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APBB1 reinforces cancer stem cell and epithelial-to-mesenchymal transition by regulating the IGF1R signaling pathway in non-small-cell lung cancer cells

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ABSTRACT

Amyloid β precursor protein binding family B member 1(APBB1) was first identified as a binding partner of amyloid precursor protein during brain development, but its function in the context of cancer remain unclear. Here we show for the first time that APBB1 is partly associated with intensifying cancer stem cell(CSC) and epithelial-to-mesenchymal transition (EMT) and enhancing radiation-resistant properties of lung cancer cells. We found that *APBB1* was highly expressed in ALDH1^{high} CSC-like cells sorted from A549 lung cancer cells. In APBB1-deficient H460 cells with forced overexpression of *APBB1*, the protein directly interacted with IGF1Rβ, enhanced phosphorylation of IGF1Rβ/PI3K/AKT pathway(activation) and subsequently induced the phosphorylation of GSK3β(inactivation). This phosphorylation stabilized Snail1, a negative regulator of *E-cadherin* expression, and regulated β-catenin–mediated *ALDH1* expression, which are representative markers for EMT and CSCs, respectively. In contrast, suppression of *APBB1* expression with siRNA yielded the opposite effects in APBB1-rich A549 cells. We concluded that APBB1 partly regulates the expression of *ALDH1*. We also found that APBB1 regulates activation of nuclear factor-κB, which is involved in reducing various stresses including oxidative stress, which suggests that APBB1 is associated with γ-radiation sensitivity. Our findings imply that APBB1 plays an important role in the maintenance of EMT-associated CSC-like properties and γ-radiation resistance via activation of IGF1Rβ/AKT/GSK3β pathway in lung cancer cells, highlighting APBB1 as a potential target for therapeutic cancer treatment.

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1. Introduction

Cancer stem cells(CSCs), also known as tumor-initiating cells of small population, have been suggested as being responsible for tumorigenesis and tumor recurrence because of their pluripotency, self-renewing ability, and resistance to therapeutic treatments [1,2]. Although the CSC model suggests that CSCs can arise from normal stem cells, studies have shown that the epithelial-to-mesenchymal transition(EMT) process, which is a key stage in metastatic dissemination, can play an important role in generating CSC-like properties in non tumorigenic cancer cells [3,4]. Among the specific CSC markers that have been reported, aldehyde dehydrogenase 1(ALDH1) is an important biological marker characterizing CSC-like property in many cancer cells [5–7]. Therefore, the characteristic features and underlying molecular mechanisms for determining CSC-like characteristics in ALDH1^{high} cells should be identified for potential therapeutic application.

Amyloid β precursor protein binding family B member 1(APBB1 or Fe65) is a brain-enriched adaptor protein that has multiple protein-interacting domains including the N-terminal WW domain and two phosphotyrosine-binding(PTB) domains at the C-terminal

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[8]. The PTB2 domain of APBB1 interacts with amyloid precursor protein (APP), an important molecule in Alzheimer's disease, and is involved in diverse biological processes during neuronal differentiation as well as in neurodegenerative diseases. The functions of APBB1 have been well established in brain development including neurogenesis and synaptogenesis [9–11]. Moreover, APBB1 has been reported to play an essential role in the cellular response to genotoxic stress-induced DNA damage [12,13]. A recent study suggested that phosphorylation of the Ser288 residue within the APBB1 molecule is essential for inhibition of gene transcription by the APBB1-APP complex [14]. Earlier studies of APBB1 primarily focused on neuronal functions or disease, but recent investigations have suggested that APBB1 plays a role in cancer cells as well. APBB1 has been shown to interact with the endogenous ERα through its PTB2 domain and to potentiate tumor growth in breast cancer cells [15]. However, APBB1 also suppressed EMT of ERα-negative tumors by promoting the acetylation of cortactin through Tip60 [16]. Overall, little is known about the possible functions of APBB1 in cancer cells.

Type 1 insulin-like growth factor receptor(IGF1R), a membrane receptor tyrosine kinase, is composed of two extracellular α subunits, which are responsible for ligand binding, and two intracellular β subunits. Binding of IGF to IGF1R leads to autophosphorylation of tyrosine residues in the kinase domain of the β subunit, which sequentially activates docking proteins, such as insulin receptor substrates(IRS) or Src homology/collagen domain protein(SHC) that promote cell growth, migration, and survival [17,18]. In addition, the IGF1R signaling pathway has been shown to be potentially involved in the acquisition of therapeutic resistance in lung cancer cells [19]. Here, we show for the first time that APBB1 acts as a potential regulator that partly controls cell stemness, EMT, and radiation-resistant properties via activation of the IGF1Rβ/AKT/GSK3β signal pathway in non–small-cell lung cancer(NSCLC) cells.

2. Materials and methods

2.1. Cell culture and irradiation

Human NSCLC cells were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). A549 and H460 cell lines were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum(Hyclone, Logan, UT, USA) and 1% penicillin/streptomycin. Cells were maintained at 37 °C under a humidified atmosphere with 95% air/5% CO_2. For γ-ray irradiation, cells were plated in 35-mm dishes at a density of 1×10^3 cells/plate the day before treatment and were irradiated with a single dose of radiation(6 Gy) using a ^{60}Co γ-ray source(Korea Atomic Energy Research Institute) at a dose rate of 0.2 Gy/min.

2.2. cDNA synthesis and PCR amplification (APBB1)

Total RNA was isolated from cancer cell lines using RNA extraction TRIzol reagent (Invitrogen, Carlsbad, CA, USA). cDNA was then synthesized using a cDNA synthesis kit(Intron Biotechnology, Gyungki-do, Korea) and served as templates for polymerase chain reaction(PCR) amplification with the following forward and reverse primers: APBB1-forward, 5′-GTGGGTCGGGCTCCAG-3′; APBB1-reverse, 5′-ATTGCGATT CTGGTCACGGT-3′; ALDH1A1-forward, 5′-ATATAAGCTTATGTCATCCTCAGG CACGCCA-3′; ALDH1A1-reverse, 5′-ATATGAATTCTTATGAGTTCTTCTGAGAG AT-3′; ALDH1A3-forward, 5′-GCCCTGGAGACGATGGATAC-3′; ALDH1A3-reverse, 5′-TCCACTGCCAAGTCCAAGTC-3′; GAPDH-forward, 5′-ATGGGGAAGG TGAA GG-3′; GAPDH-reverse, 5′-TTACTCCTTGGAGGCC-3′. For the construction of *APBB1* expression vector, a 2132-bp insert of human *APBB1* is amplified from human lung cancer cell cDNA and cloned into pcDNA3.1(+) vector using following primers containing restriction enzyme site sequences: APBB1-forward [*Bam*HI], 5'-GCGGGATCCATGTCTGTTCCATCATCACTG-3'; APBB1-reverse [*Eco*RV], 5'-ATATGATATCTCATGGGGTATGGGCCCC-3'.

2.3. Western blot analysis

Western blot analysis was performed with primary antibodies specific for human APBB1, IGF1Rβ, p-IGF1Rβ, Twist, Snail, Zeb1, β-catenin, GAPDH, PI3K, p-PI3K, and Sox-2(Santa Cruz Biotechnology, Dallas, TX, USA); AKT, p-AKT, GSK3β, p-GSK3β, NFκB, p-NFκB, ATM, p-ATM, γH2AX, E-cadherin, and β-actin(Cell Signaling Technology, Beverly, MA, USA); N-cadherin(BD Biosciences, San Jose, CA, USA); Oct3/4(Millipore, Billerica, MA, USA); and anti-ALDH1A1 and ALDH1A3 (Abcam, Cambridge, UK). Proteins were separated on a 10% or 15% sodium dodecyl sulfate–polyacrylamide gel and transferred to a nitrocellulose membrane(Hybond; Amersham Pharmacia, Piscataway, NJ, USA). Membranes were blocked with 5% nonfat dry milk in TBST(50 mM tris-HCl, pH7.6, 150 mM NaCl, and 0.1% Tween 20) for 1 h at room temperature. Membranes were incubated overnight in a cold chamber with specific antibodies. After being washed three times with TBS, the membrane was incubated with secondary antibody for 1 h and visualized with the WEST-ZOL enhanced chemiluminescence detection kit(Intron Biotechnology).

2.4. Silencing RNA targeting of APBB1

Cells were transfected with Stealth™ RNAi targeting *APBB1*(Invitrogen; primer sequences: 5′-GGAUGAGACACUAAAGCUATT-3′/5′-UAGCUUUAGUGUCUCAUC CTC-3′) and *β-catenin*(primer sequences: 5′-CGUUCUCCUCAGAUGGUGU(dTdT)-3′/5′-ACACCAUCUGAGGAGAACG(dTdT)-3′, Bioneer, Daejeon, Korea) or with Stealth RNAi Negative Control Medium GC(Invitrogen) using Lipofectamine RNAi MAX reagent(Invitrogen). The cells were incubated for 72 h after transfection, and then APBB1 or β-catenin was determined by RT-PCR or Western blot analysis.

2.5. Flow cytometric analysis and CSCs sorting

The ALDEFLUOR reagent system(STEMCELL Technologies, Vancouver, BC, Canada) was used to isolate and characterize populations of ALDH1^{high} cells in A549 cells according to the manufacturer's instructions. Cells(1×10^6) were resuspended in assay buffer containing the fluorescent ALDH1 substrate, ALDEFLUOR™, and incubated for 30 min at 37 °C. Baseline fluorescence was established by incubation of the specific ALDH1 inhibitor, diethylaminobenzaldehyde(DEAB). Then the ALDEFLUOR™ fluorescence was measured with Cytomics FC 500 flow cytometer (Beckman Coulter Counter, Fullerton, CA, USA).

2.6. Cell migration and invasion assay

Cell migration assays were performed using Transwell cell culture chamber inserts with 8.0-μm pores(Costar, Cambridge, MA, USA) according to the manufacturer's recommendations. For invasion assay, the upper chambers were coated with Matrigel gel(Sigma-Aldrich, St Louis, MO, USA). Cells were incubated for 24 h(migration assay) or 48 h(invasion assay) at 37 °C in a humidified atmosphere of 95% air/5% CO_2. After the indicated incubation times, cells on the upper surface of the membrane were removed by wiping with a cotton tips. The migrant cells attached to the lower surface were stained with 2% crystal violet and incubated for 5 min. The membrane was washed several times with phosphate-buffered saline, and the cells within the filter were

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