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Functional roles of 3a protein in the pathogenesis of SARS

Key Message

The SARS-CoV 3a protein can induce apoptosis through a caspase-8-dependent pathway in VeroE6 cells.

Introduction

Severe acute respiratory syndrome (SARS) affected more than 8000 individuals and resulted in about 800 deaths in 26 countries. The genomes of different strains of SARS-CoV have been sequenced and found to contain 15 open reading frames (ORFs) encoding the replicase, four major structural proteins and several proteins of unknown function.[1-3]

The *3a* locus encodes one of the ORFs of unknown function and is located between two structural genes encoding the spike and the envelope proteins of SARS-CoV. Interestingly, the *3a* ORF is not found in any coronaviruses identified to date. This suggests that the 3a protein is a newly emerged protein in coronaviruses.

Previous studies have shown that many coronaviruses, including murine hepatitis virus, avian infectious bronchitis virus and transmissible gastroenteritis coronavirus, are able to induce apoptosis of host cells,[4] but little is known about this ability in SARS-CoV. Apoptosis was observed in liver specimens from patients with SARS-associated viral hepatitis, just as lymphopenia is commonly observed in SARS patients (postulated to be due to apoptosis induced by SARS-CoV infection).[5,6] Furthermore, SARS-CoV can induce a cytopathic effect and apoptosis in cell-culture models, such as VeroE6 cells.[7]

Aims and objectives

1. To identify the molecular mechanism underlying the 3a-induced apoptosis in SARS-CoV infected VeroE6 and human cells.
2. To identify potential inhibitors of such 3a-induced apoptosis.

Methods

The cDNA coding for the SARS-CoV 3a protein was cloned into mammalian expression vectors pcDNA4 and pEGFP and expressed in VeroE6 cells. Apoptosis induced by the 3a protein expression was detected by a DNA fragmentation assay, chromatin-condensation analysis and immunostaining and terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling (TUNEL) assay. To dissect the signalling pathway mediating the 3a-induced apoptosis, the expression of apoptosis-related proteins were determined by western blot analysis. We have also employed the commercially available antibody array to identify more protein targets involved in 3a-induced apoptosis.

Results

To investigate whether the 3a protein could induce apoptosis, Vero E6 cells were transfected with pEGFP-3a. Extensive chromatin condensation was observed in green fluorescent protein-positive cells. To examine whether the 3a protein induced DNA fragmentation, Vero E6 cells were transfected transiently with pcDNA4-3a. Extensive low-molecular-mass apoptotic DNA fragments were observed on day 3 onwards after transfection. The apoptotic effect of the 3a protein was finally confirmed by the TUNEL assay.

To delineate the pathway by which the 3a protein might be involved in the

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induction of apoptosis, we examined the expression levels of Bcl-2 family proteins and caspase-8, which are common mediators of the mitochondrion- and receptor-mediated pathways, respectively. Cleavage of procaspase-8 was increased in 3a-transfected Vero E6 cells. However, there were no effects on the endogenous levels of Bcl-2 family proteins (such as Bcl-2 and Bad) and on proliferating-cell nuclear antigen.

Using the commercially available antibody array, many apoptosis-related genes, including β-catenin, cytochrome c, caspase 4, glycogen synthase kinase 3 beta, Fas-associated death domain protein, p53 binding protein 2, and protein kinase R were upregulated in VeroE6 cells expressing the SARS-CoV 3a protein. These may be novel target genes triggered by the SARS-CoV 3a protein.

Discussion

Our study has shown that expression of the 3a protein can induce chromatin condensation and low-molecular-mass apoptotic DNA fragmentation from 3 days post-transfection. These data were consistent with the results of the TUNEL assay, showing a significant amount of internucleosomal DNA cleavage. Since caspase-8 was activated in 3a-induced apoptosis, we postulate that expression of the 3a protein induces apoptosis through a pathway similar to the death-receptor signalling cascades. Finally, additional proteins targets were identified by antibody array for future investigation of SARS-CoV induced apoptosis.

Conclusions

The 3a protein can induce apoptosis thorough a caspase-8-dependent pathway in VeroE6 cells. An anti-apoptotic strategy can be considered in future outbreaks of SARS or SARS-related diseases.

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