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Transforming growth factor-β1 increases cell migration and β1 integrin up-regulation in human lung cancer cells

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ABSTRACT

Transforming growth factor-β1 (TGF-β1) plays a crucial role in adhesion and migration of human cancer cells. Besides, integrins are the major adhesive molecules in mammalian cells. Here we found that TGF-β1 increased the migration and cell surface expression of β1 integrin in human lung cancer cells (A549 cells). TGF-β1 stimulation increased phosphorylation of p85α subunit of phosphatidylinositol 3-kinase (PI3K) and Ser^{473} of Akt was determined. Besides, we performed that PI3K inhibitor (Ly294002) or Akt inhibitor suppressed the TGF-β1-induced migration activities of A549 cells. Treatment of A549 cells with NF-κB inhibitor (PDTC) or IκB protease inhibitor (TPCK) also repressed TGF-β1-induced cells migration and β1 integrins expression. In addition, treatment of A549 cells with TGF-β1 induced IκB kinase α/β (IKKα/β) phosphorylation, IκB phosphorylation, p65 Ser^{536} phosphorylation, and κB-luciferase activity. Furthermore, the TGF-β1-mediated increases in IKKα/β, IκBα phosphorylation and p65 Ser^{536} phosphorylation were inhibited by Ly294002 and Akt inhibitor. Co-transfection with p85α and Akt mutants also reduced the TGF-β1-induced κB-luciferase activity. Taken together, our results suggest that TGF-β1 acts through PI3K/Akt, which in turn activates IKKα/β and NF-κB, resulting in the activations of β1 integrins and contributing the migration of human lung cancer cells.

1. Introduction

Lung cancer is the leading cause of cancer-related mortality in both men and women [1], with 1.2 million new cases diagnosed every year and 1 million deaths being recorded worldwide [2]. Non-small cell lung cancer (NSCLC) affects approximately 80% of all lung cancer patients. Most patients present with locally advanced (37%) or metastatic (38%) disease at the time of diagnosis [1], and a large percentage of those diagnosed with early-stage disease eventually experience recurrence of metastatic disease. Thus, the high invasiveness of NSCLC to regional lymph nodes, liver, adrenal glands, contralateral lung, brain, and bone marrow, etc. may play a key role in its biological virulence [1].

Decades of scrutiny into the molecular bases of cancer have largely focused on what causes oncogenic transformation and the incipient emergence of tumors [3]. The invasion of tumor cells is a complex, multistage process. To facilitate the cell motility, invading cells need to change the cell–cell adhesion properties, rearrange the extracellular matrix (ECM) environment, suppress anoikis and reorganize their cytoskeletons [4]. Integrins are a family of transmembrane adhesion receptors comprising 19 α and 8 β subunits that interact noncovalently to form up to 24 different heterodimeric receptors. The combination of different integrin subunits on the cell surface allow cells to recognize and respond to a variety of different ECM proteins including fibronectin, laminin, collagen and vitronectin [5]. Because integrins are the primary receptors for cellular adhesion to ECM molecules, they act as crucial transducers of bidirectional cell signaling, regulating cell survival, differentiation, proliferation, migration and tissue remodelling [6]. Integrin

Abbreviations: TGF, transforming growth factor; ECM, extracellular matrix; PI3K, phosphatidylinositol 3-kinase; IKKα/β, IκB kinase α/β; NF-κB, nuclear factor-κB.

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has been heavily implicated in tumor development [7,8], was correlated to reduced patient survival in colon carcinoma and melanoma [9,10], and has been associated with breast cancer cell metastasis to bone [11]. In addition, in vitro studies have found that integrins facilitated prostate cancer cell adhesion and migration through several ECM substrates [12,13], and transendothelial migration [14].

In the last 20 years, a large family of secreted polypeptides, known collectively as the transforming growth factor-β (TGF-β) super-family has been revealed [15]. TGF-β, originally called "sarcoma growth factor" was discovered in 1978 [16]. TGF-β is a potent growth inhibitor for a wide variety of cells including epithelial cells, vascular endothelial cells, hematopoietic cells, and immune lymphocytes. Perturbations of the TGF-β signaling pathways result in loss of cell growth regulation which is one of the most crucial steps in oncogenesis [17]. TGF-β family consists of three closely related isoforms (TGF-β1, -β2, and -β3) that are prototypes of the larger TGF-β super-family. TGF-β family members elicit a diverse range of cellular responses including cell proliferation, migration, fibrosis, inflammation, and wound repair [15,18]. TGF-β1 knockout mice develop diffuse mononuclear cell infiltrates that prove lethal within a few weeks of birth [19]. Recent studies have suggested a fundamental role for TGF-β1 as a critical mediator of the metastasis activity of cancer cells [20]. Evidence for the role of TGF-β signaling in the complex process of cancer metastasis has recently been documented specifically in breast cancer. In mouse models of breast cancer, TGF-β promotes bone metastasis mediated by secreted factors such as parathyroid hormone-related peptide, interleukin-11 and CTGF [21,22]. Besides, Smad signaling is required for this TGF-β-induced bone metastasis of breast cancer cells [23,24]. Although the mechanisms underlying TGF-β1-mediated tumor invasion have been studied in some cancers [20], the role of TGF-β1 in the process of human lung cells migration remains large unknown.

Previous studies have shown that TGF-β1 modulates cell migration and invasion in several cancer cells [20]. TGF-β1-mediated invasion may involve activation of integrins receptors [32,33]. However, the effect of TGF-β1 on integrins expression and migration activity in human non-small cell lung cancer cells is mostly unknown. Here we found a phenomenon that TGF-β1 increased the migration and the expression of β1 integrin of human lung cancer cells. In addition, phosphatidylinositol 3-kinase (PI3K), Akt, IKKα/β and NF-κB signaling pathways were involved it.

2. Materials and methods

2.1. Materials

Protein A/G beads, anti-mouse and anti-rabbit IgG-conjugated horseradish peroxidase, rabbit polyclonal antibodies specific for p-Akt, Akt, p85α, phosphotyrosine residues (PY20), IKKα/β, IκB, p-IκBα, α-tubulin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Ly294002, Akt inhibitor (1L-6-hydroxymethyl-chiro-inositol-2-((*R*)-2-*O*-methyl-3-*O*-octadecylcarbonate)), TPCK and PDTC were purchased from Calbiochem (San Diego, CA, USA). Rabbit polyclonal antibody specific for phosphor-IKKα/β ($Ser^{180/181}$) and phosphor-p65 (Ser^{536}) were purchased from Cell Signaling (Danvers, MA, USA). The recombinant human TGF-β1 was purchased from PeproTech (Rocky Hill, NJ, USA). Rabbit polyclonal antibody specific for β1 integrin was purchased from Chemicon (Temecula, CA). The p85α (Δp85; deletion of 35 amino acids from residues 479 to 513 of p85) and Akt (Akt K179A) dominant-negative mutants were gifts from Dr. W.M.Fu (National Taiwan University, Taipei, Taiwan). The IKKα (KM) and IKKβ (KM) mutants were gifts from Dr. H. Nakano (Juntendo University, Tokyo, Japan). pSV-β-galactosidase vector and luciferase assay kit were purchased from Promega (Madison, MA, USA). All other chemicals were obtained from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Cell culture

The human lung adenocarcinoma cell lines (A549) were obtained from the American Type Culture Collection. The cells were maintained in Dulbecco's modified Eagle's medium/Nutrient Mixture Ham's F12 (DMEM/F12) medium which was supplemented with 10% heat-inactivated FCS, 2 mM-glutamine, penicillin (100 U/ml) and streptomycin (100 ng/ml) at 37 °C with 5% CO_2.

2.3. Migration assay

The migration assay was performed using Transwell (Costar, NY; pore size, 8 μm) in 24-well dishes. Before performing the migration assay, cells were pretreated for 30 min with different concentrations of inhibitors, including the Ly294002 (10 μM), Akt inhibitor (10 μM), PDTC (30 μM), TPCK (3 μM) or vehicle control (0.1% DMSO). Those concentrations of inhibitors didn't affect cell death or proliferation of A549 cells by cell viability assay (data not show). Approximately 1×10^4 cells in 200 μl of serum-free medium were placed in the upper chamber, and 300 μl of the same medium containing 10 ng/ml TGF-β1 was placed in the lower chamber. The plates were incubated for 24 h at 37 °C in 5% CO_2, then cells were fixed in methanol for 15 min and stained with 0.05% crystal violet in PBS for 15 min. Cells on the upper side of the filters were removed with cotton-tipped swabs, and the filters were washed with PBS. Cells on the underside of the filters were examined and counted under a microscope. Each clone was plated in triplicate in each experiment, and each experiment was repeated at least three times. The number of invading cells in each experiment was adjusted by the cell viability assay to correct for proliferation effects of the TGF-β1 treatment (corrected invading cell number = counted invading cell number/precentage of viable cells) [34].

2.4. Flow cytometric analysis

Human lung cancer cells were plated in six-well dishes. The cells were then washed with PBS and detached with trypsin at 37 °C. Cells were fixed for 10 min in PBS containing 1% paraformaldehyde. After rinsed in PBS, the cells were incubated with rabbit anti-human antibody against β1 integrin (1:100) for 1 h at 4 °C. Cells were then washed again and incubated with fluorescein isothiocyanate-conjugated goat anti-rabbit secondary IgG (1:150; Leinco Tec. Inc., St. Louis, MO, USA) for 45 min and analyzed by flow cytometry using FACS Calibur and CellQuest software (BD Biosciences) [35].

2.5. Western blot analysis

The cellular lysates were prepared as described previously [36]. Proteins were resolved on SDS–PAGE and transferred to Immobilon polyvinyldifluoride (PVDF) membranes. The blots were blocked with 4% BSA for 1 h at room temperature and then probed with rabbit anti-human antibodies against IκBα, p-IκB, IKKα/β or p-Akt (1:1000) for 1 h at room temperature. After three washes, the blots were subsequently incubated with a donkey anti-rabbit peroxidase-conjugated secondary antibody (1:1000) for 1 h at room temperature. The blots were visualized by enhanced chemiluminescence using Kodak X-OMAT LS film (Eastman Kodak, Rochester, NY). Lanes were digitized by UN-SCAN-IT gel™ (a gel & graph digitizing softwave, version 6.1 silk scientific corporation; Utah, USA).