The modulation of MiR-155 and MiR-23a manipulates *Klebsiellapneumoniae* Adhesion on Human pulmonary Epithelial cells via Integrin α5β1 Signaling

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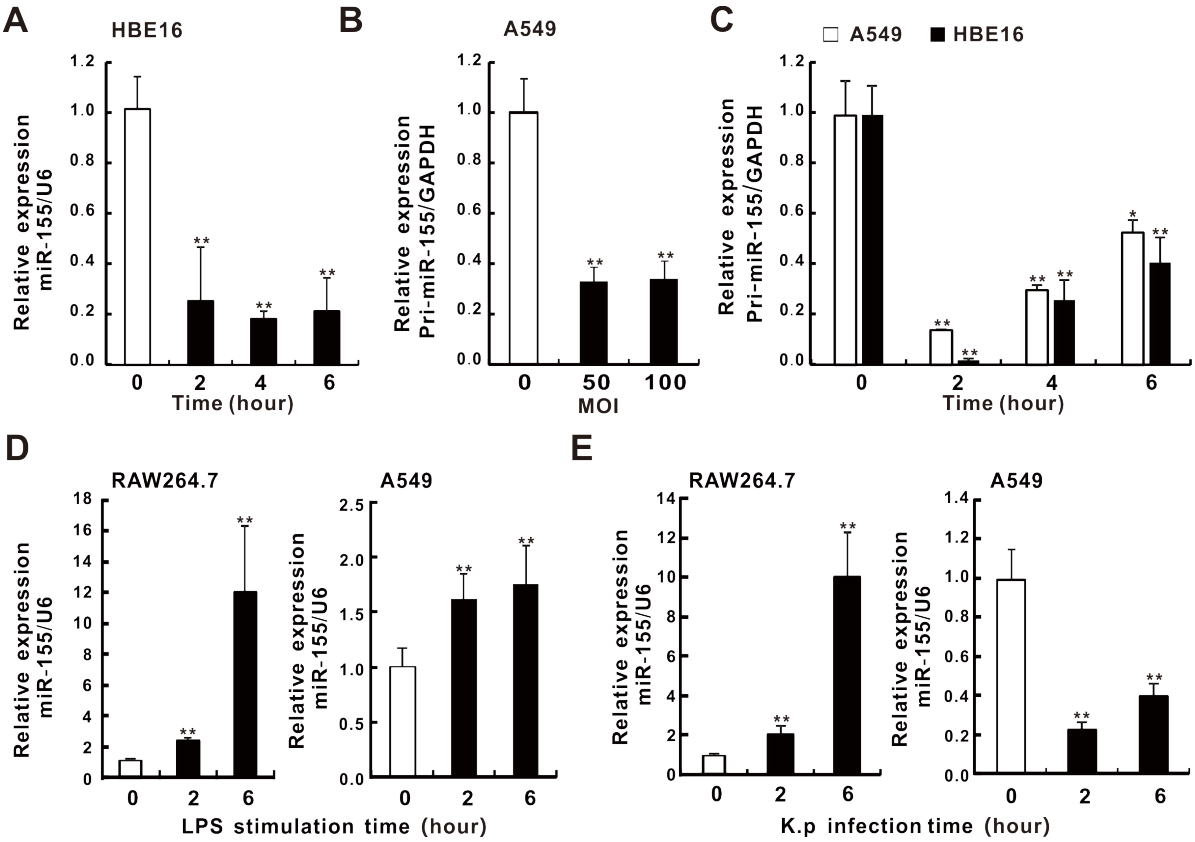
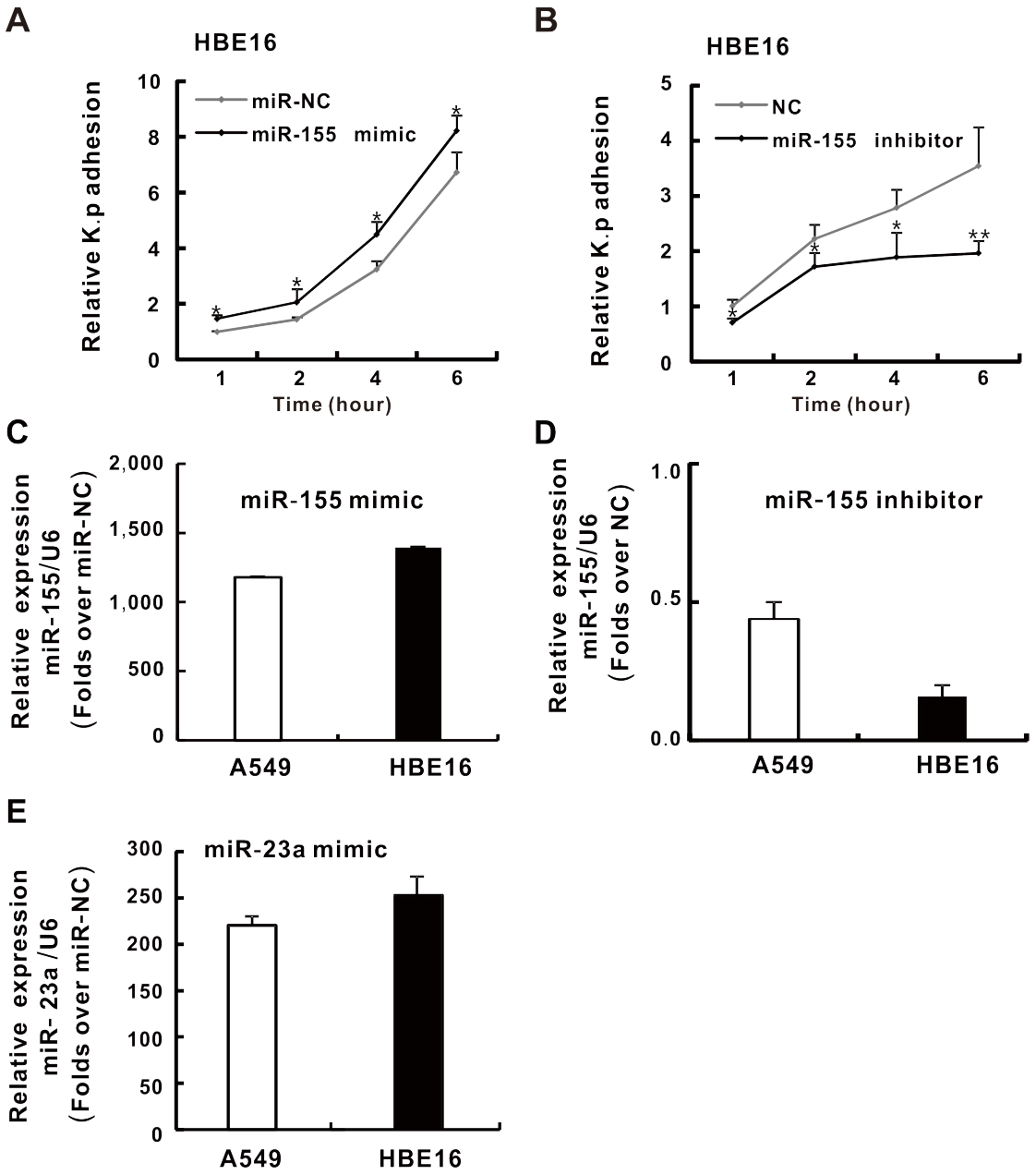
FigS1. Relative expression of miR-155 and pri-miR-155 under *K. pneumoniae*or LPS stimulation.

A549 and HBE16 cells were exposed to increasing doses of *K. pneumoniae (*MOI=0, 50, 100) for 2 hours, or fixed amount of bacteria (MOI=100) at indicated time points (0hr, 2hr, 4hr and 6hr). The expression levels of miR-155 (A)and pri-miR-155 (B and C) were examined by RT-qPCR. RAW264.7 and A549 cells were stimulated by LPS (10 ng/mL) or exposed to fixed amount of bacteria (MOI=100) at indicated time points (0hr, 2hr and 6hr), the expression level of miR-155 (D and E) was examined by RT-qPCR. The Relative expression was normalized to U6 and then converted to the fold change over uninfected or unstimulated. (Data are the mean ± SD and represent three individual experiments. *p<0.05, **p<0.01 compared with *K. pneumoniae*or LPS unstimulated).



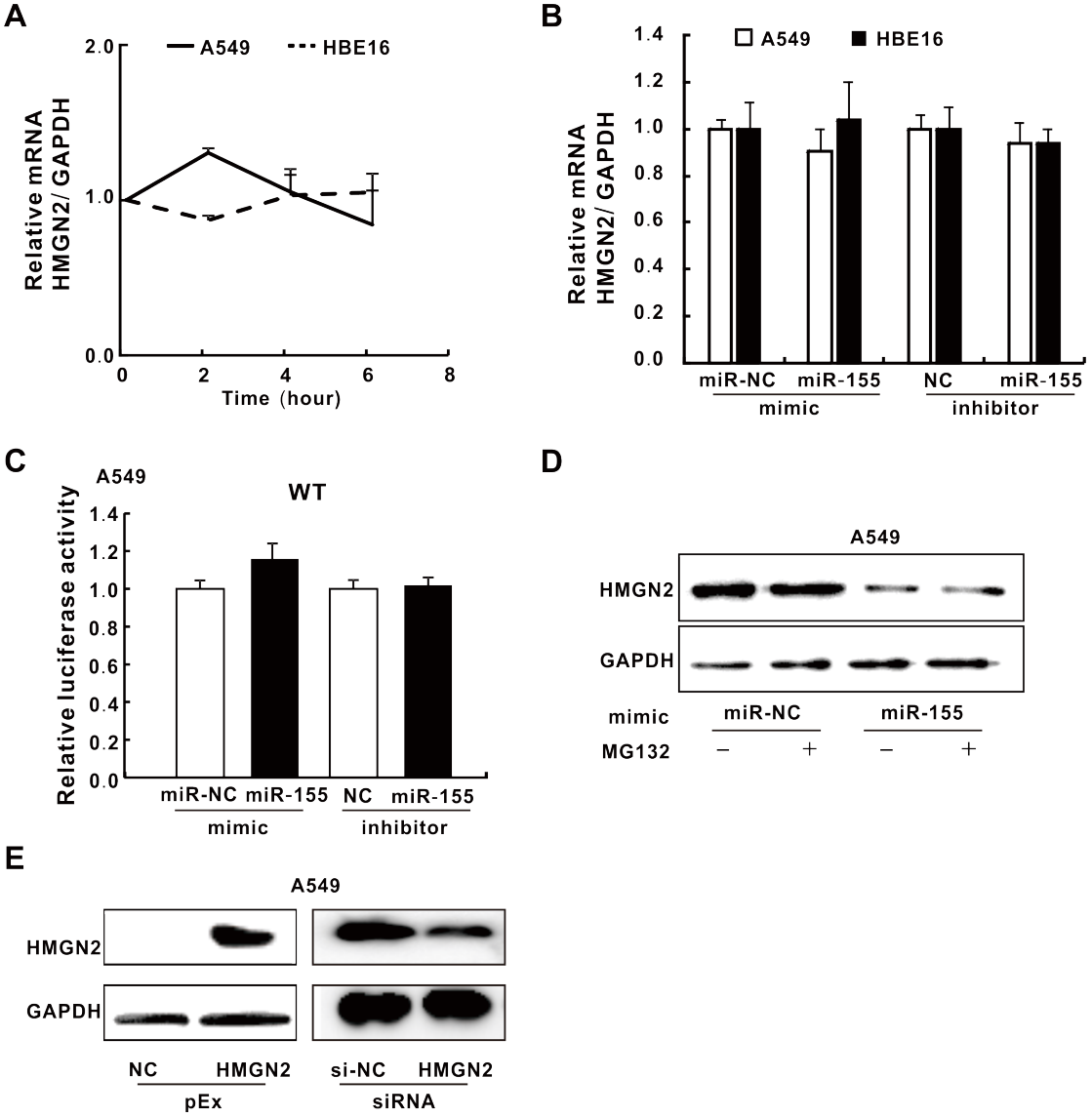
FigS2. MiR-155 regulated the adhesion of *K. pneumoniae*.

HBE16 cells were transfected with miR-155 mimic (A), inhibitor (B) and according negative control (miR-NC or NC) 24 hours prior to 100 MOI of *K. pneumoniae*exposure. The relative *K. pneumoniae* adhesion at indicated time points was determined by colony counts and represented after normalizing to 1 hour bacterial adhesion of miR-NC or NC. Relative expression of indicated miRNA after transfecting miR-155 mimic (C), inhibitor (D) or miR-23a mimic (E) in A549 or HBE16 cells (Folds over miR-NC or NC). (Data are the mean ± SD and represent three individual experiments. *p<0.05, **p<0.01).



FigS3. HMGN2 was potential target of miR-155 and miR-23a.

(A) RT-qPCR analysis showing the change of HMGN2 mRNA level in *K. pneumoniae* infected A549 or HBE16 cells at different infection time points (MOI=100). (B) RT-qPCR analysis showing the effect of miR-155 mimic or inhibitor on the mRNA levels of HMGN2 in *K. pneumoniae* infected A549 and/or HBE16 cells (MOI=100 for 2 hours, same as B, C, D, E, and F). (C)A549 cells were co-transfected with miR-155 mimic or inhibitor and HMGN2 3' UTR wild-type (WT) luciferase reporter construct. Luciferase activity was measured 24hr after transfection. (D) A549 cells transfected with miR-155 mimic were treated with proteasome inhibitor (MG132, 20 μM, 3 hours) the relative HMGN2 protein expression were assessed by Western blot. (E) HMGN2 protein levels of A549 cells transfected with pEx-HMGN2 or si-HMGN2 (Data are the mean ± SD and represent three individual experiments. **p<0.01).



FigS4. MiR-155 promoted integrin-Rac1 pathway in *K. pneumoniae*-infected HBE16 cells.

(A) RT-qPCR analysis showingthe integrin α5 and β1 mRNA expression of HBE16 cells transfected with miR-155 mimic or miR-NC prior to *K. pneumoniae* infection (MOI=100 for 2 hours, same as B, D and E). Western blot analysis showing the levels of integrin α5, integrin β1(B), Rac1-GTP, total Rac1 (D) and F-actin (E) in HBE16 cells treated as (A).(C) FN–cell Adhesion assay was performed to evaluate the effect of miR-155 mimic on HBE16 cells to associate with fibronectin coated plates. (Data are the mean ± SD and represent three individual experiments. *p<0.05, **p<0.01 compared with miR-NC).