The Role of ESE-1 in Tumorigenesis and Metastasis in Human Non-Small Cell Lung Cancer (NSCLC) Cells

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Introduction

Epithelium-specific Ets-1 (ESE-1) protein belongs to the superfamily of ETS transcription factors and is mainly expressed in epithelial-rich tissues such as lung tissue. Role of ESE-1 in cancer is complex. ESE-1 expression is associated with poor prognosis in breast cancer and colorectal cancer patients while downregulation of ESE-1 expression was related to reduce survival in ovarian cancer and ESE-1 inhibits the invasion of oral squamous cell carcinoma. However, a role of ESE-1 in lung tumorigenesis is questionable. Therefore, the aim of this study is to investigate if ESE-1 modulates lung cancer development using human NSCLC cells. Here, we report that ESE-1 possesses the anti-tumorigenic activities in NSCLC.

Abstract

Objectives

Lung cancer is the leading cause of cancer mortality in the United State due to poor prognosis. Identification of effective molecular target is essential for lung cancer prevention and therapy. Epithelial Specific ETS-1 (ESE-1) is a transcription factor that belongs to ETS superfamily and associated with development of several types of cancer. However, the significance of ESE-1 in lung cancer remains unanswered. The objective of the current study was to investigate if ESE-1 expression influences tumorigenic and metastatic activity of human non-small cell lung cancer (NSCLC).

Methods

Soft agar, FACS analysis, apoptosis, invasion and migration assays were prepared to investigate the phenotype of ESE-1 stably overexpression cells. Xenograft study were performed to identify the formation and development of tumor. ESE-1 promoters were transfected into H1299 and H1703 cells and the luciferase activity was measured using a dual-luciferase assay kit. EMT was induced by treatment of TGF-β to smad2- and smad3 knockdowned A549 cells. ESE-1 expression was measured using Western blotting.

Results

Figure 1. Basal expression of ESE-1 in human lung cancer cells.

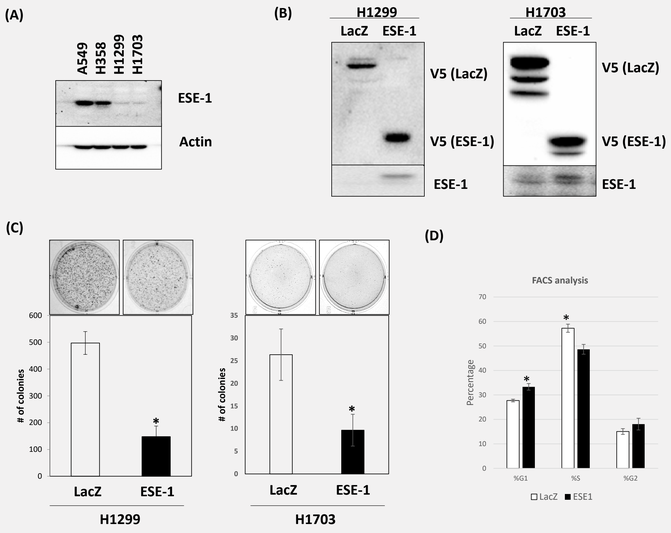

Figure 1. (A) A549, H358, H1299, and H1703 cell lysates were harvested and subjected to WB analysis for ESE-1 and actin. (B) H1299 and H1703 stable cell lines expressing V5-tagged LacZ (control) and V5-tagged ESE-1 were established and cell lysates were harvested and subjected to WB analysis for V5 and ESE-1. (C) Soft agar assay was performed as described in *Materials and Methods*. The image was taken by ChemiDoc MP Imaging system after 2 weeks. (D) Cell cycle distribution was performed by staining the cells with propidium iodide and subsequent FACS analysis. (E) Apoptosis was measured using Cell death Detection ELISA. (F) The cells were harvest and subjected to WB analysis for CDK4, CDK6, p27, Bcl2 and Actin (Left). Data represent mean ± SD from three replicates (Right).

Figure 2. ESE-1 represses cell migration and invasion of lung cancer cells.

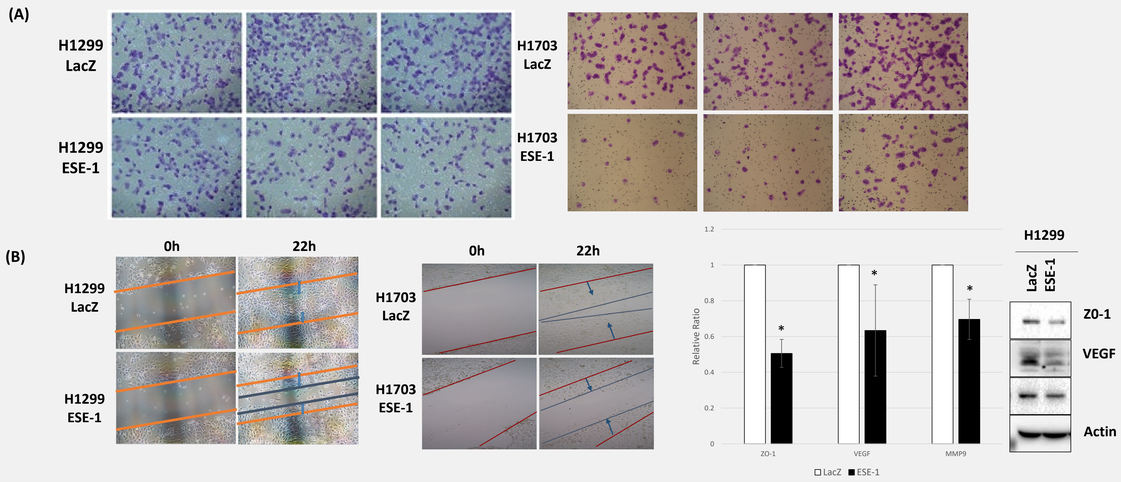

Figure 2. (A) H1299 and H1703 stable cells expressing LacZ (control) and ESE-1 were subjected to trans-well assay. After 12 h incubation, cells were stained, and the image was taken using the microscope. (B) Stable LacZ and ESE-1 cells subjected to wound healing assay for 0 and 22 h. (C) H1299 stable cells LacZ and ESE-1 were harvest and subjected to WB analysis for ZO-1, VEGF, MMP9 and Actin.

Figure 3. ESE-1 suppresses formation and development of tumor *in vivo*.

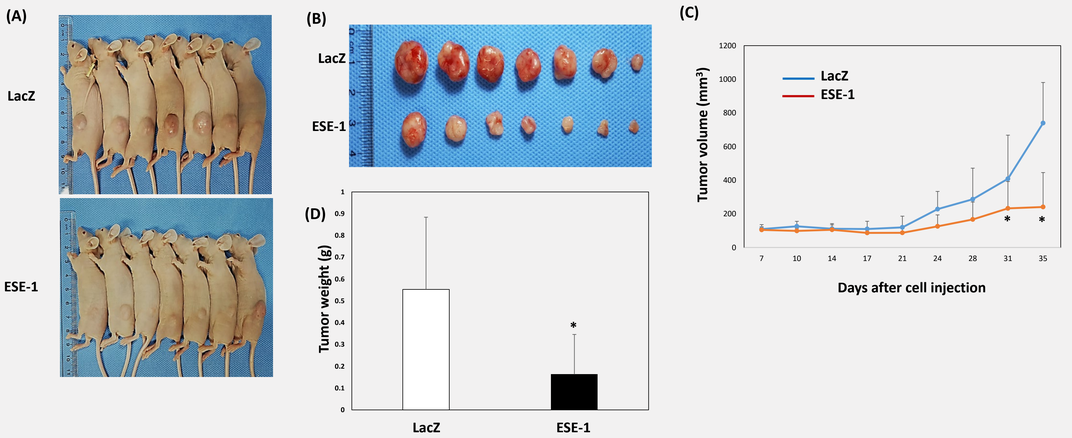

Figure 3. H1299 stable cell lines overexpressing LacZ and ESE-1 were subcutaneously injected into the nude mice. On 35 days after injection, the mice were sacrificed. (A) Representative pictures of xenograft tumors. (B) Comparison of extracted tumors. (C) Mean tumor volume, Data are expressed as mean±SD of 7 mice. (D) Mean tumor weight.. *, P<0.05 versus control

Figure 4. Exogenous ESE-1 overexpression represses NF-κB transcription activity in H1299 and H1703 cells.

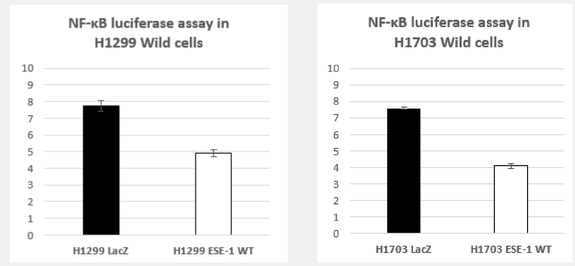

Figure 4. (A) H1299 and H1703 wild cells were transiently transfected by using ESE-1 (control and ESE-1), NF-κB promoter, and pRL-null vectors with PolyJet DNA transfection reagent (SignaGen) for 48 h. The luciferase activity was measured and normalized to the pRL-null luciferase activity with a dual-luciferase assay kit (Promega).

Figure 5. TGF-β-induced ESE-1 downregulation is Smad2/3 dependent in human lung cancer cells.

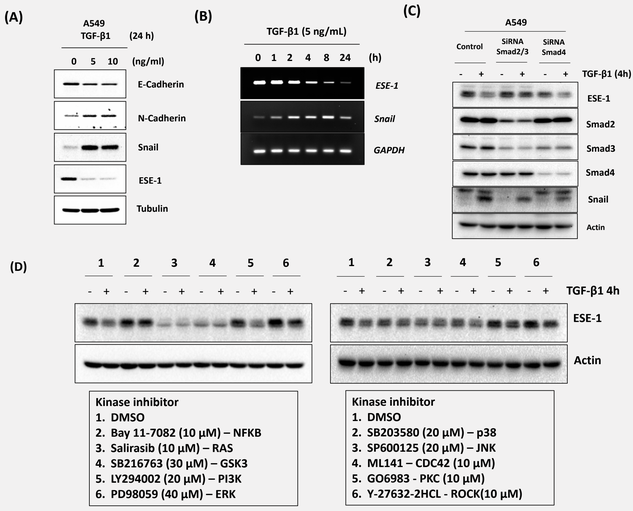

Figure 3. (A) A549 treated with TGF-β1 for indicated doses and time. WB were preformed. (B) A549 cells were treated with TGF-β1 using indicated time. RNA was isolated and (RT)-PCR was performed.
(C) A549 cells were transfected with control and Smad siRNA with Lipofectamine 3000 and then treated with TGF-β (5 ng/ml) for 4h. Lysates were subjected to western blotting. (D) A549 cells were treated with selective inhibitors for 1.5h and then co-treated with TGF-β 4 hours. Lysates were subjected to western blotting.

Conclusion

- **ESE-1 has the anti-tumorigenic and anti-metastatic activities in human NSCLC cells.**

Acknowledgement

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