Knocking Down of P65 Component of NF-B Effectively Down Regulates Pro-inflammatory Cytokines in Human Pulmunary Epithelial Cells Infected with Influenza Virus H9N2

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Background/Objective

H9N2 Influenza viruses have been periodically isolated from patients showing clinical findings of influenza. Some reports indicated that H9N2 viruses have repetitively infected humans although causing a mild disease. However, the pathogenicity of the avian influenza H9N2 viruses is unclear. Since transcription factor NF-κB regulates the production of many pro-inflammatory cytokines produced by macrophages and epithelial cells, we proposed that knocking down of NF-κB can reduce stimulatory effects of influenza virus on some downstream genes in human lung epithelial cells.

Method

Different RNA PolII or PolIII based plasmids were constructed to design shRNA plasmids inducing RNAi for gene function analysis. Here we used a RNA Pol III promoter, H1, to knockdown p65 segment of NF-kB. Immunocytochemistry,real-time quantitative PCR (qPCR) and western blot analysis was used to confirm the effective knockdown of p65 segment. Also, qPCR and ELISA was employed to detect and quantify the production of pro-inflammatory cytokines; IL-1β, IL-6, IL-8 and TNF-α in A549 cell line infected with H9N2 influenza virus. We examined the propagation features of H9N2 virus in A549 cell line to obtain an insight to its ability to stimulate production of pro-inflammatory cytokines, and down regulation of NF- B on its replication efficiency and cytokine production in human respiratory tract cells.

Result

Immunocytochemistry, qPCR and western blot analysis confirmed the effective knockdown of p65 segment of NF- B. Moreover, knocking down of p65 segment of NF- B, down-regulates production of both IL-6, IL-8 and TNF-α at both mRNA and protein levels. On the other hands the amount of IL-1β produced in A549 pulmonary epithelial cells was too low and changes were not significant.

Conclusion

All together results showed that down-regulation of NF-kB gene not only directly reduce virus propagation but has also decreased stimulatory effects of virus on production of pro-inflammatory cytokines and therefore may prevents from cytokine storm and disease pathogenicity.