Poly I:C Suppresses TGF-β1-Induced Akt Phosphorylation and Reduces the Motility of A549 Lung Carcinoma Cells

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Abstract

Backgrounds: Transforming growth factor (TGF)-β is shown to play a critical role in cancer progression by inducing epithelial mesenchymal transition (EMT). Polyinosinic-polycytidylic acid (polyI:C), a synthetic agonist for toll-like receptor (TLR) 3, has been successfully used to treat some cancer patients as a vaccine adjuvant, but its direct action on the proliferation or migration of cancer cells, such as lung cancer cells, undergoing EMT remains unknown.

Methods and results: By an in vitro cell proliferation assay, polyI:C showed no effect on the growth of TGF-β1-treated A549 human lung cancer cells at the concentration range up to 10 mg/ml; however, it markedly suppressed the motility in a cell scratch and a cell invasion assay. By Western blotting, polyI:C dramatically decreased TGF-β1-induced Ak strain transforming (Akt) phosphorylation and increased phosphatase and tensin homologue (PTEN) expression without affecting the Son of mothers against decapentaplegic (Smad) 3 phosphorylation or the expression level of E-cadherin, N-cadherin or Snail, indicating that polyI:C suppressed cell motility independently of the ‘cadherin switching’. The Akt inhibitor perifosine inhibited TGF-β1-induced cell invasion, and the PTEN-specific inhibitor VO-OHpic appeared to reverse the inhibitory effect of polyI:C.

Conclusion: Our results indicate that polyI:C has the capacity to suppress the motility of TGF-β1-treated A549 cells by targeting the phosphatidylinositol 3-kinase /Akt pathway partly via PTEN and suggest that polyI:C may be used to prevent or reduce the metastasis of lung cancer cells.

Full Text

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Figures

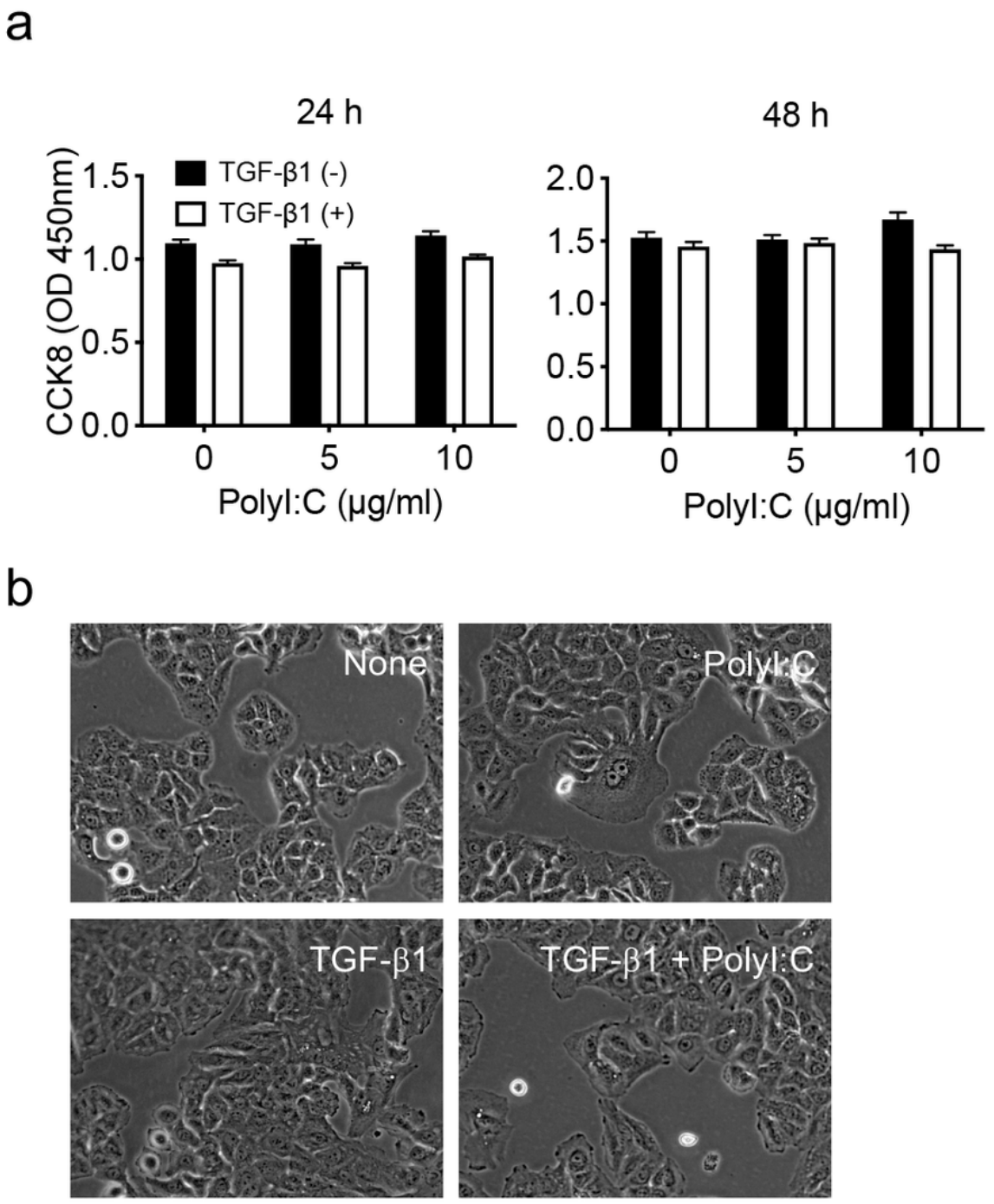

Figure 1

The effects of polyI:C on the proliferation and shape change of A549 cells treated with TGFb1. (a) A549 cells (1.5 x 104) were cultured with 10 ng/ml TGF-b1 plus different doses of polyI:C for 24 or 48 hours and the number of cells in each well was quantitated using CCK-8. Data is presented as mean ± SEM. **p < 0.001, n=12. (b) A549 cells (2.5 x 105) were incubated in 60-mm plastic dishes as indicated for 24

hours and changes in cell morphology were examined under microscopy (x200). TGF-b1; 10 ng/ml, polyI:C; 10 mg/ml.

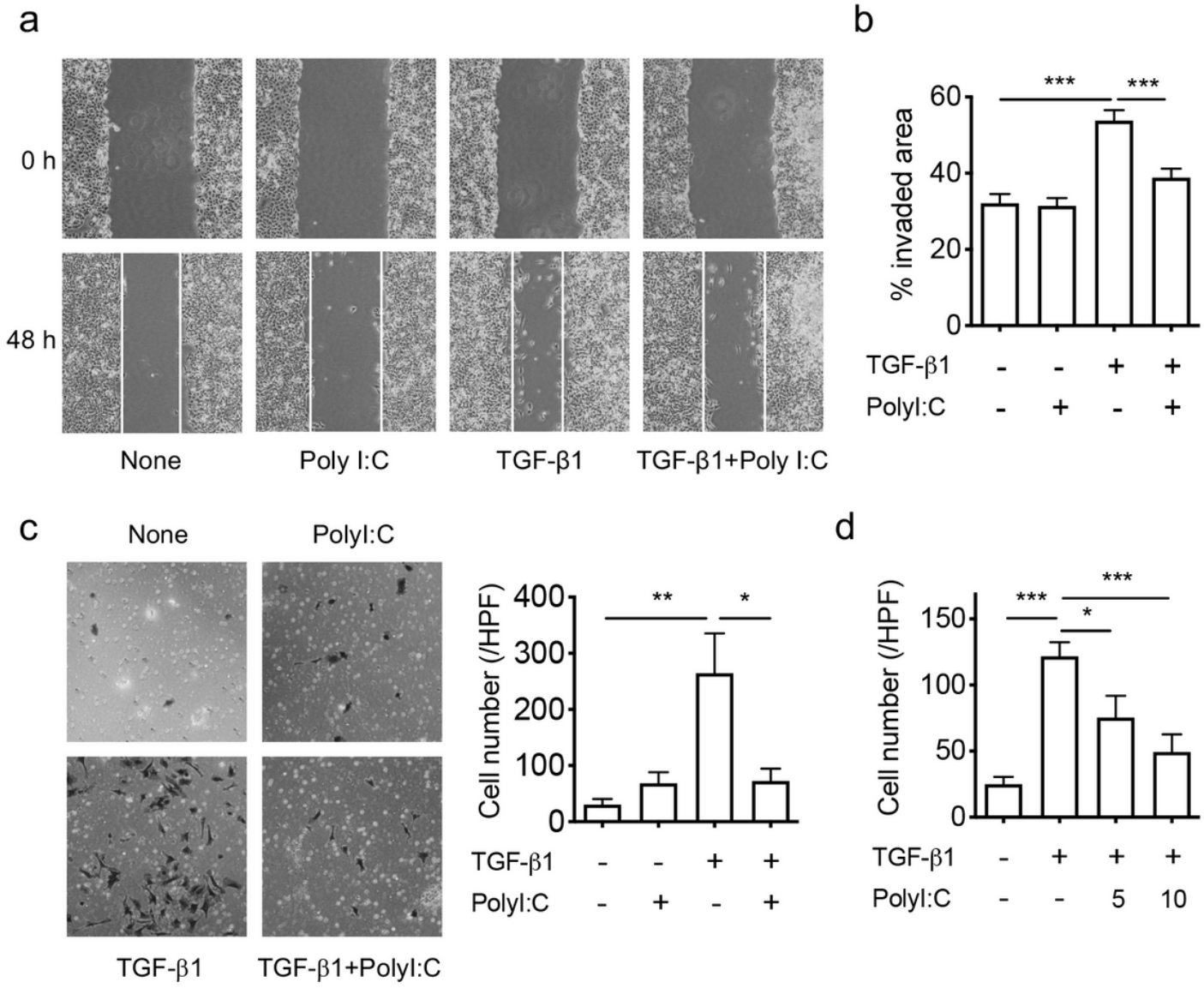

Figure 2

The effects of polyI:C on TGF-b1-induced migration and invasion of A549 cells. (a) A549 cells (2 x 106) were incubate at 37ºC for overnight in RPMI-1640 containing 10% HI-FBS. Wounds were made by gently scratching cell monolayers with sterile yellow pipette tips, and monolayers were cultured for 48 hours in RPMI 1640 supplemented with 0.5% HI-FBS containing 10 mg/ml polyI:C alone, 10 ng/ml TGFb1 alone or both. Wounded areas were observed under microscopy and photos were taken. x100. (b) Wounded areas were quantitated and represented as the percentage of wounded area at time 0. Data is presented as mean ± SEM of four measures of each wounded area. The summary of ten independent experiments. ***p < 0.001. (c) A549 cells (2.5 x 105) were suspended in 0.5 ml RPMI-1640 containing 0.5% HI-FBS and 10 mg/ml polyI:C, 10 ng/ml TGF-b1 or both and placed in the inner wells of BioCoatTM Matrigel® Invasion Chamber and incubated at 37ºC for 22 hours. Membranes were fixed, stained and the number of

invaded cells was counted under phase contrast microscopy. Data is presented as mean ± SEM. The summary of two independent experiments. *p < 0.05, **p < 0.01. n = 8. (d) Different doses of polyI:C and 10 ng/ml TGF-b1 were used. Data is presented as mean ± SEM. The summary of two independent experiments. *p < 0.05, ***p < 0.001.

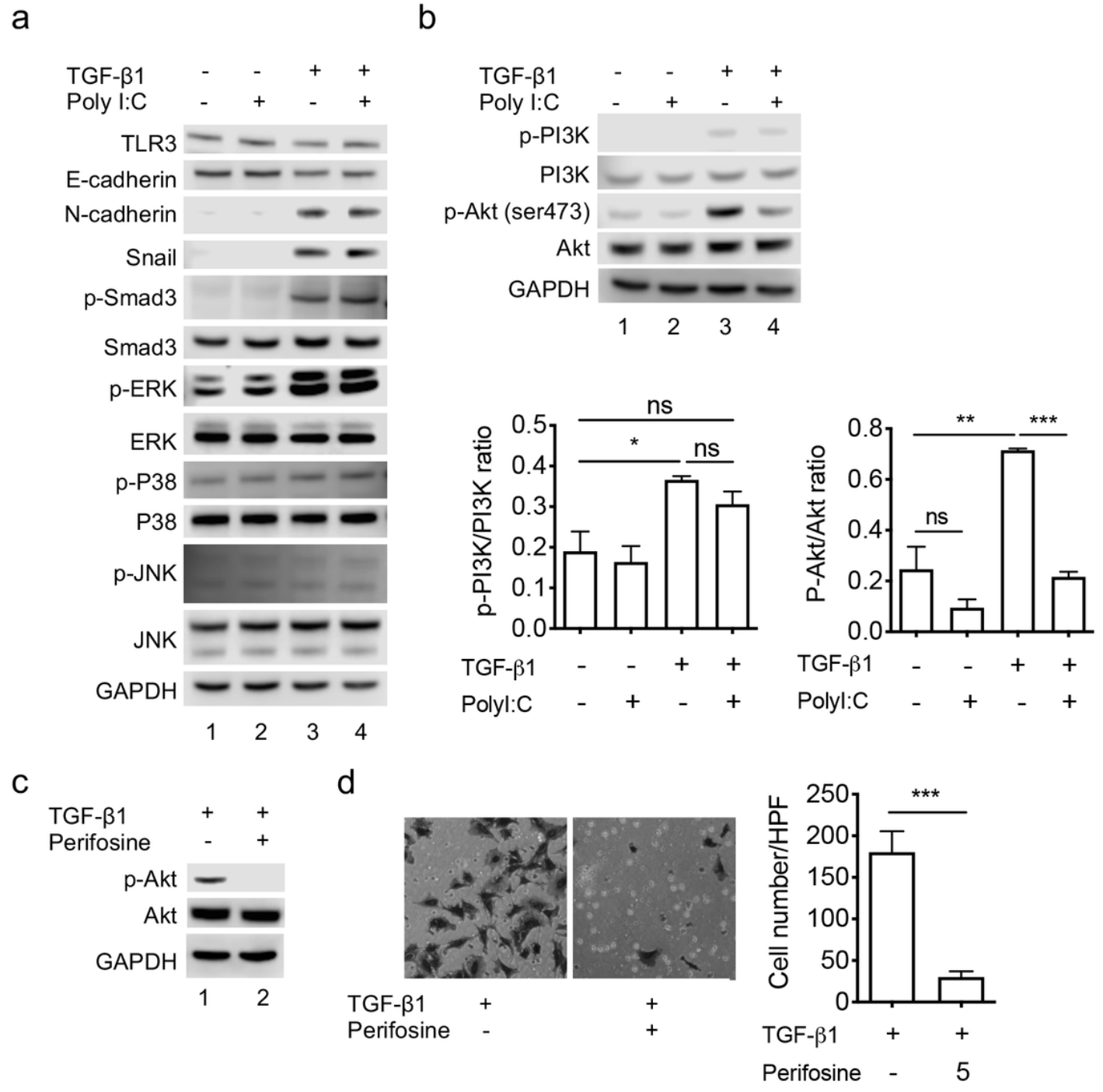

Figure 3

The effects of polyI:C on the expression of TLR3 and EMT markers and on the phosphorylation of Smad3 and MAPKs. (a) One million A549 cells were incubated in 60-mm plastic dishes for 24 hours at 37ºC in

RPMI 1640 with 10% HI-FBS. After the incubation, cells were incubated for additional 24 hours in fresh medium containing 0.5% HI-FBS with 10 ng/ml TGF-b1, 10 mg/ml polyI:C or both. The level of each protein and the level of phosphorylation were evaluated by Western blotting. (b) The level of phosphorylation in each cell lysate was evaluated by Western blotting, followed by analyses using densitometry (lower panels). Data is present as mean ± SEM. A summary of three independent experiments. **p < 0.01, ***p < 0.001. (c) A549 cells (1 x 106) were suspended in RPMI-1640 containing 10% HI-FBS. After a 24-hour incubation at 37ºC, medium was replaced by fresh medium containing 0.5% HI-FBS and cells were incubated in the presence of 10 ng/ml TGF-b1 with or without 5 mM perifosine at 37ºC for 24 hours. The level of phosphorylated Akt at Ser 473 was evaluated by Western blotting. (d) Two hundred and fifty thousand A549 cells were suspended in 0.5 ml RPMI 1640 containing 0.5% HI-FBS and incubated for 24 hours with 10 ng/ml TGF-b1 in the presence or absence of 5 mM perifosine. Results are 14 representative of two independent experiments. Data is present as mean ± SEM per HPF. ***p < 0.001. n = 8.

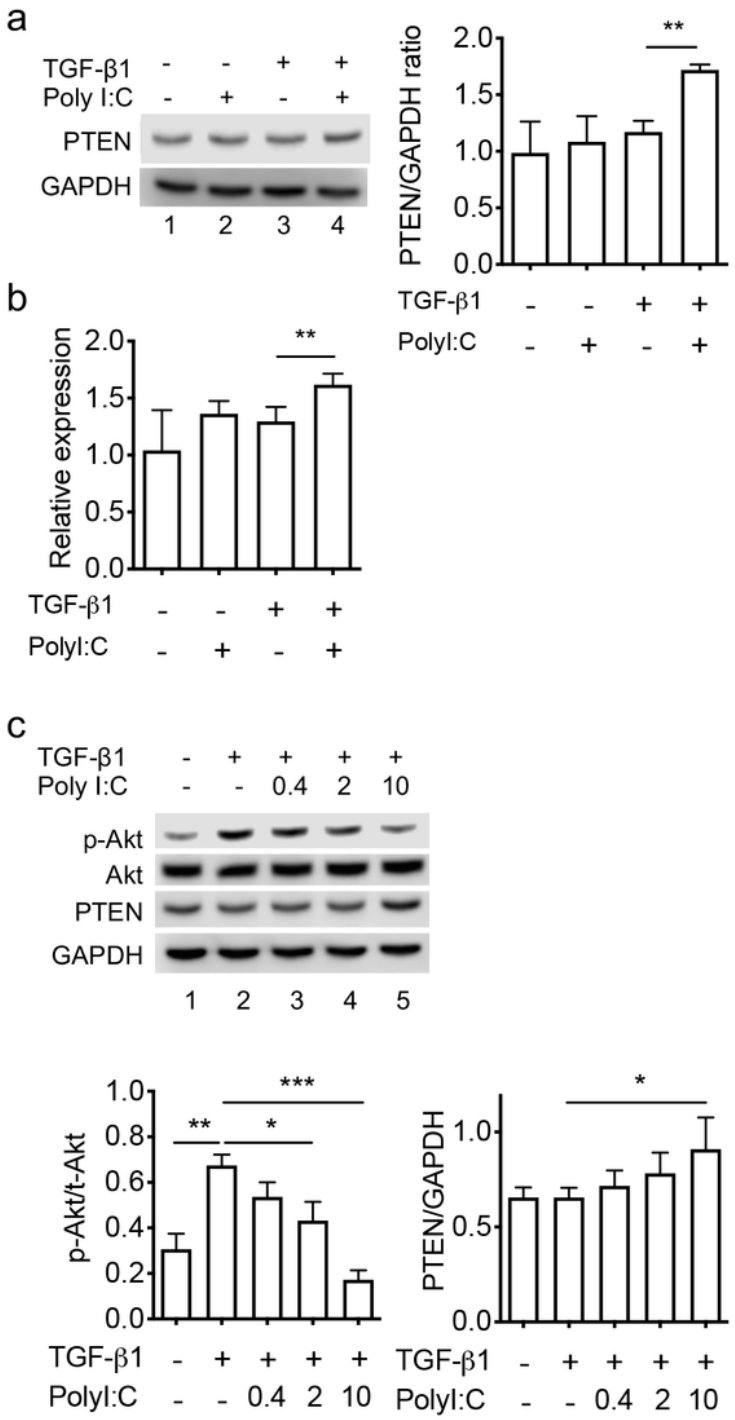

Figure 4

The effects of polyI:C on the PTEN/Akt signaling pathway. (a) A549 cells (1 x 106) were incubated in 60-mm plastic dishes at 37ºC for 24 hours. Medium was replaced by fresh medium containing 0.5% HI-FBS and 10 ng/ml TGF-b1, 10 mg/ml polyI:C or both for 24 hours. The level of PTEN in each cell lysate was evaluated by Western blotting, followed by densitometry (right panel). Data is present as mean ± SEM. The summary of three independent experiments. **p < 0.01. (b) Expression of PTEN mRNA was analyzed

by quantitative RT-PCR. The summary of two independent experiments. Data is present as mean ± SEM. **p < 0.01. (c) A549 cells (1 x 106) were suspended in RPMI-1640 medium containing 10% HI-FBS. After 24 hours incubation at 37ºC, medium was replaced by fresh medium containing 0.5% HIFBS and 10 ng/ml TGF-b1 with different doses of polyI:C. The level of each protein in each cell lysate and the level of phosphorylation were evaluated by Western blotting, followed by densitometry (lower panels). Data is present as mean ± SEM. The summary of four independent experiments. *p < 0.05, **p < 0.01, ***p < 0.0001.

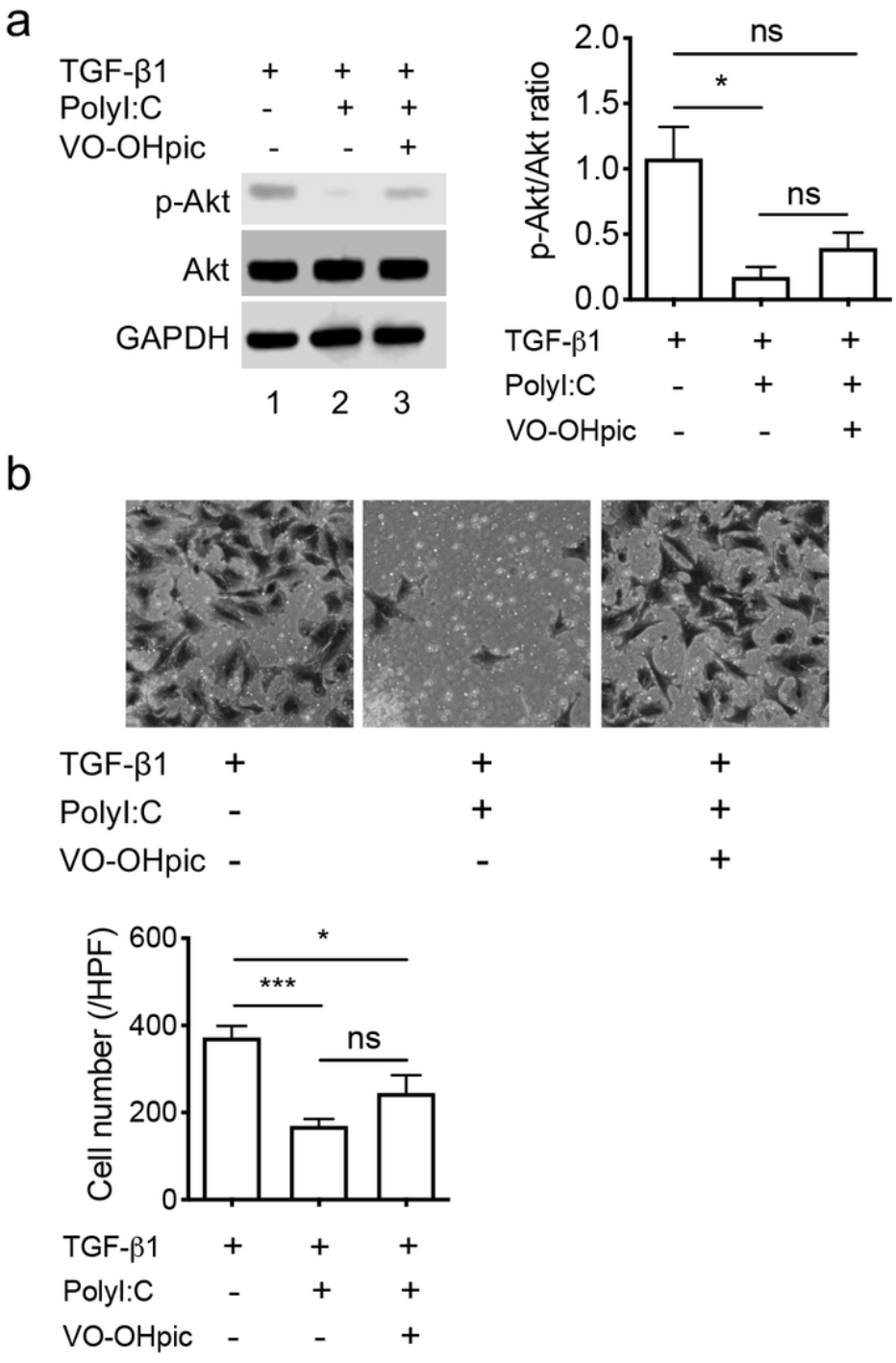

Figure 5

The effects of VO-OHpic on the inhibition of Akt phosphorylation and invasion of TGF-β1- treated cells by polyI:C. (a) A549 cells (1 x 106) were suspended in RPMI-1640 medium containing 10% HI-FBS. After 24 hours incubation at 37ºC, the medium was replaced by fresh medium containing 0.5% HI-FBS and incubated at 37ºC for 24 hours as indicated. TGF-b1; 10 ng/ml, polyI:C; 10 mg/ml, VO-OHpic; 10 μM. The level of Akt and the phosphorylation at Ser 473 were evaluated by Western blotting. Data is present as mean ± SEM. *p < 0.05. Results are representative of three independent experiments. (b) A549 cells (2.5 x 105) were pre-treated with 10 μM VO-OHpic for 1 hour at 4°C and then seeded in Matrigel® Invasion Chamber. After a 22-hr incubation with 10 ng/ml TGF-β1 with or without 10 μg/ml polyI:C, cells that migrated through the membranes of the inserts were counted under phase contrast microscopy. Data is presented as mean ± SEM per HPF. *p < 0.05, ***p < 0.001. Results are representative of three independent experiments.

Supplementary Files

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- YamaguchietalSupplementarydata.pdf