RESEARCH ARTICLE

TIAM2 Enhances Non-small Cell Lung Cancer Cell Invasion and Motility

Zheng-Yuan Zhao*, Chen-Guang Han, Jun-Tao Liu, Chang-Lei Wang, Yi Wang, Li-Ya Cheng

Abstract

Background: TIAM2, a Rac guanine nucleotide exchange factor, is closely associated with cell adherence and migration. Here, we aimed to investigate the role of TIAM2 in non-small cell lung cancer (NSCLC) cells. Materials and Methods: A small interference RNA (siRNA) was introduced to silence the expression of TIAM2. Invasion and motility assays were then performed to assess the invasion and motility potential of NSCLC cells. GST-pull down assays were used to detect activation of Rac1. Results: TIAM2 was highly expressed in NSCLC cells. Knockdown of TIAM2 inhibited the invasion and motility, and suppressed activation of Rac1. Further experiments demonstrated that knockdown of TIAM2 could up-regulate the expression of E-cadherin, and down-regulate the expression of MMP-3, Twist and Snail. Conclusions: Our data suggest that TIAM2 can promote invasion and motility of NSCLC cells. Activation of Rac1 and regulation of some EMT/invasion-related genes may be involved in the underlying processes.

Keywords: TIAM2 - invasion - motility - non-small cell lung cancer - EMT - Rac1 - MMP-3

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Introduction

Lung cancer is the most common cancer in the world, and has become one of the leading causes of cancer death. Lung cancer can be further divided into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) (Smith et al., 2013). NSCLC is the most common type, accounting for approximately 85% of lung cancers (Debevec et al., 2007). Thus, investigating the underlying mechanisms of NSCLC may provide a better understanding of lung cancer.

Tumor invasion and metastasis are responsible to most cancer deaths, and quantities of molecules and pathways are involved in the processes (Yilmaz et al., 2007). As a member of the Rho family GTPases, Rac1 plays an important role in the regulation of cancer invasion and metastasis. It is reported that activation of Rac1 up-regulates the expression of matrix metalloproteinases (MMPs), which are associated with cell invasion and metastasis (Parri et al., 2010). Studies also found that Rac1 activation induces the formation of lamellipodia (Ridley et al., 1992), and regulates the epithelial to mesenchymal transition (EMT) process (Hage et al., 2009). Many studies have shown that TIAM1 and TIAM2, which belong to TIAM-family guanine nucleotide exchange proteins, can activate Rac1 GTPase (Shepherd et al., 2011). TIAM1 expression is closely related to lung cancer development and metastasis (Wang et al., 2012), and can be used as a marker for the prognosis of hepatocellular carcinoma patients (Ding et al., 2009). Involvement of TIAM1 in the invasion and metastasis has been reported in many cancer cell lines, such as colon, breast and lung cancer cell lines (Hou et al., 2004; Minard et al., 2004; Liu et al., 2006). However, little study focuses on the role of TIAM2 in the tumor progression. Therefore, in this study, we aimed to determine whether TIAM2, the homolog of TIAM1, was involved in the regulation of cell invasion and motility in NSCLC cells, and tried to elucidate the underlying mechanisms.

Materials and Methods

Cell lines and culture conditions

The cell lines were purchased from American Type Culture Collection (Manassas, VA, USA), and cultured in the DMEM medium (GIBCO, Grand Island, NY, USA) supplemented with 10% FBS. All cells were cultured in a humidified atmosphere of 5% CO_2 at 37 °C.

Antibodies

The antibodies of TIAM1, TIAM2, E-cadherin, and β-actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The antibodies of Rac1, Twist and Snail were purchased from Cell Signaling Technology (Danvers, MA, USA).

siRNA and transfection

To silencing the expression of TIAM2 in A549 and

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H1299 cells, a TIAM2 siRNA was designed and obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). A scramble siRNA was also purchased from Santa Cruz Biotechnology and used as control siRNA. Cells were transfected with siRNAs using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Invasion assay

Cells were trypsinized and suspended in the serum-free medium at the concentration of 6×10^6 cells/ml. Transwell plates were obtain from Costar (San Diego, CA, USA), and the filters were coated with matrigel (BD, Franklin Lakes, NJ, USA) before used. Two hundred microliter cell suspensions were placed in the upper chambers, and 600 μl DMEM medium with 20% FBS were placed in the low chambers. Sixteen hours later, the invaded cells were fixed with 4% formaldehyde and stained with crystal violet. Seven fields of the invaded cells were observed under a microscope at × 100 magnification.

Motility assay

Two million cells were seeded in the 6-well plate. Ten hours later, the cell monolayers were scratched straightly by a micropipette tip. After washed with PBS for three times, the cells were incubated in serum-free medium for 16 hours. The width of cell wound was measured at 0 h and 16 h to assess the cell motility ability.

Western blotting

Cells were lysed with the RIPA lysis buffer, and the protein concentrations were determined by BCA assay. After boiled with loading buffer, equal amounts of protein were separated by SDS-PAGE gel, and transferred onto a PVDF membrane (Bio-Rad, Hercules, CA, USA). The membrane was incubated with primary antibodies at 4 °C overnight, and then probed with secondary antibodies for 1 hour at room temperature. The immunoreactive bands were obtained to film after incubated with chemiluminescence (Applygen Technologies Inc, Beijing, China) for 3 min.

GST pull-down assay

Cells were lysed with the RIPA lysis buffer, and equal amounts of protein were incubated with GST-PAK1-CD fusion protein, which were obtained from plasmid pGEX-PAK1-CRIB. The Glutathione-Sepharose beads (Amersharm Pharmacia Biotech) were also added to the protein mixture. Twelve hours later, the beads were washed. Then the proteins were collected and boiled with loading buffer, and separated by 15% SDS-PAGE gel. The bands were detected by immunoblotting with antibodies against Rac1.

Reverse transcription and real-time PCR

Trizol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract the total RNA from A549 and H1299 cells. Reverse transcription was performed with the cDNA synthesis kit (Promega, Madison, Wisconsin, USA) to obtain the cDNA. Real-time PCR was carried out with 50 ng cDNA and primers of MMP-3 (sense: 5'-ATGGACAAAGGATACAACAGGGA-3'; antisense: 5'-TGTGAGTGAGTGATAGAGTGGG-3'). The expression of β-actin (sense: 5'-CCAACCGCGAGAAGATGA-3'; antisense: 5'-CCAGAGGCGTACAGGGATAG-3') was also detected to normalize the level of MMP-3. The 2 –ΔΔCt method was performed to quantify the mRNA level of MMP-3 in A549 and H1299 cells (Livak et al., 2001).

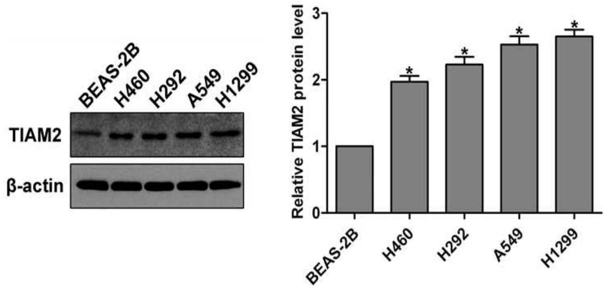

Figure 1. TIAM2 was Highly Expressed in NSCLC Cells. The protein levels of TIAM2 in H460, H292, A549, H1299 and BEAS-2B cells were detected by western blotting. Three independent experiments were performed. $*p<0.05$

ELISA assay

Cell supernatant was collected and centrifuged for 10 min at 10 000 rpm to remove cell debris. MMP-3 ELISA kit (Calbiochem, Darmstadt, Germany) was used to test the protein level of MMP-3 in the cell supernatant. Each sample was assayed in duplicate. The cells were lysed with RIPA lysis buffer, and the protein concentrations were detected using a BCA protein assay. Then the concentration of MMP-3 in the cell supernatant was determined by normalizing to the total protein of cells.

Statistical analysis

All data were presented as Means ± SD. Student's t test was performed to determine the significant difference between two means with the software of SPSS 17.0. *P*-values less than 0.05 were considered statistically significant.

Results

TIAM2 is highly expressed in NSCLC cells

To examine the expression of TIAM2, we performed western blotting in normal lung epithelial cell line (BEAS-2B) and non-small cell lung cancer cell lines (H460, H292, A549, H1299). Compared with the expression of TIAM2 in BEAS-2B cells, we found that TIAM2 was highly expressed in all the examined NSCLC cells (Figure 1), implying that TIAM2 may participate in the progression of NSCLC.

TIAM2 promotes cell invasion of NSCLC cells

To investigate the role of TIAM2 in NSCLC cells, we silenced the expression of TIAM2 in A549 and H1299 cells using siRNA technology. Then we examined the knockdown efficiency of TIAM2 by western blotting. As TIAM1 is the homolog of TIAM2, we also tested the expression of TIAM1 in A549 and H1299 cells. The result showed that the TIAM2 siRNA could achieve prominent effect on knockdown of TIAM2, but have little effect on TIAM1 expression (Figure 2A). After knockdown

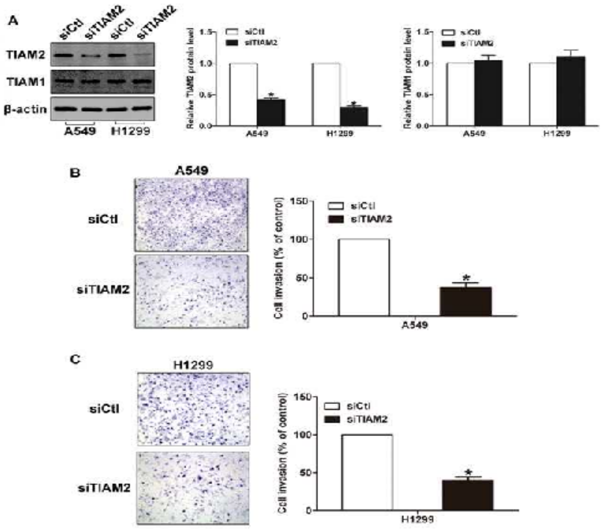

Figure 2. Knockdown of TIAM2 Suppressed Cell Invasion of NSCLC Cells. (A) A549 and H1299 cells were transfected with a TIAM2 siRNA to silence the expression of TIAM2 (siTIAM2). Cells were transfected with a scramble siRNA and used as control siRNA (siCtl). The expressions of TIAM2 and TIAM1 were detected by western blotting. (B) After knockdown of TIAM2, invasion assay was performed in A549 cells. (C) After knockdown of TIAM2, invasion assay was performed in H1299 cells. Three independent experiments were performed. *$p<0.05$

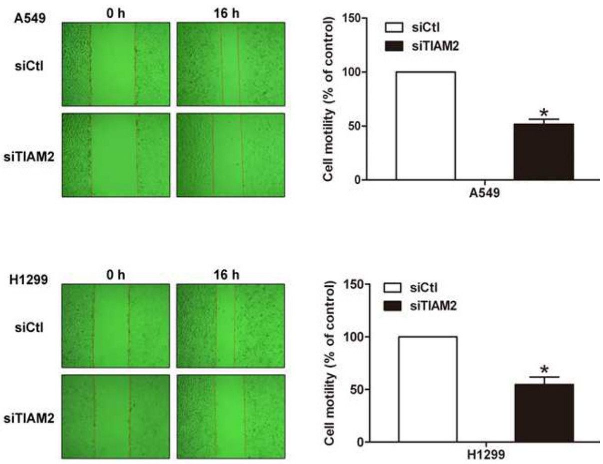

Figure 3. Knockdown of TIAM2 Inhibited Cell Motility of NSCLC Cells. (A) Effect of TIAM2 on the motility of A549 cells. (B) Effect of TIAM2 on the motility of H1299 cells. Three independent experiments were performed. *$p<0.05$

of TIAM2, invasion assay was carried out in A549 and H1299 cells. The result demonstrated that knockdown of TIAM2 suppressed the invasion of A549 and H1299 (Figure 2B and 2C), suggesting that TIAM2 can promote cell invasion in NSCLC cells.

TIAM2 enhances motility of NSCLC cells

Next, we carried out cell motility assay in A549 and H1299 cells to detect the effect of TIAM2 on cell migration. We found that compared with control siRNA cells, TIAM2 siRNA cells exerted lower relative motility (Figure 3A and 3B). The result indicates that TIAM2 is involved in the regulation of cell motility in NSCLC cells.

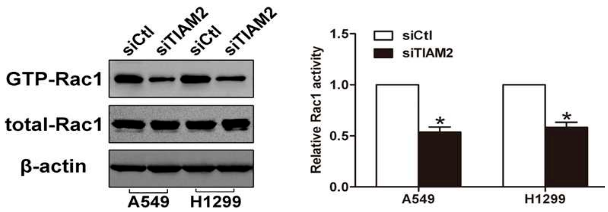

Figure 4. TIAM2 Induced the Activation of Rac1 in A549 and H1299 Cells. After transfected with TIAM2 siRNA (siTIAM2) or control siRNA (siCtl), GST-pull down assay was carried out to detect the activation of Rac1. Three independent experiments were performed. *$p<0.05$

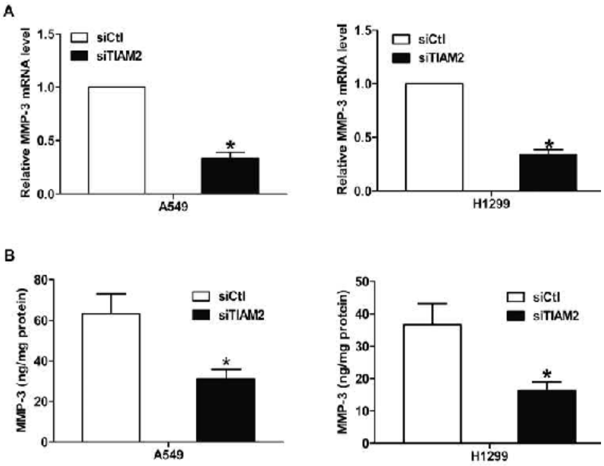

Figure 5. TIAM2 Promoted the Expression of MMP-3 in A549 and H1299 Cells. (A) After knockdown of TIAM2, the mRNA expression of MMP-3 was tested by real-time PCR in A549 and H1299 cells. (B) After knockdown of TIAM2, the protein level of MMP-3 was examined by ELISA assay in A549 and H1299 cells. Three independent experiments were performed. *$p<0.05$

TIAM2 is required for the activation of Rac1

Rac1 acts as a crucial player in cell invasion and migration of cancers (Heasman et al., 2008). Using GST-pull down assay, we demonstrated that Rac1 was significantly activated in control siRNA cells of A549 and H1299. After knockdown of TIAM2, the activation of Rac1 was inhibited, confirming that Rac1 activation is dependent on TIAM2 expression (Figure 4).

TIAM2 regulates the expression of MMP-3 in NSCLC cells

Matrix metalloproteinases (MMPs) are enzymes that can degrade extracellular matrix (ECM) and promote cell invasion of cancers (Martin et al., 2007). In our study, after knockdown of TIAM2, the mRNA expression of MMP-3 was tested by real-time PCR. Interestingly, we found that knockdown of TIAM2 decreased the expression of MMP-3 in A549 and H1299 cells (Figure 5A). Furthermore, we examined the protein level of MMP-3 by ELISA assay, and confirmed that knockdown of TIAM2 down-regulated the expression of MMP-3 in NSCLC cells (Figure 5B).

TIAM2 mediates the expression of some EMT-related genes

EMT process is crucial for cell invasion, and can be regulated by Rac1 activation and MMPs expression (Radisky et al., 2005). Here, we examined whether TIAM2

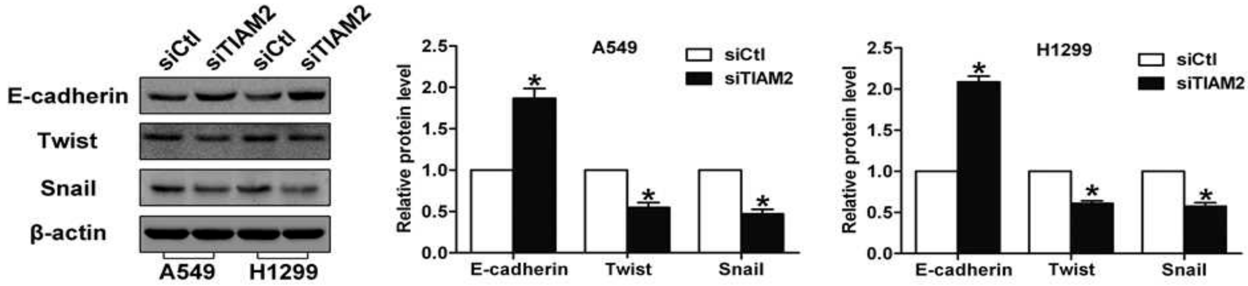

Figure 6. TIAM2 Regulated the Expression of E-cadherin, Twist and Snail. After knockdown of TIAM2, the expressions of E-cadherin, Twist and Snail were detected by western blotting. Three independent experiments were performed. **p*<0.05

was involved in the EMT process. Using western blotting, we found that after knockdown of TIAM2, the expression of E-cadherin was increased, whereas the protein levels of Snail and Twist were decreased in A549 and H1299 cells (Figure 6), suggesting that TIAM2 can mediate the expression of some EMT-related genes in NSCLC cells.

Discussion

Tumor invasion and metastasis, which account for approximately 90% of cancer-caused deaths, are complex and multistep biological processes (Mehlen et al., 2006). Lots of molecular pathways are up-regulated or activated during the processes (Spano et al., 2012). Therefore, investigation of these molecules may provide new aspects in the diagnosis and treatment of cancers. As a member of TIAM-family guanine nucleotide exchange proteins, TIAM2 has been reported to play an important role in cell adherence and migration, and participate in the human malignancies. In this study, we found that TIAM2 was highly expressed in NSCLC cells. We also found that TIAM2 could promote the invasion and motility of NSCLC cells. Further experiments proved that knockdown of TIAM2 inhibited the activation of Rac1, and down-regulated the expression of MMP-3. Additionally, knockdown of TIAM2 affected the expression of some EMT-related genes such as E-cadherin, Twist and Snail. All of these results suggested that TIAM2 was involved in the regulation of NSCLC cell invasion and migration.

TIAM-family guanine nucleotide exchange proteins, including TIAM1 and TIAM2, are critical players in cell development (Shepherd and Fuentes, 2011). The involvement of TIAM1 in the invasion and metastasis has been well studied in many tumors (Minard et al., 2004). As the homology of TIAM1, TIAM2 has been shown to regulate the reorganization of actin cytoskeletal in neuronal cells (Matsuo et al., 2002; Goto et al., 2011). Recent studies have found that TIAM2 promotes proliferation and invasion in liver cancer cells (Chen et al., 2012). In this study, using invasion assay and motility assay, we demonstrated that TIAM2 promoted cell invasion and motility in NSCLC cells.

Rac signaling is involved in the regulation of diverse tumor processes, such as tumorigenesis, survival, apoptosis and metastasis (Sun et al., 2006). Activation of Rac1 GTPase can induce EMT, and regulate the expression of MMPs (Mack et al., 2011). It is reported that TIAM2 is required for the activation of Rac1 GTPase in neuronal and skin papilloma cells (Matsuo et al., 2002; Rooney et al., 2010). Here, we found that TIAM2 could induce the activation of Rac1 in NSCLC cells, further confirming the role of TIAM2 in the regulation of Rac1 activation. Belonging to the matrix metalloproteinase family, MMP-3 is involved in the invasion and metastasis of various types of human cancers, including gastric (Liu et al., 2011), colon (Baba et al., 2004) and lung cancer (Petrella et al., 2012). In our study, we found that TIAM2 could promote the expression of MMP-3 at both mRNA and protein levels, indicating that the TIAM2-enhanced cell invasion and motility may be partially dependent on MMP-3 expression. EMT process has been proved to participate in the regulation of cell adherence and motility, and affect the invasion and metastasis of tumors (Radisky et al., 2005). Recent study has shown that TIAM2 regulates the expression of E-cadherin, N-cadherin and Vimentin in liver cancer cells (Chen et al., 2012). Using western blotting, we found that knockdown of TIAM2 could increase the expression of E-cadherin, and decrease the expression of Twist and Snail in A549 and H1299 cells, indicating that TIAM2 could induce EMT in NSCLC cells.

In conclusion, our study suggested that TIAM2 enhanced cell invasion and motility via activation of Rac1 and regulation of some EMT/invasion-related genes. Therefore, TIAM2 may act as a potential molecular target for the treatment of lung cancer.

Acknowledgements

The author(s) declare that they have no competing interests.

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