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ScienceDirect
Virology 354 (2006) 132–142
VIROLOGY
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The human severe acute respiratory syndrome coronavirus (SARS-CoV) 8b protein is distinct from its counterpart in animal SARS-CoV and down-regulates the expression of the envelope protein in infected cells

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Received 10 April 2006; returned to author for revision 15 June 2006; accepted 17 June 2006
Available online 31 July 2006

Abstract

The severe acute respiratory syndrome coronavirus (SARS-CoV), isolated from humans infected during the peak of epidemic, encodes two accessory proteins termed as 8a and 8b. Interestingly, the SARS-CoV isolated from animals contains an extra 29-nucleotide in this region such that these proteins are fused to become a single protein, 8ab. Here, we compared the cellular properties of the 8a, 8b and 8ab proteins by examining their cellular localizations and their abilities to interact with other SARS-CoV proteins. These results may suggest that the conformations of 8a and 8b are different from 8ab although nearly all the amino acids in 8a and 8b are found in 8ab. In addition, the expression of the structural protein, envelope (E), was down-regulated by 8b but not 8a or 8ab. Consequently, E was not detectable in SARS-CoV-infected cells that were expressing high levels of 8b. These findings suggest that 8b may modulate viral replication and/or pathogenesis.

Keywords: Severe acute respiratory syndrome (SARS); Coronavirus (CoV); Accessory proteins; Envelope (E) protein; 8a; 8b; 8ab

Introduction

A novel coronavirus was identified as the aetiological agent for the recent severe acute respiratory syndrome (SARS) epidemic (Drosten et al., 2003; Poon et al., 2004). In addition to the replicase polyproteins (pp1a and pp1ab) and structural proteins (spike (S), membrane (M), nucleocapsid (N) and envelope (E)), 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., open reading frames (ORFs) 3a, 3b, 6, 7a, 7b, 8a, 8b and 9b) (Marra et al., 2003; Snijder et al., 2003; Tan et al., 2005). Although it was recently demonstrated that most of these so-called accessory proteins are not essential for viral replication in cell culture or in the murine model (Yount et al., 2005), the exact contributions of these proteins to viral replication or pathogenesis in the natural host have not been established.

Interestingly, epidemiological studies have revealed that the part of the viral genome that encodes for two of these accessory proteins, 8a and 8b, shows major variations. In one of these studies, Guan and co-workers (2003) analyzed SARS-CoV isolates obtained from animals in a live-market in Guangdong and found that all the animal isolates contain a 29-nucleotides (nt) sequence, which is absent in most of the human isolates (Fig. 1A). As a result of this, the ORF8a and ORF8b (also termed as ORF10 and ORF11, respectively) in the human isolates become one ORF, termed as ORF8ab. ORF8ab encodes a protein of 122 amino acids (aa), whose N terminus is identical to 8a and C terminus is identical to 8b (Fig. 1B). Another extensive study of 63 SARS-CoV isolates obtained from the SARS epidemic in China also showed that there are major variations in this region of the viral genome (The Chinese SARS Molecular Epidemiology Consortium, 2004). In this study, the course of the epidemic was divided into the early, middle and late phase with the early phase

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0042-6822/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2006.06.026

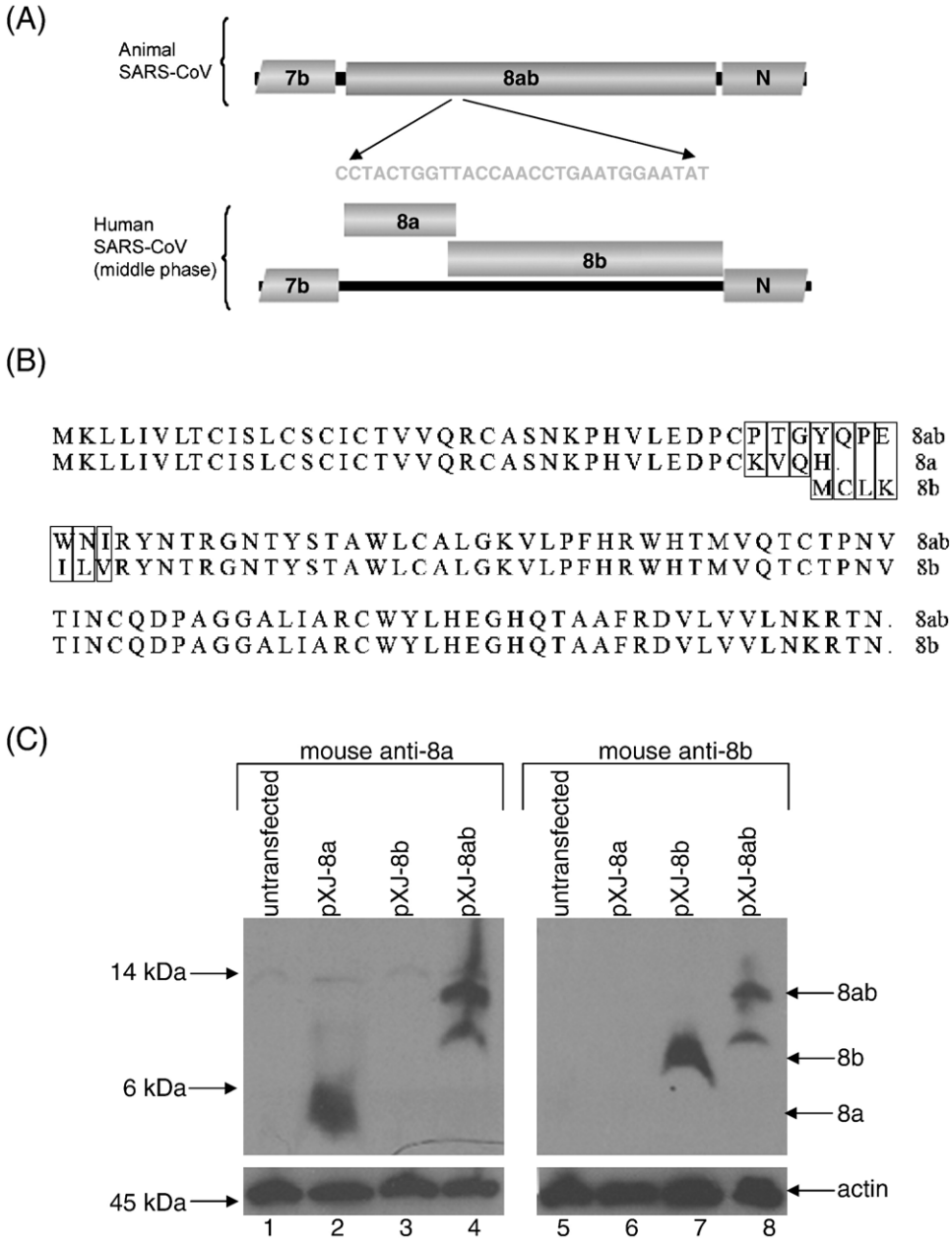

Fig. 1. Expressions of SARS-CoV 8a, 8b and 8ab proteins. (A) Schematic diagram showing the genetic differences in the ORF8 region of the SARS-CoV isolated from animals and humans infected during the middle phase of the SARS epidemic in 2003 (modified from Guan et al., 2003). The animal isolates have an extra 29-nucleotides insertion such that the subgenomic RNA encodes for a single protein, termed 8ab, whereas that of the human isolates (from the middle phase) encodes two proteins, 8a and 8b. Human isolates from early and late phases of the epidemic also have the 29-nucleotides insertion found in the animal SARS-CoV. (B) Alignment of the sequences of 8a, 8b and 8ab proteins used in this study. Mismatches between 8a and 8ab or 8b and 8ab are boxed. The 8ab is reconstructed from a human isolate from the middle phase (SIN2774) by insertion of the 29-nucletoides found in a human isolate from the early phase (GZ02). (C) Western blot analysis was performed to detect 8a, 8b and 8ab proteins expressed in Vero E6 cells using cDNA constructs. The experiments were performed with either mouse anti-8a polyclonal antibody (upper panel, lanes 1–4) or mouse anti-8b polyclonal antibody (upper panel, lanes 5–8). Equal amounts of cells were used in each lane as verified by the level of endogenous actin (bottom panel).

defined as the period of first emergence of SARS in Guangdong Province. The middle phase referred to all events up to the first cluster of SARS cases in the Metropole hotel in Hong Kong and the late phase referred to all cases following this cluster. Interestingly, the clustering of patients with different patterns of variations in ORF8 region was correlated with the different phases of the epidemic. These findings were subsequently verified by researchers who studied the SARS-CoV isolated in different countries (Chiu et al., 2005; Lan et al., 2005; Qin et al., 2003; Wang et al., 2004, 2005).

Although these mutations in the ORF8 region do not appear to have any adverse effect on the survival of the virus, it is conceivable that the 8a, 8b and 8ab proteins may have different stabilities and/or functions and hence would contribute differently to viral replication and/or pathogenesis *in vivo*. In order to understand how the changes in the ORF8 region of the viral