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Over-expression of severe acute respiratory syndrome coronavirus 3b protein induces both apoptosis and necrosis in Vero E6 cells

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Abstract

The genome of the severe acute respiratory syndrome coronavirus encodes for eight accessory viral proteins with no known homologues in other coronaviruses. One of these is the 3b protein, which is encoded by the second open reading frame in subgenomic RNA 3 and contains 154 amino acids. Here, a detailed time-course study was performed to compare the apoptosis and necrosis profiles induced by full-length 3b, a 3b mutant that was deleted by 30 amino acids from the C terminus (3bΔ124-154) and the classical apoptosis inducer, Bax. Our results showed that Vero E6 cells transfected with a construct for expressing 3b underwent necrosis as early as 6 h after transfection and underwent simultaneous necrosis and apoptosis at later time-points. At all the time-points analysed, the apoptosis induced by the expression of 3b was less than the level induced by Bax but the level of necrosis was comparable. The 3bΔ124-154 mutant behaves in a similar manner indicating that the localization of the 3b protein does not seems to be important for the cell-death pathways since full-length 3b is localized predominantly to the nucleolus, while the mutant is found to be concentrated in the peri-nuclear regions. To our knowledge, this is the first report of the induction of necrosis by a SARS-CoV protein.

1. Introduction

Severe acute respiratory syndrome (SARS) originated in early November 2002 in Guangdong province, People's Republic of China. The disease soon spread worldwide infecting more than 8000 people, with more than 700 fatalities (World Health Organization, http://www.who.int/csr/sars/country/en/). The causative agent of SARS was identified as a novel coronavirus, now known as SARS-CoV (for reviews, see Berger et al., 2004; Christian et al., 2004; Peiris et al., 2004). SARS-CoV contains a RNAgenome of~30 kilobases, which encodes for up to 14 potential open reading frames (ORFs) (Marra et al., 2003; Rota et al., 2003). In addition to the replicase polyproteins (pp1a and pp1ab) and structural proteins, spike, membrane, nucleocapsid and envelope, which are common to all members of the genus coronavirus, the SARS-CoV genome also encodes eight putative proteins with no significant sequence homology to viral proteins of other known coronaviruses (i.e.ORF3a, 3b, 6, 7a, 7b, 8a, 8b and 9b) (Marra et al., 2003; Snijder et al., 2003; Tan et al., 2005a). It has not yet been established which of the SARS-CoV accessory proteins are essential for viral replication and/or for viral–host interactions. However, the

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