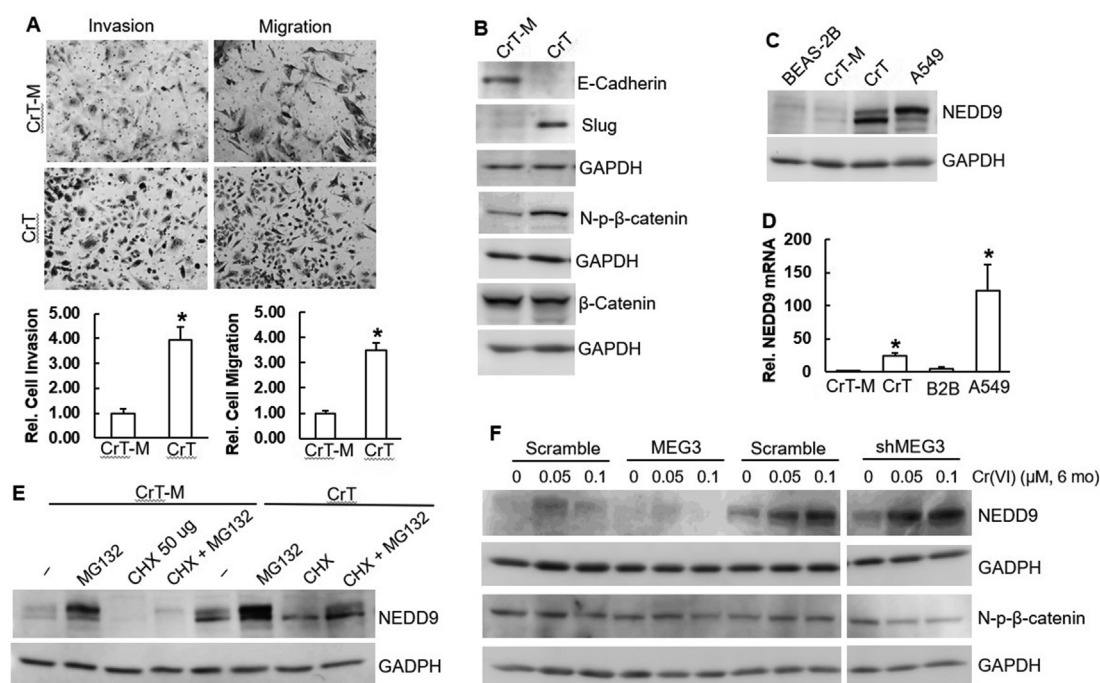

Figure 1. Increased invasion and migration and upregulation of NEDD9 in Cr(VI)-transformed cells. Normal human bronchial epithelial (BEAS-2B) cells were chronically exposed to a low dose of Cr(VI) for 6 months. A cell transformation (Soft agar) assay was performed. Single colonies from the cell transformation assay were isolated and expanded in culture. These cells were used as Cr(VI)-transformed cells (CrT). Passage-matched normal BEAS-2B cells without exposure to Cr(VI) were used as control (CrT-M). (A) Both invasion and migration were determined using a transwell assay. Images represent one of three images captured in each group. The numbers of cells in invasion and migration were counted. (B) and (C), Whole protein lysates from Cr(VI)-transformed cells (CrT), passage-matched normal BEAS-2B cells (CrT-M), BEAS-2B cells, and A549 cells were harvested for immunoblotting analysis. (D) RNAs were isolated to determine NEDD9 mRNA levels using real-time PCR. (E) Both CrT-M and CrT cells were treated with MG132 or cycloheximide or their combination for 6 h. The whole-cell lysates were harvested for the determination of NEDD9 levels using immunoblotting analysis. (F) BEAS-2B cells with stable overexpression or knockdown of MEG3 were established. The cells were exposed to Cr(VI) at 0.05 and 0.1 μM for 6 months. Whole-cell lysates were harvested for immunoblotting analysis. *, $p < 0.05$, compared to that in passage-matched normal BEAS-2B cells (CrT-M).

2.4. Immunoblotting analysis

The cells were cultured in 6-well plates. After 90% confluence, the cells were washed with PBS and then lysed using a boiling buffer. The whole-cell lysates were sonicated. Protein concentrations were measured. Proteins were separated by SDS-PAGE gels followed by incubation with primary antibodies overnight. The blots were then probed with secondary antibodies. Proteins were visualized using ECF substrate.

2.5. Real-time qPCR

RNA was extracted and purified using Qiagen RNeasy mini kit. cDNA for MEG3 and NEDD9 mRNA was synthesized using a SuperScript first-strand synthesis kit. cDNA for miR-145 was synthesized using a miR-Script PCR kit. Primers were designed using Primer-Blast with forward sequence (F) and reverse (R) as followed: NEDD9: F-GATGGGTGTCTC-CAGCCTAA, R-GGATCTGGTGGGAGTCTTCA; MEG3: F-AGACCCGCCCTCTGACTGAT, R-AGGAGCCCACTTCCCACA; GAPDH: F-AGAAGGCTGGGGCTCATTTG, R-AGGGGCCATCCACAGTCTTC; miR-145-5p: GTCCAGTTTTCCCAGGAATCCCT. Levels of MEG3 and NEDD9 mRNA were measured using PowerUp SYBR Green master mix and GAPDH as a control. The miR-145 level was measured using a miScript PCR kit and U6 as a control. The value of cycle threshold (CT) was examined. Data were analyzed by calculation of $\Delta\Delta$CT.

2.6. Invasion and migration assays

The invasion assay was conducted using the Biocoat Matrigel Invasion Chambers (Corning, NY) according to the manufacturer's instructions. Migration assay was conducted using chamber inserts (Corning, NY). Cells were seeded in the chamber inserts at a density of 1.0–2.0 $\times$ 10^4 per well in 500 μL medium (0.1% FBS). The inserts were placed into the wells with 700 μL culture medium (10% FBS). After 48 h, the cells were washed with PBS, fixed with 3.7% formalin and methanol followed by Giemsa staining. The images were captured using an Olympus microscope. The number of invaded and migrated cells were counted and recorded.

2.7. Luciferase assay

The cells were transfected with a miR-145 luciferase reporter. After 48 h, the cells were washed with PBS. Luciferase activity was measured using the Dual-Luciferase Assay kit according to the manufacturer's protocol. The Renilla luciferase transfection control was used for normalization.

2.8. Statistical analysis

The student's test was used to evaluate the difference between the two groups. A value of $p < 0.05$ was considered significant.

3. Results

3.1. Cr(VI)-transformed cells exhibit increased invasion and migration as well as upregulation of NEDD9 expression

The results from migration and invasion assays showed that both invasion and migration were increased in Cr(VI)-transformed cells (CrT) compared to passage-matched normal BEAS-2B cells (CrT-M) (Figure 1A). Epithelial-mesenchymal transition (EMT) was associated with increased invasion and migration of cancer cells. During the EMT process, cancer cells lose epithelial markers, such as E-cadherin, and activate β-Catenin. The
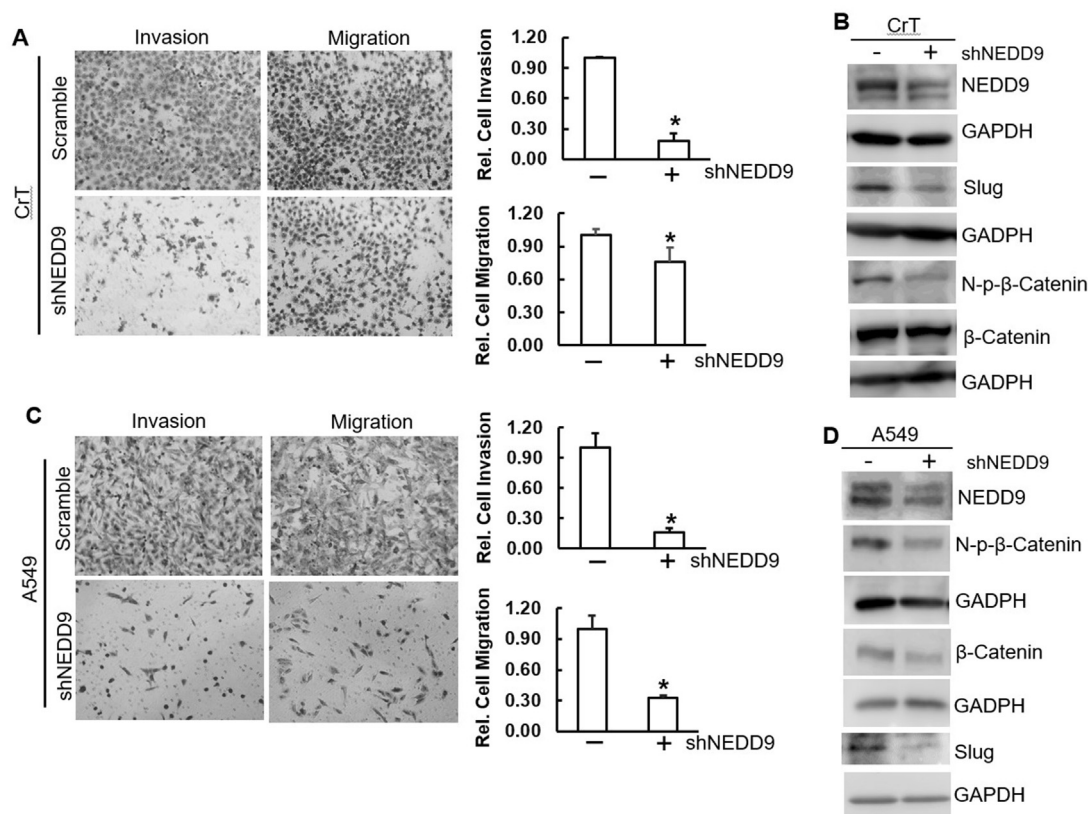

Figure 2. Knockdown of NEDD9 reduces invasion and migration in both Cr(VI)-transformed cells and human A549 lung adenocarcinoma cells. Stable knockdown of NEDD9 by using its shRNA was established in CrT and A549 cells. (A) and (C) The cells were collected for invasion and migration assays. Images represent one of three images captured in each group. The total cell numbers of invasion and migration were counted. (B) and (D) The whole-cell lysates from Cr(VI)-transformed cells and A549 cells with or without knockdown of NEDD9 were harvested for immunoblotting analysis. *, $p < 0.05$, compared to that scramble cells.

results of the present study showed that E-Cadherin was lost and β-Catenin was activated in Cr(VI)-transformed cells (Figure 1B). EMT is regulated by transcription factors, one of them is zinc finger protein SNAI2 (Slug) [26]. Slug represses the expression of E-cadherin [26]. Slug protein level was elevated in Cr(VI)-transformed cells (Figure 1B). NEDD9 has been identified as a metastatic marker. The results showed that both mRNA and protein levels of NEDD9 were elevated in Cr(VI)-transformed cells (Figure 1C and 1D). Treatment of cells with cycloheximide (CHX), a protein synthesis inhibitor, markedly reduced NEDD9 (Figure 1E). In contrast, treatment of cells with MG132, a proteasome inhibitor, increased NEDD9 (Figure 1E). Stable overexpression or knockdown of MEG3 was established in BEAS-2B cells. Those cells were chronically exposed to low doses of Cr(VI) for 6 months. The results showed that exposure of BEAS-2B cells to Cr(VI) at 0.05 or 0.1 μM was able to increase the NEDD9 protein level (Figure 1F). Overexpression of MEG3 blocked the increase in NEDD9 induced by Cr(VI) (Figure 1F). In contrast, knockdown of MEG3 caused a greater increase of NEDD9 compared to cells harboring a control vector (Figure 1F). Overexpression of MEG3 appeared to reduce β-Catenin activation compared to the scramble controls (Figure 1F). The reduction of activated β-Catenin occurred in the cells with or without Cr(VI) exposure. However, no significant changes in activated β-Catenin were observed in BEAS-2B cells with MEG3 knockdown (Figure 1F). To demonstrate the increase of NEDD9 in Cr(VI)-transformed cells is not cell-type specific, A549 cells, well-known lung cancer cells were used to measure the NEDD9 level. The results from Figure 1C and 1D showed that both mRNA and protein levels of NEDD9 in A549 cells were increased compared to those in BEAS-2B cells.

3.2. NEDD9 is a positive regulator of invasion and migration in Cr(VI)-transformed cells

To study whether upregulation of NEDD9 plays an important role in the increased invasion and migration of Cr(VI)-transformed cells, stable knockdown of NEDD9 by its shRNA in Cr(VI)-transformed cells was established. The results showed that knockdown of NEDD9 reduced both invasion and migration of Cr(VI)-transformed cells (Figure 2A). Further study showed that knockdown of NEDD9 decreased Slug level (Figure 2B). It has been reported that β-catenin activation upregulates EMT [39]. The results of the present study showed that knockdown of NEDD9 in Cr(VI)-transformed cells decreased β-catenin activation (Figure 2B). To verify the above observations in Cr(VI)-transformed cells, A549 cells with stable knockdown of NEDD9 were established. The results showed that knockdown of NEDD9 decreased invasion and migration of A549 cells (Figure 2C), β-catenin activation, and Slug protein level (Figure 2D).

3.3. MEG3 is a negative regulator of invasion and migration in Cr(VI)-transformed cells

Maternally expressed 3 (MEG3), imprinted long non-coding RNA gene, is often lost in cancer cells. The results from Figure 3A showed that exposure of BEAS-2B cells to Cr(VI) at 2.5 and 5.0 μM up to 12 h decreased MEG3 expression. Furthermore, MEG3 was lost (reduced by 99.9999%) in Cr(VI)-transformed cells (CrT) compared to that in passage-matched normal BEAS-2B cells (CrT-M) (Figure 3B). To explore whether loss of MEG3 is important in the invasion and migration of Cr(VI)-transformed cells, stable expression of MEG3 was established and MEG3 expression was measured (Figure 3C). The results showed that overexpression of MEG3 reduced both invasion and migration of Cr(VI)-transformed cells (Figure 3D), indicating that MEG3 is important in the invasion and migration of Cr(VI)-transformed cells. To test whether MEG3 itself is sufficient to regulate invasion and migration, normal BEAS-2B cells with stable knockdown of MEG3 were subjected to invasion and migration assays. MEG3 expressions were determined to confirm the success of these engineered cells (Figure 3E). Knockdown of MEG3 increased invasion and migration of BEAS-2B cells (Figure 3F),
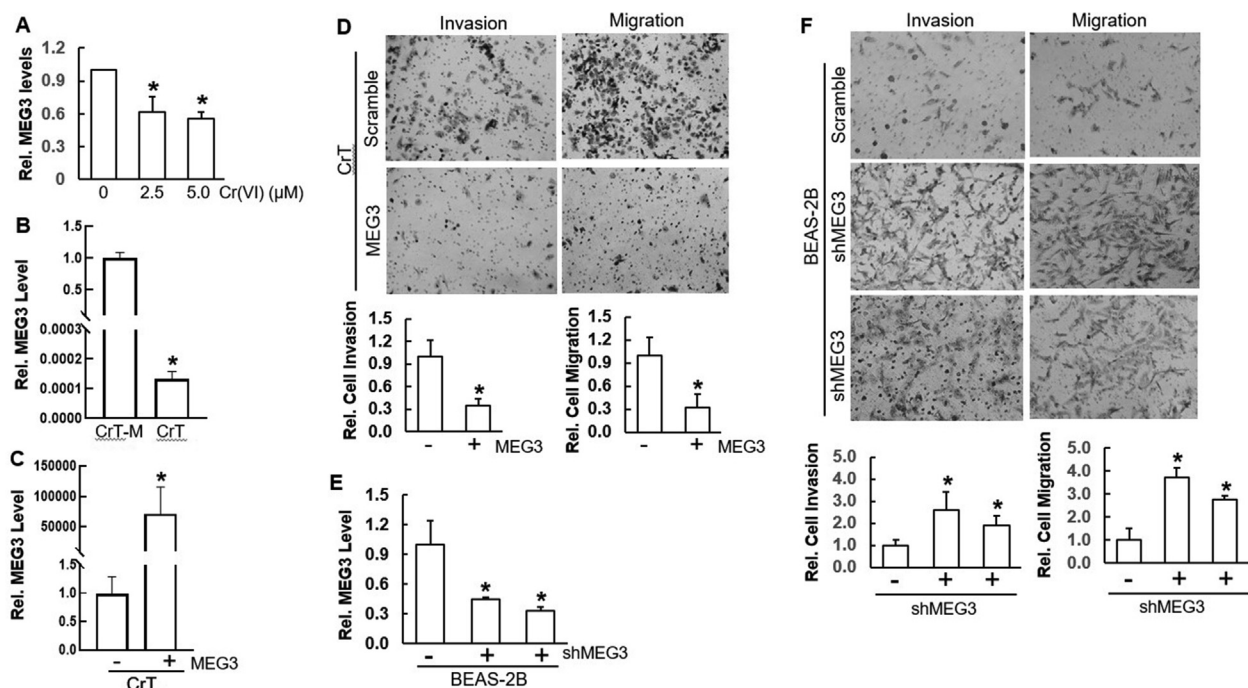

Figure 3. MEG3 negatively regulates invasion and migration of Cr(VI)-transformed cells. (A) BEAS-2B cells were exposed to various doses of Cr(VI) for 12 h. RNA was isolated for the measurement of MEG3 levels using real-time PCR analysis. *, $p < 0.05$, compared to without Cr(VI) exposure. (B) RNA was isolated from CrT-M and CrT cells. Real-time PCR was performed to measure MEG3 levels. *, $p < 0.05$, compared to that in passage-matched normal BEAS-2B cells (CrT-M). (C) and (D) Stable expression of MEG3 in CrT cells was established. The cells were harvested for the measurement of MEG3 levels using real-time PCR (C) and invasion and migration assays (D). *, $p < 0.05$, compared to that in scramble cells. (E) and (F) BEAS-2B cells with stable knockdown of MEG3 were subjected to measurement of MEG3 levels using real-time PCR analysis (E), and invasion and migration assays (F).. For those invasion and migration assays, the images represent one of three captured in each group. The total cell numbers of invasion and migration were counted, and relative invasion and migration were presented as mean ± SD (Right). *, $p < 0.05$, compared to that in scramble cells.
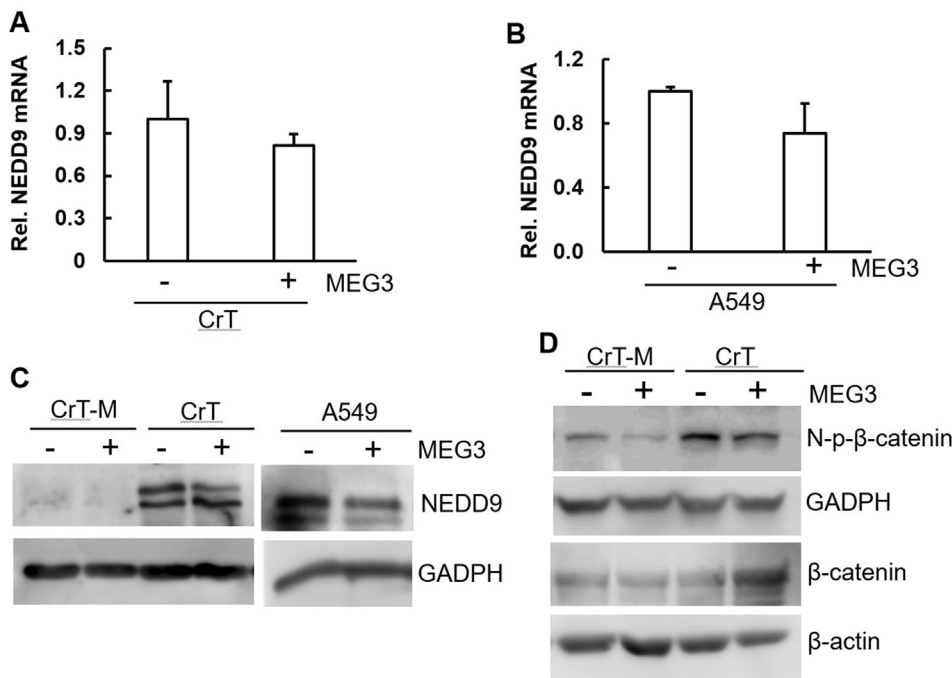
Figure 4. Overexpression of MEG3 in Cr(VI)-transformed cells reduces NEDD9 protein level and its downstream targets. (A) and (B) Cr(VI)-transformed cells and A549 cells were transfected with MEG3. After 48 h, the cells were collected, and RNA was isolated. NEDD9 mRNA level was measured using real-time PCR. (C) and (D) Cr(VI)-transformed cells (CrT) and their passage-matched normal BEAS-2B (CrT-M) and A549 cells with or without MEG3 overexpression were harvested for immunoblotting analysis.

consistent with the results observed in Cr(VI)-transformed cells. Taken together, these results suggest that MEG3 was a negative regulator of invasion and migration.

3.4. MEG3 negatively regulates NEDD9 in Cr(VI)-transformed and A549 cells

NEDD9 was upregulated in Cr(VI)-transformed cells (Figure 1C and 1D) and knockdown of NEDD9 decreased invasion and migration of

Cr(VI)-transformed cells (Figure 2A). The results from Figure 3D showed that overexpression of MEG3 reduced invasion and migration in Cr(VI)-transformed cells. We hypothesized that MEG3 may regulate NEDD9. Our results showed that MEG3 did not change NEDD9 mRNA levels in Cr(VI)-transformed cells or A549 cells (Figure 4A and 4B), but it reduced NEDD9 protein levels in these two types of cells (Figure 4C). Furthermore, MEG3 decreased activated β-catenin (Figure 4D). These results indicated that MEG3 was an upstream regulator of NEDD9 and β-catenin.
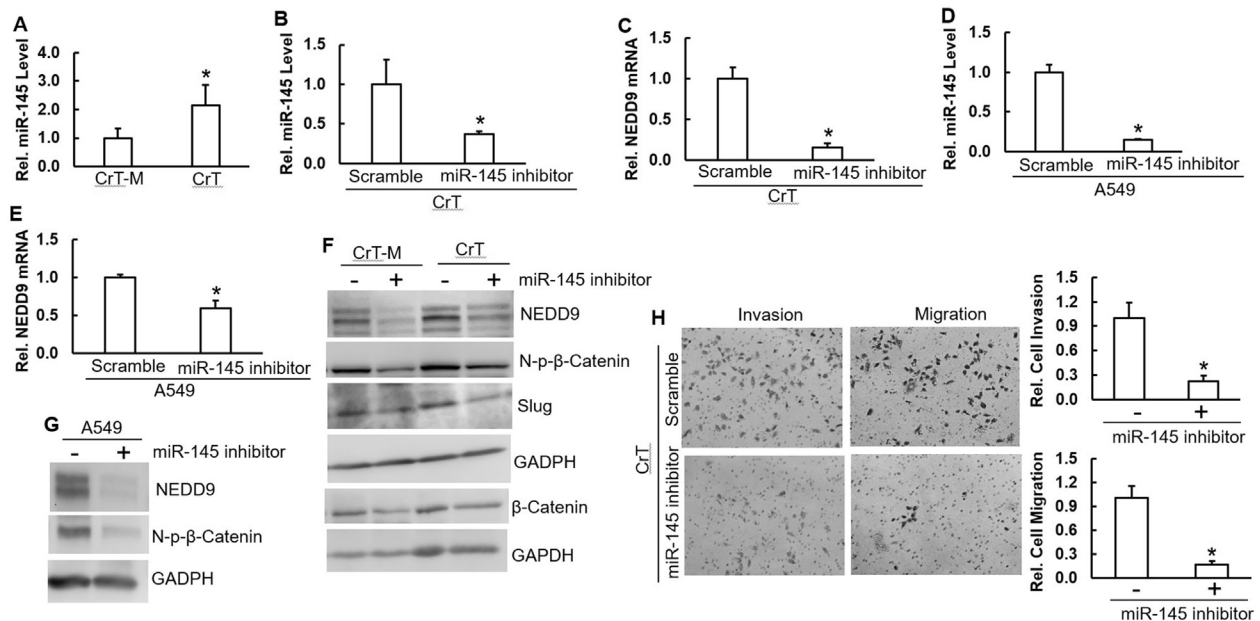

Figure 5. Inhibition of miR-145 reduces NEDD9 and invasion and migration of Cr(VI)-transformed cells. (A)–(E) Both Cr(VI)-transformed cells (CrT) and A549 cells with or without stable expression of miR145 inhibitor were collected for RNA isolation. Real-time PCR was performed to measure miR145 levels (A, B, and D) and NEDD9 mRNA levels (C and E). (F) and (G) Whole protein lysates were isolated in Cr(VI)-transformed cells (CrT) (F) and A549 cells (G) with or without stable expression of miR-145 inhibitor. Immunoblotting analysis was performed to measure NEDD9 protein levels. (H) Cr(VI)-transformed cells (CrT) with or without stable expression of miR-145 inhibitor were used for invasion and migration assays. Images represent one of three images captured in each group. The total cell numbers of invasion and migration were counted. The relative invasion and migration were presented as mean $\pm$ SD. (A) *, $p < 0.05$, compared to that in passage-matched normal BEAS-2B cells. (B–E) and (H) *, $p < 0.05$, compared to that in scramble cells.
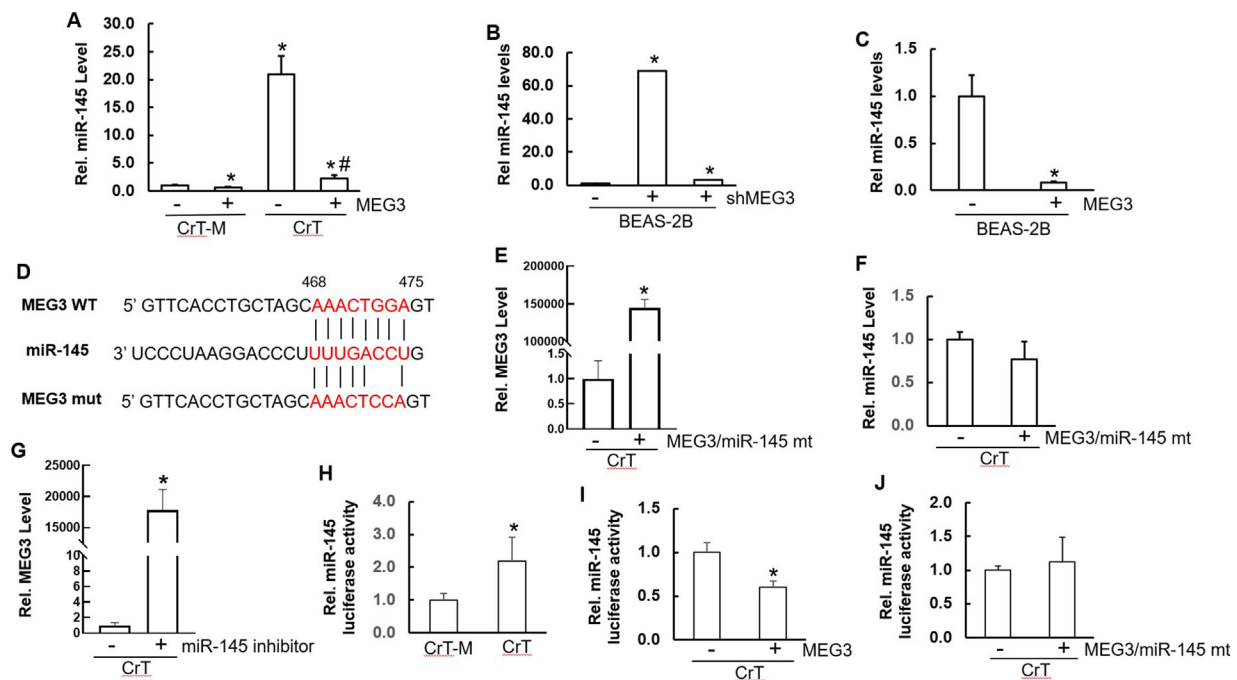
Figure 6. MEG3 is a negative regulator of miR-145 and miR-145 feedbacks to MEG3. (A)–(C) miR-145 levels were measured in Cr(VI)-transformed cells (CrT) and their passage-matched normal BEAS-2B cells (CrT-M) with or without MEG3 overexpression (A) and in BEAS-2B cells with or without knockdown or overexpression of MEG3 (B and C). (A) * and #, p < 0.05, compared to that in passage matched normal BEAS-2B cells without MEG3 overexpression and Cr(VI)-transformed cells without MEG3 overexpression, respectively. (B) and (C) *, p < 0.05, compared to that in scramble cells. (D) Point mutation of MEG3 at the binding site to miR-145 was constructed. (E) and (F) Cr(VI)-transformed cells (CrT) with or without overexpression of MEG3 with miR-145 mutation (MEG3/miR-145 mt) were harvested to measure MEG3 level (E) and miR-145 level (F) using real-time PCR. (G) RNA was isolated in the Cr(VI)-transformed cells with or without expression of miR-145 inhibitor. MEG3 level was measured using real-time PCR. (H)–(J) Cr(VI)-transformed cells (CrT) and their passage-matched normal BEAS-2B cells (CrT-M) and CrT cells with or without MEG3 overexpression or with or without expression of MEG3/miR-145 mt were transfected with a miR-145 luciferase reporter. After 48 h, cells were harvested, and luciferase activities were measured. (E), (G), and (I), *, p < 0.05, compared to that in scramble cells. (H), *, p < 0.05, compared to that in passage-matched normal BEAS-2B cells.

3.5. Inhibition of miR-145 reduces NEDD9 and invasion and migration in Cr(VI)-transformed cells

miR-145 plays an important role in tumorigenesis and metastasis of various cancers [40]. miR-145 expression was assessed in Cr(VI)-transformed cells. The results showed that miR-145 was elevated in Cr(VI)-transformed cells compared to that in passage-matched normal BEAS-2B cells (Figure 5A). To further examine whether upregulation of miR-145 contributes to the increased migration and invasion in Cr(VI)-transformed cells, a plasmid of miR-145 inhibitor was transfected into Cr(VI)-transformed cells followed by antibiotics selection to construct a cell that stably expresses an inhibitor of miRNA-145. The results from Figure 5B showed that miR-145 levels were lowered by 60% with the miR-145 inhibitor compared to the scramble control. Inhibition of miR-145 in Cr(VI)-transformed cells reduced NEDD9 mRNA and protein levels (Figure 5C and 5F). Decreased both Slug and β-Catenin activation were also observed in the Cr(VI)-transformed cells with stable expression of miR-145 inhibitor (Figure 5F). Similarly, A549 cells with stably expressing miR-145 inhibitor were also established and miR-145 levels were verified (Figure 5D). Similar results as those in Cr(VI)-transformed cells were obtained in A549 cells (Figure 5E and 5G). Not surprisingly, inhibition of miR-145 in Cr(VI)-transformed cells reduced invasion and migration (Figure 5H). These results demonstrated that miR-145 positively regulated NEDD9 and invasion and migration of Cr(VI)-transformed cells.

3.6. MEG3 negatively regulates miR-145 and miR-145 feedbacks to MEG3

In Cr(VI)-transformed cells, MEG3 was lost (Figure 3B) and miR-145 was upregulated (Figure 5A). Overexpression of MEG3 decreased miR-

145 level in both Cr(VI)-transformed cells (CrT) and their passage-matched normal BEAS-2B cells (CrT-M) (Figure 6A). Consistent with these findings, the knockdown of MEG3 in BEAS-2B cells elevated the miR-145 level (Figure 6B), while overexpression of MEG3 decreased it (Figure 6C). These results demonstrated that MEG3 negatively regulates miR-145. A binding site of MEG3 on miR-145 located between 468 and 475 nucleotides of MEG3 was identified using miRcode, miRWalk, and TargetScan database (Figure 6D). A plasmid with two points mutation at the MEG3 binding site was constructed. To confirm the success of the construct, stable expression of MEG3 mutant in Cr(VI)-transformed cells was established, and MEG3 and miR-145 expressions were measured. The results showed that the presence of this mutation (MEG3/MiR-145 mt) in Cr(VI)-transformed cells increased MEG3 but had no effect on miR-145 (Figure 6E and 6F). In an additional experiment, miR-145 luciferase activity was determined. The results showed that miR-145 luciferase activity was increased in Cr(VI)-transformed cells compared to that in passage-matched normal BEAS-2B cells (Figure 6H). Overexpression of MEG3 reduced the miR-145 luciferase activity (Figure 6I). The miR-145 luciferase activities remained in a similar level in Cr(VI)-transformed cells with or without overexpression of MEG3/miR-145 mt (Figure 6J). These results suggest that the regulation of MEG3 by miR-145 was through its binding to miR-145. Interestingly, inhibition of miR-145 dramatically increased MEG3 level in Cr(VI)-transformed cells (Figure 6G), demonstrating that miR-145 fed back to increase MEG3.

3.7. MEG3 regulates invasion and migration through miR-145

To study whether MEG3-regulated invasion and migration were mediated by miR-145, stable expression of MEG3/miR-145 mt in Cr(VI)-transformed cells were established. The results showed that
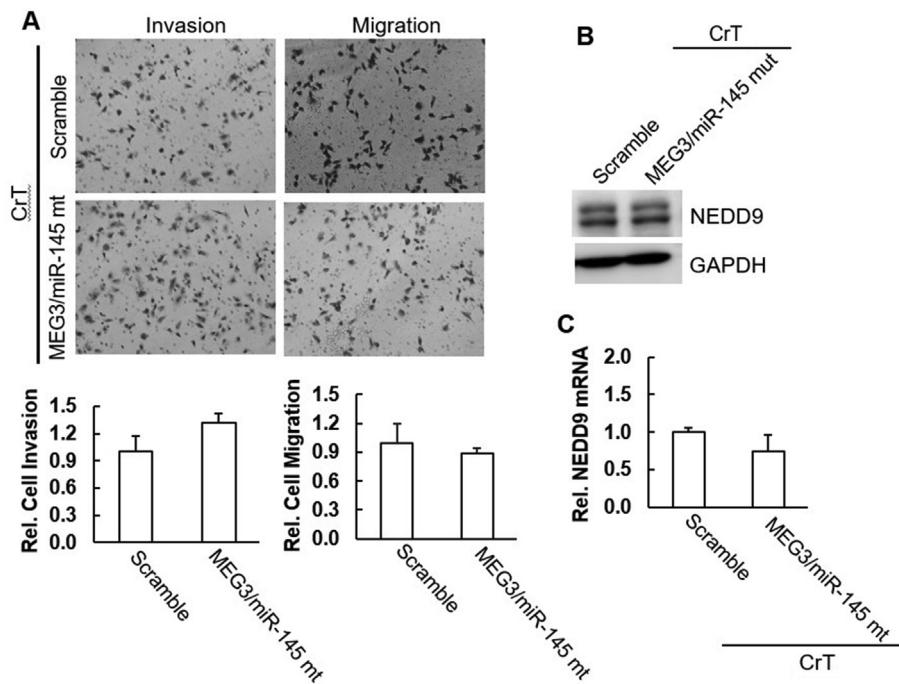
Figure 7. MEG3/miR-145 axis plays an important role in the migration and invasion of Cr(VI)-transformed cells. (A) Cr(VI)-transformed cells (CrT) with or without overexpression MEG3/miR-145 mt were subjected to invasion and migration assays. Images represent one of three images captured in each group. The total cell numbers of invasion and migration were counted. The relative invasion and migration were presented as mean ± SD. (B) Whole protein lysates were isolated in Cr(VI)-transformed cells (CrT) with or without overexpression of MEG3/miR-145 mt. Immunoblotting was used to determine the NEDD9 protein level. (C) RNA was isolated in Cr(VI)-transformed cells (CrT) with or without overexpression of MEG3/miR-145 mt. Real-time PCR was used to measure NEDD9 mRNA level.

overexpression of MEG3/miR-145 mt was unable to alter the invasion or migration of Cr(VI)-transformed cells (Figure 7A). NEDD9 protein or mRNA level remained similar in Cr(VI)-transformed cells with or without overexpression of MEG3/miR-145 mt (Figure 7B and 7C), indicating that MEG3 regulated NEDD9 and invasion and migration through its binding to miR-145.

4. Discussion

Cr(VI) compounds are confirmed human carcinogens. While researchers have made great progress in understanding Cr(VI) carcinogenesis, the mechanisms of invasion and migration in Cr(VI)-transformed cells remain unclear. Previous studies have demonstrated that Cr(VI)-transformed cells are tumorigenic [14,41]. The present study investigated (a) invasion and migration in Cr(VI)-transformed cells and (b) the mechanisms in these processes. This study provided new insights into exploring the invasion and migration of Cr(VI)-transformed cells by focusing on MEG3/miR145/NEDD9 signaling pathways.

NEDD9, a member of the Cas protein family, has been reported to be a metastasis marker in various cancers, including lung [18]. Our results showed both mRNA and protein levels of NEDD9 were elevated in Cr(VI)-transformed cells. MG132, a proteasomal inhibitor, and cycloheximide, an proteinase inhibitor, were used to treat the Cr(VI)-transformed cells. The results further confirm that upregulation of NEDD9 occurred at the transcriptional level. Knockdown of NEDD9 decreased invasion and migration in Cr(VI)-transformed cells, suggesting that NEDD9 plays an important role in the aggressiveness of Cr(VI)-transformed cells.

Mesenchymal and amoeboid invasion are major mechanisms of invasion and migration in cancer cells [42]. Previous studies indicated that NEDD9 regulated mesenchymal migration by acting as a scaffolding protein to pull cells through the extracellular matrix [42]. The results from the present study showed that β-catenin was activated in Cr(VI)-transformed cells, and that knockdown of NEDD9 by its shRNA decreased β-catenin activation, indicating that NEDD9 is a positive regulator of β-catenin. β-catenin expression was increased in EMT. Canonically, phosphorylated β-catenin is associated with APC, Axin, CK1, PP2A, GSK-3β, and β-TrCP, causing proteasomal degradation of β-catenin [43]. In many types of cancers where Wnt signaling was activated, non-phosphorylated β-catenin (active form) stimulated the expression of genes associated with the T-cell factor (TCF)/lymphoid enhancer factor (LEF), particularly EMT [44]. In normal epithelial cells, Slug transcription is low, resulting in high expression of E-cadherin, which allows the formation of epithelial adherent junctions. During the process of EMT, the loss of E-cadherin, which is caused by increased Slug expression, is an early and crucial step. The present study observed that EMT marker Slug was upregulated and that E-Cadherin was lost in Cr(VI)-transformed cells compared to passage-matched normal cells. Knockdown of NEDD9 in Cr(VI)-transformed cells decreased Slug level. E-Cadherin complexes with β-Catenin in normal cellular adhesion junctions [45]. When E-Cadherin was lost, β-Catenin was released and activated Wnt signaling associated with cancer survival and migration [45]. Cr(VI)-transformed cells exhibited decreased E-Cadherin and increased active non-phosphorylated β-Catenin, indicating that loss of E-Cadherin likely promoted β-Catenin release and facilitated EMT. Taken together, these observations suggested that activation of β-catenin, a transcription factor with TCF/LEF for transcription of Slug in β-Catenin driven EMT, plays an important role in the invasion and migration of Cr(VI)-transformed cells.

miRNAs, short noncoding RNAs, are encoded in protein-coding transcription units or are generated from noncoding transcription units. miRNA loci are located in the intron regions and exon regions of non-coding transcripts and protein-coding transcription units. One of the largest miRNA clusters is located in the DLK1/MEG3 imprinted region, which is exclusively controlled by MEG3 [46]. The canonical mechanism of miRNAs regulation on gene expression is through their binding to the 3′ untranslated region (UTR) of their target genes. Non-canonical mechanisms include (a) binding to the open reading frame or 5′-UTR of the target genes, causing downregulation or upregulation of the target genes [47]; (b) direct binding to the DNA [48,49], regulating gene expression at the transcriptional level; and (c) binding to ribonucleo-proteins, interfering with the RNA binding function [50].

miRNAs play an important role in the developmental processes including neuronal cell fate, metabolism, cell proliferation, and apoptosis [51]. miRNAs act as tumor suppressors or oncogenes in cancer biology [52]. miR-145, located on chromosome 5q, is involved in multiple human cancers. Downregulation of miR-145 was observed in colorectal cancer [53,54], non-small-cell lung cancer [55], breast cancer [56], cervical cancer [57], prostate cancer [58], glioma [59], and bladder

cancer [60]. In the contrast, miR-145 was upregulated in esophageal cancer cells, miR-145 directly targeted SMAD5, promoting proliferation and metastasis [61]. An earlier study showed that miR-145 promoted the invasion of esophageal adenocarcinoma cells [62]. It has been reported that miR-145-3p was increased in the plasma of workers occupationally exposed to Cr(VI) [63]. The results from the present study showed that miR-145-5p expression was increased in the Cr(VI)-transformed cells compared to that in passage-matched normal BEAS-2B cells. However, miR-143, the cluster of miR-145, has been reported to be reduced in Cr(VI)-transformed cells [64]. The results from a previous study showed that miR-21 was upregulated in Cr(VI)-transformed cells and inhibition of miR-21 elevated programmed cell death 4 (PDCD4), a tumor suppressor, resulting in suppression of malignant cell transformation and invasion of Cr(VI)-transformed cells [15].

It has been reported that miR-145 was expressed at much lower levels and NEDD9 was highly expressed in glioblastomas compared to normal brain tissue. Overexpression of miR-145 reduced NEDD9 and knockdown of NEDD9 increased miR-145, leading to inhibition of invasion in glioblastoma cells [65]. Inhibition of miR-145 in Cr(VI)-transformed cells reduced NEDD9 level, demonstrating that NEDD9 was the downstream target of miR-145. Using TargetScan, NEDD9 was predicted to be one of the miR-145-5p targets, and position 47–54 of NEDD9 3′ UTR was sequentially paired with miR145 (http://www.targetscan.org/cgi-bin/targetscan/vert_71/targetscan.cgi?mirg=hsa-miR-145-5p). To study whether the observations obtained in Cr(VI)-transformed cells are cell type-specific, in the present study A549 cells with stable expressing miR-145 inhibitor were established. The results showed inhibition of miR-145 in A549 cells decreased mRNA and protein levels of NEDD9 which were consistent with the results from Cr(VI)-transformed cells. Because the present study has shown that knockdown of NEDD9 decreased invasion and migration in A549 cells, it is anticipated that miR-145 promotes invasion and migration of A549 cells. However, a previous study reported that miR-145 suppressed the invasion and migration of A549 cells [66]. Given the observation of reduction of both mRNA and protein levels of NEDD9 by miR-145 inhibition, it is speculated that miR-145 may regulate NEDD9 through transcriptional mechanisms, such as mRNA degradation. The precise mechanism needs to be investigated further.

The loss of MEG3 expression has been observed in various human cancers. Competing endogenous RNA (ceRNAs) function as molecular suppressors for microRNAs through their binding sites. MEG3, a ceRNA, regulates the biological functions of cancer cells. It has been reported that ectopic expression of MEG3 reduced malignant cell transformation induced by chronic exposure to nickel, a confirmed human carcinogen, while knockdown of MEG3 facilitates the malignant cell transformation by this metal [37]. Further studies demonstrated that the downregulation of MEG3 induced by nickel was due to its promoter hypermethylation [37]. The present study has shown that MEG3 was lost in Cr(VI)-transformed cells. Overexpression of MEG3 reduced both invasion and migration of Cr(VI)-transformed cells, accompanied by reduced NEDD9 protein level and β-catenin activation. Further study showed that overexpression of MEG3 decreased miR-145 level and that inhibition of miR-145 increased MEG3 expression in Cr(VI)-transformed cells, indicating that MEG3 and miR-145 are reciprocal regulators. A previous study indicated that MEG3 binds to miR-27a, resulting in inhibition of bladder cancer invasion [36]. Furthermore, the present study identified the binding site of MEG3 to miR-145 and a plasmid of MEG3 mutation at the binding site to miR-145 was constructed. The results showed that the MEG3 mutation does not exhibit any effect on invasion and migration of Cr(VI)-transformed cells, or NEDD9, suggesting that invasion and migration inhibited by MEG3 may be through its binding to miR-145. Consistent with these findings, a recent study showed that in the human kidney 2 (HK-2) cells downregulation of MEG3 increased miR-145, and that miR-145 mimic or miR-145 inhibitor reduced or elevated MEG3 luciferase activity, respectively. The present study has
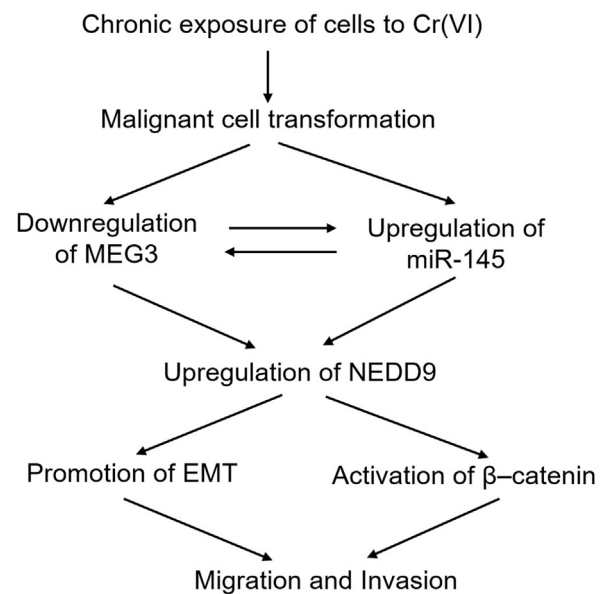

Figure 8. Scheme of the mechanism of MEG3/miR-145 in the regulation of invasion and migration of Cr(VI)-transformed cells. Chronic exposure of BEAS-2B cells to Cr(VI) causes malignant cell transformation. Those transformed cells exhibit increased invasion and migration. Loss of MEG3 causes an increase of miR-145 and miR-145 feedbacks to MEG3 in Cr(VI)-transformed cells, resulting in upregulation of NEDD9. Elevation of NEDD9 activates Wnt/β-catenin and EMT pathway, leading to increased invasion and migration of Cr(VI)-transformed cells.

also shown that knockdown of MEG3 in BEAS-2B cells increased invasion and migration, suggesting that MEG3 functions as a tumor suppressor.

In summary, the present study observed that MEG3 was downregulated and that miR-145 was upregulated in Cr(VI)-transformed cells. Overexpression of MEG3 reduced miR-145 level and inhibition of miR-145 elevated MEG3 expression. Overexpression of MEG3 or inhibition of miR-145 decreased invasion and migration in Cr(VI)-transformed cells, accompanied by reduction of NEDD9 and EMT marker Slug. The present study suggested that the downregulation of MEG3 and upregulation of miR-145 play an important role in invasion and migration in Cr(VI)-transformed cells. The overall mechanism of MEG3/miR-145 on the regulation of invasion and migration of Cr(VI)-transformed cells was summarized in Figure 8.

Declarations

Author contribution statement

Zhuo Zhang: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Jingxia Li: Performed the experiments; Analyzed and interpreted the data.

Yan Bo: Performed the experiments.

Huailu Tu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Dr. Chao Huang: Contributed reagents, materials, analysis tools or data.

Dr. Max Costa: Conceived and designed the experiments; Analyzed and interpreted the data.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

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References

[1] IARC Monogr. Eval. Carcinog. Risks Hum., Nickel, Weld. 49, 1990, pp. 1–648.

[2] W. Machle, F. Gregorius, Cancer of the respiratory system in the United States chromate producing industry, Publ. Health Rep. 63 (1948) 1114–1127.

[3] S. Langard, One hundred years of chromium and cancer: a review of epidemiological evidence and selected case reports, Am. J. Ind. Med. 17 (1990) 189–215.

[4] S. Langard, Role of chemical species and exposure characteristics in cancer among persons occupationally exposed to chromium compounds, Scand. J. Work. Environ. Health 19 (1993) 81–89.

[5] S. De Flora, Threshold mechanisms and site specificity in chromium (VI) carcinogenesis, Carcinogenesis 21 (2000) 533–541.

[6] L. Simonato, A.C. Fletcher, A.A. Anderson, K. Anderson, N. Becker, J. Chang, An historical perspective study of European stainless steel, mild steel, and shipyard welders, Br. J. Ind. Med. 48 (1991) 145–154.

[7] T. Sorahan, D.C. Burges, L. Hamilton, J.M. Harrington, Lung cancer mortality in nickel/chromium platers, 1946-1995, Occup. Environ. Med. 55 (1998) 236–242.

[8] Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological Profile for Chromium, US Dept Health Hum Serv, 1992.

[9] C. Pellerin, S.M. Booker, Reflection on hexavalent chromium: health hazards of an industrial heavy weigh, Environ. Health Perspect. 108 (2000) A402–A407.

[10] T.J. Woodruff, D.A. Axelrad, J. Caldwell, R. Morello-Frosch, A. Rosenbaum, Public health implications of 1990 air toxics concentrations across the United States, Environ. Health Perspect. 106 (1998) 245–251.

[11] The IARC Monographs. http://monographs.iarc.fr/ENG/Monographs/PDFs/index.php.

[12] D. Kim, J. Dai, L.Y. Fai, H. Yao, Y.O. Son, L. Wang, P. Pratheeshkumar, K. Kondo, X. Shi, Z. Zhang, Constitutive activation of epidermal growth factor receptor promotes tumorigenesis of Cr(VI)-transformed cells through decreased reactive oxygen species and apoptosis resistance development, J. Biol. Chem. 290 (2015) 2213–2224.

[13] D. Medan, S. Luanpitpong, N. Azad, L. Wang, B. Jiang, M.E. Davis, J.B. Barnett, L. Guo, Y. Rojanasakul, Multifunctional role of Bcl-2 in malignant transformation and tumorigenesis of Cr(VI)-Transformed lung cells, PLoS One 7 (2012), e37045.

[14] J. Dai, Y. Ji, W. Wang, D. Kim, L.Y. Fai, L. Wang, J. Luo, Z. Zhang, Loss of fructose-1,6-bisphosphatase induces glycolysis and promotes apoptosis resistance of cancer stem-like cells: an important role in hexavalent chromium-induced carcinogenesis, Toxicol. Appl. Pharmacol. 331 (2017) 164–173.

[15] P. Pratheeshkumar, Y.O. Son, S.P. Divya, L. Turcios, R.V. Roy, J.A. Hitron, L. Wang, D. Kim, J. Dai, P. Asha, Z. Zhang, X. Shi, Hexavalent chromium induces malignant transformation of human lung bronchial epithelial cells via ROS-dependent activation of miR-21-PDCD4 signaling, Oncotarget 7 (2016) 51193–51210.

[16] N. Tikhmyanova, J.L. Little, E.A. Golemis, CAS proteins in normal and pathological cell growth control, Cell. Mol. Life Sci. 67 (2010) 1025–1048.

[17] M.S. Guerrero, J.T. Parsons, A.H. Bouton, Cas and NEDD9 contribute to tumor progression through dynamic regulation of the cytoskeleton, Genes Cancer 3 (2012) 371–381.

[18] J.X. Chang, F. Gao, G. Zhao, G. Zhang, Role of NEDD9 in invasion and metastasis of lung adenocarcinoma, Exp. Ther. Med. 4 (2012) 795–800.

[19] E. Izumchenko, MaheM.K. Singh, O.V. Plotnikova, N. Tikhmyanova, J.L. Little, I.G. Serebriiskii, S. Seo, M. Kurokawa, B.L. Egleston, A. Klein-Szanto, E.N. Pugachева, R.R. Hardy, M. Wolfson, D.C. Connolly, E.A. Golemis, NEDD9 promotes oncogenic signaling in mammary tumor development, Cancer Res. 69 (2009) 7198–7206.

[20] F. Rambow, A. Bechadergue, F. Luciani, G. Gros, M. Domingues, J. Bonaventure, G. Meurice, J. Marine, L. Larue, Regulation of melanoma progression through the TCF4/miR-125b/NEDD9 cascade, J. Invest. Dermatol. 136 (2016) 1229–1237.

[21] Z. Wang, M. Shen, P. Lu, X. Li, S. Zhu, S. Yue, NEDD9 may regulate hepatocellular carcinoma cell metastasis by promoting epithelial-mesenchymal-transition and stemness via repressing Smad7, Oncotarget 8 (2017) 1714–1724.

[22] C.U. Afsar, M. Karabulut, S. Karabulut, S.T. Ozal, M. Cikot, M. Serilmez, F. Tas, Clinical significance of serum NEDD9 levels in patients with pancreatic cancer, Biomolecules 8 (2018) 169.

[23] K. Morimoto, T. Tanaka, Y. Nitta, K. Ohnishi, H. Kawashima, T. Nakatani, NEDD9 crucially regulates TGF-beta-triggered epithelial-mesenchymal transition and cell invasion in prostate cancer cells: involvement in cancer progressiveness, Prostate 74 (2014) 901–910.

[24] H. Meng, J. Wu, Q. Huang, X. Yang, K. Yang, Y. Qiu, J. Ren, R. Shen, H. Qi, NEDD9 promotes invasion and migration of colorectal cancer cell line HCT116 via JNK/EMT, Oncol. Lett. 18 (2019) 4022–4029.

[25] J.P. Thiery, J.P. Sleeman, Complex networks orchestrate epithelial-mesenchymal transitions, Nat. Rev. Mol. Cell Biol. 7 (2006) 131–142.

[26] H. Son, A. Moon, Epithelial-mesenchymal transition and cell invasion, Toxicol. Res. 26 (2010) 245–252.

[27] C. Kong, C. Wang, L. Wang, M. Ma, C. Niu, X. Sun, J. Du, Z. Dong, S. Zhu, J. Lu, B. Huang, NEDD9 is a positive regulator of epithelial-mesenchymal transition and promotes invasion in aggressive breast cancer, PLoS One 6 (2011), e22666.

[28] J. Ahn, V. Sanz-Moreno, C.J. Marshall, The metastasis gene NEDD9 product acts through integrin beta3 and Src to promote mesenchymal motility and inhibit amoeboid motility, J. Cell Sci. 125 (2012) 1814–1826.

[29] Y. He, Y. Luo, B. Liang, L. Ye, G. Lu, W. He, Potential applications of MEG3 in cancer diagnosis and prognosis, Oncotarget 8 (2017) 73282–73295.

[30] S. Ghafouri-Fard, M. Taheri, Maternally expressed gene 3 (MEG3): a tumor suppressor of long noncoding RNA, Biomed. Pharmacother. 118 (2019), 109129.

[31] T.L. Kruer, S.M. Dougherty, L. Reynolds, E. Long, T. de Silva, W.W. Lockwood, B.F. Clem, Expression of the lncRNA maternally expressed gene 3 (MEG3) contributes to the control of lung cancer cell proliferation by the Rb pathway, PLoS One 11 (2016), e0166363.

[32] M. Sun, R. Xia, F. Jin, T. Xu, Z. Liu, W. De, X. Liu, Downregulated long noncoding RNA MEG3 is associated with poor prognosis and promotes cell proliferation in gastric cancer, Tumor Biol. 35 (2014) 1065–1073.

[33] M. Moradi, H. Fallahi, Z. Rahimi, Interaction of long noncoding RNA MEG3 with miRNAs: a reciprocal regulation, J. Cell. Biochem. 120 (2019) 3339–3352.

[34] F.A. Karreth, P.P. Pandolfi, ceRNA cross-talk in cancer: when ce-bling rivalries go awry, Cancer Discov. 3 (2013) 1113–1121.

[35] Y. Tay, J. Rinn, P.P. Pandolfi, The multilayered complexity of ceRNA crosstalk and competition, Nature 505 (2014) 344–352.

[36] C. Huang, X. Liao, H. Jin, F. Xie, F. Zheng, J. Li, C. Zhou, G. Jiang, X. Wu, C. Huang, MEG3, as a competing endogenous RNA, binds with miR-27a to promote PHLPP2 protein translation and impairs bladder cancer invasion, Mol. Ther. Nucleic Acids 16 (2019) 51–62.

[37] C. Zhou, C. Huang, J. Wang, H. Huang, J. Li, Q. Xie, Y. Liu, J. Zhu, Y. Li, D. Zhang, Q. Zhu, C. Huang, LncRNA MEG3 downregulation mediated by DNMT3b contributes to nickel malignant transformation of human bronchial epithelial cells via modulating PHLPP1 transcription and HIF-1α translation, Oncogene 36 (2017) 3878–3889.

[38] J. Zhu, Z. Tian, Y. Li, X. Hua, D. Zhang, J. Li, H. Jin, J. Xu, W. Chen, B. Niu, X. Wu, S. Comincini, H. Huang, C. Huang, ATG7 promotes bladder cancer invasion via autophagy-mediated increased ARHGDIB mRNA stability, Adv. Sci. 6 (2019), 1801927.

[39] W.K. Kim, Y. Kwon, M. Jang, M. Park, J. Kim, S. Cho, D.G. Jang, W.B. Lee, S.H. Jung, H.J. Choi, B.S. Min, T. Kim, S.P. Hong, Y.K. Paik, H. Kim, β-catenin activation down-regulates cell-cell junction-related genes and induces epithelial-to-mesenchymal transition in colorectal cancers, Sci. Rep. 9 (2019), 18440.

[40] W.X. Xu, J. Liu, F. Deng, D. Wang, X. Li, T. Tian, J. Zhang, Ji. Tang, MiR-145: a potential biomarker of cancer migration and invasion, Am. J. Transl. Res. 11 (2019) 6739–6753.

[41] Z. Wang, H. Lin, Y. Li, H. Tao, P. Yang, J. Xie, D. Maddy, K. Kondo, C. Yang, Chronic hexavalent chromium exposure induces cancer stem cell-like property and tumorigenesis by increasing c-myc expression, Toxicol. Sci. 172 (2019) 252–264.

[42] N.V. Krakhmal, M.V. Zavyalova, E.V. Denisov, S.V. Vtorushin, V.M. Perelmuter, Cancer invasion: patterns and mechanisms, Acta Nat. 7 (2015) 17–28.

[43] J.L. Stamos, W.I. Weis, The beta-catenin destruction complex, Cold Spring Harbor Perspect. Biol. 5 (2013) a007898.

[44] H. Clevers, Wnt/beta-catenin signaling in development and disease, Cell 27 (2016) 469–480.

[45] X. Tian, Z. Liu, B. Niu, J. Zhang, T. Tan, S.R. Lee, Y. Zhao, D.C.H. Harris, G. Zheng, E-Cadherin/β-Catenin complex and the epithelial Barrier, J. Biomed. Biotechnol. 2011 (2011), 567305.

[46] H. Royo, J. Cavaille, Non-coding RNAs in imprinted gene clusters, Biol. Cell. 100 (2008) 149–166.

[47] U.A. Ørom, F.N. Nielsen, A.H. Lund, MicroRNA-10a binds the 5' UTR of ribosomal protein mRNAs and enhances their translation, Mol. Cell 4 (2008) 460–471.

[48] D.H. Kim, P. Saetrom, O. Snøve, J.J. Rossi, MicroRNA directed transcriptional gene silencing in mammalian cells, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 16230–16235.

[49] B. Khraiwesh, M.A. Arif, G.I. Seumel, S. Ossowski, D. Weigel, R. Reski, W. Frank, Transcriptional control of gene expression by MicroRNAs, Cell 140 (2010) 112–122.

[50] M. Beitzinger, G. Meister. MicroRNAs, From decay to decoy, Cell 140 (2010) 612–614.

[51] A.M. Ardekani, M.M. Naeini, The role of MicroRNAs in human diseases, Avicenna J. Med. Biotechnol. (AJMB) 2 (2010) 161–179.

[52] L. Benetatos, G. Vartholomatos, Deregulated microRNAs in multiple myeloma, Cancer 118 (2012) 878–887.

[53] N. Sheng, G. Tan, W. You, H. Chen, J. Gong, D. Chen, H. Zhang, Z. Wang, MiR-145 inhibits human colorectal cancer cell migration and invasion via PAK4-dependent pathway, Cancer Med. 6 (2017) 1331–1340.

[54] H. Tang, K. Li, J. Zheng, X. Dou, Y. Zhao, L. Wang, microRNA-145 regulates tumor suppressor candidate 3 and mitogen-activated protein kinase pathway to inhibit the progression of colorectal cancer, J. Cell. Biochem. 120 (2019) 8376–8384.

[55] Y. Zhang, Q. Lin, MicroRNA-145 inhibits migration and invasion by down-regulating FSCN1 in lung cancer, Int. J. Clin. Exp. Med. 8 (2015) 8794–8802.

[56] H. Zhao, X. Kang, X. Xia, L. Wo, X. Gu, Y. Hu, X. Xie, H. Chang, L. Lou, X. Shen, miR-145 suppresses breast cancer cell migration by targeting FSCN-1 and inhibiting epithelial-mesenchymal transition, Am. J. Transl. Res. 8 (2016) 3106–3114.

[57] A. Gonzalez-Torres, E.G. Banuelos-Villegas, N. Martinez-Acuna, E. Sulpice, X. Gidrol, L.M. Alvarez-Salas, MYPT1 is targeted by miR-145 inhibiting viability, migration and invasion in 2D and 3D HeLa cultures, Biochem. Biophys. Res. Commun. 507 (2018) 348–354.

[58] W. Guo, D. Ren, X. Chen, X. Tu, S. Huang, M. Wang, L. Song, X. Zou, X. Peng, HEF1 promotes epithelial mesenchymal transition and bone invasion in prostate cancer under the regulation of microRNA-145, J. Cell. Biochem. 114 (2013) 1606–1615.

[59] X. Wan, Q. Cheng, R. Peng, Z. Ma, Z. Chen, Y. Cao, B. Jiang. Rock1, A novel target of miR-145, promotes glioma cell invasion, Mol. Med. Rep. 9 (2014) 1877–1882.

[60] L.L. Mei, W.J. Wang, Y.T. Qiu, X.F. Xie, J. Bai, Z.Z. Shi, miR-145-5p suppresses tumor cell migration, invasion and epithelial to mesenchymal transition by regulating the Sp1/NF-kappaB signaling pathway in esophageal squamous cell carcinoma, Int. J. Mol. Sci. 18 (2017) 1833.

[61] Q. Zhang, H. Gan, W. Song, D. Chai, S. Wu, MicroRNA-145 promotes esophageal cancer cells proliferation and metastasis by targeting SMAD5, Scand. J. Gastroenterol. 53 (2018) 769–776.

[62] M.F. Derouet, G. Liu, G.E. Darling, MiR-145 expression accelerates esophageal adenocarcinoma progression by enhancing cell invasion and anoikis resistance, PLoS One 9 (2014), e115589.

[63] J. Jia, T. Li, C. Yao, J. Chen, L. Feng, Z. Jiang, L. Shi, J. Liu, J. Chen, J. Lou, Circulating differential miRNAs profiling and expression in hexavalent chromium exposed electroplating workers, Chemosphere 260 (2020), 127546.

[64] J. He, X. Qian, R. Carpenter, Q. Xu, L. Wang, Y. Qi, Z. Wang, L. Liu, B. Jiang, Repression of miR-143 mediates Cr (VI)–Induced tumor angiogenesis via IGF-IR/IRS1/ERK/IL-8 pathway, Toxicol. Sci. 134 (2013) 26–38.

[65] M.C. Speranza, V. Frattini, F. Pisati, D. Kapetis, P. Porrati, M. Eoli, S. Pellegatta, G. Finocchiaro, NEDD9, a novel target of miR-145, increases the invasiveness of glioblastoma, Oncotarget 3 (2012) 723–734.

[66] Y. Pan, C. Ye, Q. Tian, S. Yan, X. Zeng, C. Xiao, L. Wang, H. Wang, miR-145 suppresses the proliferation, invasion, and migration of NSCLC cells by regulating the BAX/BCL-2 ratio and the caspase-3 cascade, Oncol. Lett. 15 (2018) 4337–4343.