Peptides 90 (2017) 1–9

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Atrial natriuretic peptide: A novel mediator for TGF-β1-induced epithelial-mesenchymal transition in 16HBE-14o and A549 cells

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ARTICLE INFO

Article history:
Received 15 September 2016
Received in revised form 9 February 2017
Accepted 9 February 2017
Available online 13 February 2017

Keywords:
Atrial natriuretic peptide
Epithelial-mesenchymal transition
Bronchial epithelial cells
Alveolar epithelial cells

ABSTRACT

Atrial natriuretic peptide (ANP) is increasingly expressed on airway and inhibits pulmonary arterial remodeling. However, the role of ANP in remodeling of respiratory system is still unclear. The role of ANP on airway remodeling and the possible mechanism was explored in this study. Both human bronchial epithelial 16HBE-14o cells and alveolar epithelial A549 cells were stimulated by TGF-β1, ANP, cGMP inhibitor, PKG inhibitor, and cGMP analogue. The expressions of epithelial markers, mesenchymal markers, and Smad3 were assessed by quantitative real-time PCR and western blotting. Immunohistochemical staining was employed to assess Smad3 expression once it was silenced by siRNA in 16HBE-14o or A549 cells. Our results showed that the mRNA and protein expressions of E-Cadherin were decreased, whereas α-SMA expressions were increased after induction by TGF-β1 in 16HBE-14o and A549 cells. The E-Cadherin expressions were increased and α-SMA expressions were decreased after ANP stimulation. Inhibition of cGMP or PKG decreased E-Cadherin expression but increased α-SMA expression, which could be reversed by cGMP analogue. Moreover, the phosphorylated Smad3 expression was consistent with α-SMA expression. After smad3 was silenced, Smad3 was mostly expressed in cytoplasm instead of nucleus as non-silenced cells during epithelial-mesenchymal transition (EMT). In conclusion, ANP inhibits TGF-β1-induced EMT in 16HBE-14o and A549 cells through cGMP/PKG signaling, by which it targets TGF-β1/Smad3 via attenuating phosphorylation of Smad3. These findings suggest the potential of ANP in the treatment on pulmonary diseases with airway remodeling.

1. Introduction

Natriuretic peptides have attracted concerns in human bronchi, particular brain natriuretic peptide, which may be involved in chronic respiratory disorders by regulating the function of bronchial epithelium and airway smooth muscle [1]. In contrast, a member of natriuretic peptides secreted by cardiomyocytes, namely atrial natriuretic peptide (ANP), in the respiratory disorders comes into the light of researchers. ANP is a polypeptide hormone that controls blood pressure via reducing water and sodium loads in circulatory system [2]. ANP exerts biological effect through binding to natriuretic peptide receptor A and activating cGMP/PKG signaling [3,4]. Other than cardiomyocytes, ANP could also be secreted and degraded in lung, which may play a local regulatory role in the pathogenesis of pulmonary diseases [5,6]. A double blind and randomized trial demonstrated that high-dose inhaled ANP serves as a bronchodilator in asthmatic patients [7], but the underlying mechanisms remained unclear.

More recently, ANP was noted to inhibit pulmonary arterial remodeling via cGMP signaling in response to hypoxia stress [8]. ANP supressed TGF-β1-induced myofibroblast transformation, proliferation, and expression of extracellular matrix molecules in cardiac fibroblasts, suggesting that ANP may repress mesenchymal transition. In respiratory system, airway epithelial-mesenchymal transition (EMT) contributes to the pathophysiology of airway remodeling in asthma, chronic obstructive pulmonary disease and lung cancer, and may determine the efficiency of therapeutic efficacy in these diseases [9–11]. Therefore, we investigated the role of

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http://dx.doi.org/10.1016/j.peptides.2017.02.002
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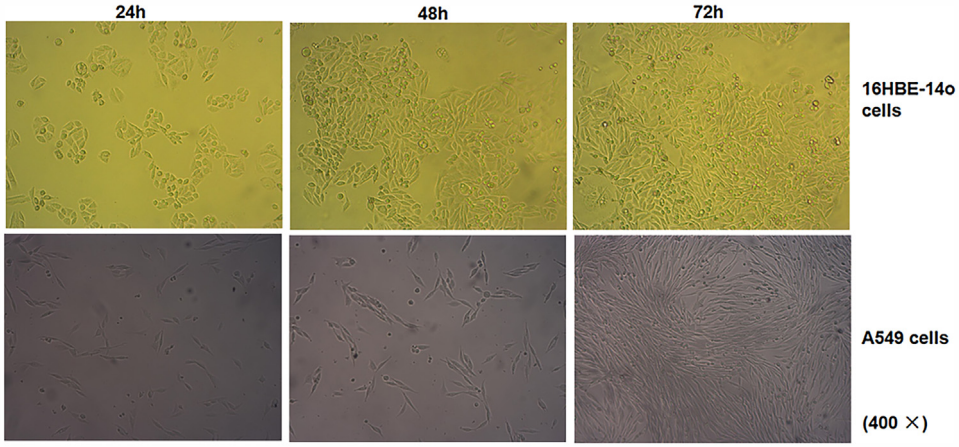

Fig. 1. Epithelial-mesenchymal transition (EMT) morphology of human bronchial epithelial cells and alveolar epithelial cells after TGF-β1stimulation. Human bronchial epithelial cells (16HBE-14o) and human alveolar epithelial cells (A549) were co-cultured with 10 ng/ml TGF-β1 for 0 h, 24 h, 48 h, and 72 h, respectively. Cells in control group were cultured without TGF-β1. After 24 h, 16HBE-14o and A549 cells became spindle fibroblast-like morphology with an elongated shape and reduced cell–cell contact, which indicated to EMT.

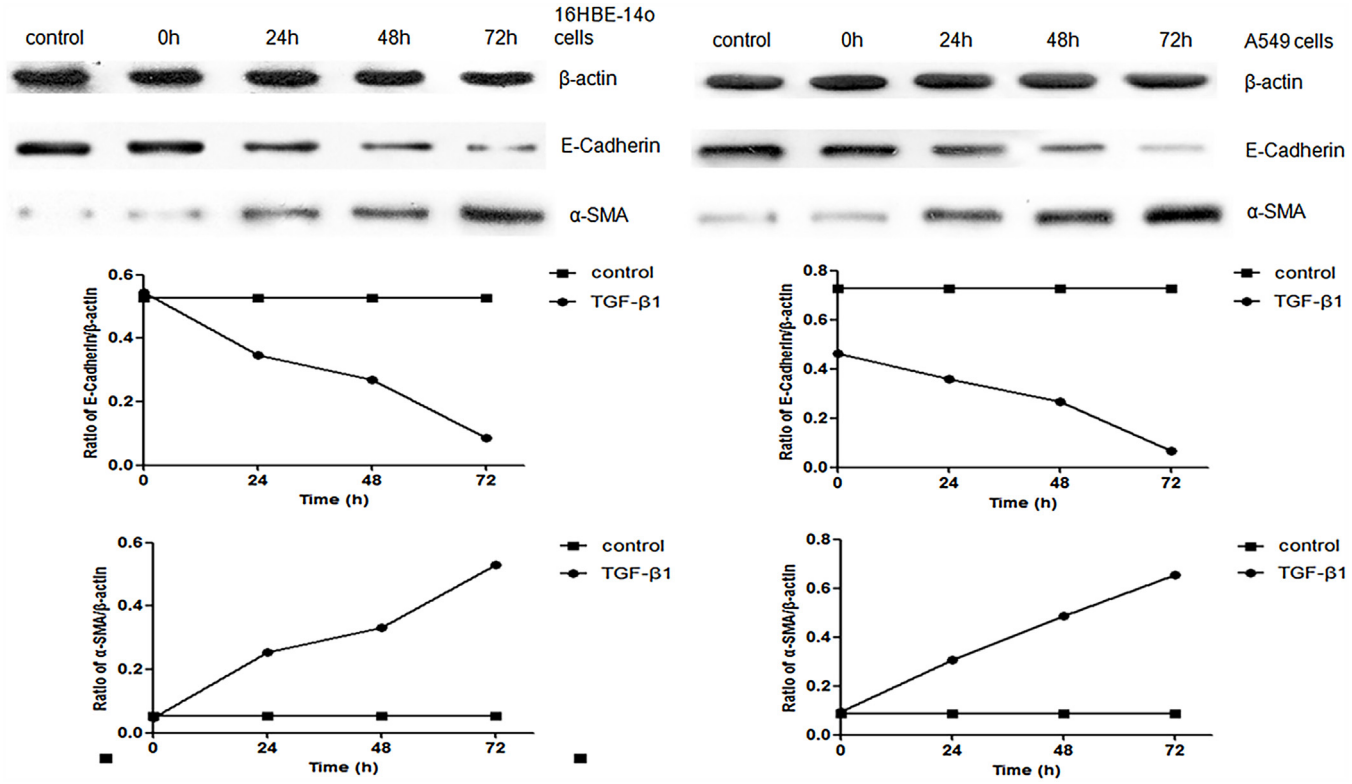

Fig. 2. The expressions of epithelial marker and EMT marker in human bronchial epithelial cells and alveolar epithelial cells after TGF-β1 stimulation. In both 16HBE-14o and A549 cells, the expression of epithelial marker E-Cadherin protein was decreased after stimulation with TGF-β1, whereas the mesenchymal marker α-SMA protein expression was increased in both 16HBE-14o and A549 cells. The expression of E-Cadherin and α-SMA proteins was in a time-dependent manner of TGF-β1 stimulation.

ANP in human airway EMT using human bronchial epithelial cells and alveolar epithelial cells.

2. Methods

2.1. Cell culture

Human 16HBE-14o and A549 cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in 1640RPM medium (Hyclone) with 10% fetal bovine serum (FBS, Gibco) at 37 °C in 5% CO2, and were subdivided into eight groups as follows: control group without any treatment, TGF-β1 group with 10 ng/ml TGF-β1, ANP group with 1uM ANP for 30 min and followed by 10 ng/ml TGF-β1, A71915 group with 1uM A71915 for 30 min and followed by 1uM ANP, 8-Br-cGMP group with 1 mM 8-Br-cGMP for 30 min and then 10 ng/ml TGF-β1, Rp-8-Br-cGMP group with 50 μM Rp-8-Br-cGMP for 30 min and then treated as 8-Br-cGMP group, KT + ANP group with 1uM KT5823 for 30 min and then treated as ANP group, KT + 8-Br-cGMP group with 1uM KT5823 for 30 min and then treated as 8-Br-cGMP group.