Cellular Microbiology (2007) **9**(11), 2552–2561
doi:10.1111/j.1462-5822.2007.01034.x
First published online 20 August 2007

Microreview

Regulation of cell death during infection by the severe acute respiratory syndrome coronavirus and other coronaviruses

Yee-Joo Tan,* Seng Gee Lim and Wanjin Hong
Collaborative Anti-Viral Research Group, Institute of Molecular and Cell Biology, 61 Biopolis Drive, Proteos, Singapore 138673.

Summary

Both apoptosis and necrosis have been observed in cells infected by various coronaviruses, suggesting that the regulation of cell death is important for viral replication and/or pathogenesis. Expeditious research on the severe acute respiratory syndrome (SARS) coronavirus, one of the latest discovered coronaviruses that infect humans, has provided valuable insights into the molecular aspects of cell-death regulation during infection. Apoptosis was observed *in vitro*, while both apoptosis and necrosis were observed in tissues obtained from SARS patients. Viral proteins that can regulate apoptosis have been identified, and many of these also have the abilities to interfere with cellular functions. Occurrence of cell death in host cells during infection by other coronaviruses, such as the mouse hepatitis virus and transmissible porcine gastroenteritis virus, has also being extensively studied. The diverse cellular responses to infection revealed the complex manner by which coronaviruses affect cellular homeostasis and modulate cell death. As a result of the complex interplay between virus and host, infection of different cell types by the same virus does not necessarily activate the same cell-death pathway. Continuing research will lead to a better understanding of the regulation of cell death during viral infection and the identification of novel antiviral targets.

Received 13 February, 2007; revised 24 July, 2007; accepted 25 July, 2007. *For correspondence. E-mail mcbtanyj@imcb.a-star.edu.sg; Tel. (+65) 65869625; Fax (+65) 67791117.

Introduction

The regulation of cell death during a viral infection is an important determinant in the struggle between virus and host for survival. Two forms of cell death have been extensively described, one is necrosis, defined as a passive and non-physiological type of death caused by accidental and acute damage to the cell, and the other is apoptosis, defined as an active and genetically regulated process of cell suicide by which an organism eliminates senescent, abnormal and potentially harmful cells. These two forms of cell death are distinguishable by their morphological and biochemical effects on the cell. However, recent studies have shown that necrosis is also highly regulated and can play essential roles in maintaining homeostasis in healthy cells as well as in the elimination of infectious pathogens (Assuncao Guimaraes and Linden, 2004; Nelson and White, 2004; Festjens *et al*., 2006; Zong and Thompson, 2006).

A novel coronavirus (termed as severe acute respiratory syndrome coronavirus, SARS-CoV) was the cause of a viral outbreak which caused profound disturbances worldwide in 2003 (Fouchier *et al*., 2003; Marra *et al*., 2003; Peiris *et al*., 2003; Rota *et al*., 2003). Coronaviruses are a family of enveloped, single-, positive-stranded RNA viruses with very large genomic size of ~30 kb and have been known to infect many animal species as well as humans (Siddell, 1995). One of the most common abnormalities in SARS-CoV-infected patients is lymphopenia (Peiris *et al*., 2003; Chng *et al*., 2005; Chen *et al*., 2006), which could be caused by the depletion of T lymphocytes by apoptosis. Indeed, several laboratories have successfully detected SARS-CoV in the lymphocytes isolated from infected patients, suggesting that the virus can infect lymphocytes (Wang *et al*., 2004; Gu *et al*., 2005). However, there is still no evidence that infection of lymphocytes is the direct cause for lymphopenia in SARS patients. In addition, both apoptosis and necrosis have been observed in various infected tissues obtained during autopsy studies on SARS casualties (Ding *et al*., 2003; Lang *et al*., 2003; Chau *et al*., 2004; Chong *et al*., 2004;

Wei *et al*., 2007). Thus, cell death has been observed during SARS-CoV infection *in vivo*.

This review summarizes current knowledge on the molecular aspects of cell-death regulation during SARS-CoV infection and the contributions of viral proteins and viral–host interactions to this process. Together with studies on other coronaviruses, these investigations provide important insights into the regulation of apoptosis and necrosis during viral infection and contribute to the development of antiviral therapeutics.

Regulation of apoptosis and necrosis during SARS-CoV infection

The main cause of death among SARS casualties was respiratory failure as a result of severe lung injury. Histopathological examinations revealed extensive damages to the alveolar and bronchial epithelial cells and macrophages, and these are likely to be caused by multiple factors, including cytopathic effects mediated by replication of the SARS-CoV and the overproduction of immune mediators (see a recent review by Chen and Subbarao, 2007). Besides lymphopenia (as described above), there is currently a lack of information on the role of cell death during the earlier stages of infection, as most of these data were obtained during autopsy studies on fatal cases and would therefore reflect the terminal stages of the disease. Extra-pulmonary spreading of the virus has also been reported, and in some of these organs, apoptosis and necrosis have been observed. In one study, extensive apoptosis was observed in the hepatocytes of three SARS patients who had liver impairment, suggesting that liver damages in these patients may be mediated by apoptosis (Chau *et al*., 2004). Apoptosis was also observed in the thyroid glands obtained from five fatal SARS cases, suggesting that pathogenesis in the thyroid glands may be related to apoptosis induction (Wei *et al*., 2007). In various studies, necrosis was also observed in lymphoid tissues and lymph nodes (Ding *et al*., 2003; Lang *et al*., 2003; Gu *et al*., 2005).

The occurrence of apoptosis during SARS-CoV infection *in vitro* (i.e. in cell culture systems) has been reported by several groups (Mizutani *et al*., 2004; Tan *et al*., 2004; Yan *et al*., 2004; Ren *et al*., 2005; Bordi *et al*., 2006). In these studies, the Vero cell line (or a subclone of Vero known as Vero E6), which is a green monkey kidney cell line that supports SARS-CoV replication and shows extensive cytopathic effects upon infection, was used. The induction of apoptosis was dependent on viral replication and could be inhibited by caspase inhibitors or the overexpression of the pro-survival protein, Bcl-2 (Ren *et al*., 2005; Bordi *et al*., 2006). Although necrosis was not observed in SARS-CoV-infected Vero E6 cells (Yan *et al*., 2004), it has been observed in different tissues obtained from SARS-CoV-infected patients (Ding *et al*., 2003; Lang *et al*., 2003; Chong *et al*., 2004). It is not clear whether necrosis in these tissues represented secondary necrosis reflecting the degradative changes that apoptotic cells undergo at the later stages of apoptosis, but at least one SARS-CoV protein [open reading frame (ORF) 3b] has been shown to induce necrosis (Khan *et al*., 2006).

Apoptosis and necrosis were also observed during the infection of Vero cells by the infectious bronchitis virus (IBV), an avian coronavirus (Liu *et al*., 2001). Interestingly, necrosis was also observed during infection and could be a more dominant factor for viral-induced cell death, as neither the death of infected cells nor the productive replication of IBV was severely affected by the inhibition of apoptosis by the general caspase inhibitor, z-VAD-FMK. Like the ORF 3b protein of SARS-CoV, the ORF 3b protein of IBV is localized to the nucleus (Shen *et al*., 2003), although it has not yet been determined whether the latter can induce apoptosis or necrosis. For another human coronavirus, OC43, intracerebral inoculation into mice resulted in acute encephalitis, with neuronal cell death caused by both necrosis and apoptosis (Jacomy *et al*., 2006). However, infection of MRC-5, diploid human fetal lung cells, seems to induce mainly apoptosis (Collins, 2001). Similarly, infection of monocytes/macrophages *in vitro* by 229E, yet another human coronavirus, caused mainly apoptosis, although a few necrotic cells were also observed (Collins, 2002).

In two independent studies, it was demonstrated that the inhibition of apoptosis, either by caspase inhibitors or by overexpression of the Bcl-2 protein, did not affect SARS-CoV replication in Vero cells (Ren *et al*., 2005; Bordi *et al*., 2006), suggesting that apoptosis does not play a role in facilitating viral release. However, this was only performed in the Vero cell line, and it is not known whether the inhibition of apoptosis will affect SARS-CoV replication in other cell lines or animal models. The regulation of cell death in other cell lines may be dramatically different, as some cell lines supported SARS-CoV replication but, unlike the Vero cell line, displayed minimal cytopathic effects (Gillim-Ross *et al*., 2004; Kaye *et al*., 2006). Analysis of host gene transcriptions in various cell lines also revealed significant differences in cellular responses to SARS-CoV infection. For example, Tang *et al*. (2005) reported that the upregulation of pro-apoptotic genes in SARS-CoV-infected Huh7 cells, while the opposite was observed in SARS-CoV-infected intestinal cell lines, Caco-2 and CL-14 (Cinatl *et al*., 2004).

Besides these two studies, the transcriptional profiles of apoptosis-related genes in SARS-CoV-infected Vero E6 have also been reported (Leong *et al*., 2005). Interestingly, several pathways that promote apoptosis, as well as those that prevent apoptosis, appeared to be modified during SARS-CoV infection, suggesting that the cell death may be

regulated differently at different stages of the SARS-CoV life cycle. Similar results were obtained when gene profiling was performed using peripheral blood mononuclear cells (PMBCs) from healthy donors that were inoculated *in vitro* with SARS-CoV (Ng *et al*., 2004). Other studies that used PMBCs isolated from SARS patients also revealed changes in the transcription of many genes involved in cell-death regulation (Reghunathan *et al*., 2005; Yu *et al*., 2005; Shao *et al*., 2006). One interesting gene that was found to be upregulated is lipocalin 2, which belongs to a class of secreted proteins that are thought to trigger apoptosis in immune cells via an unknown cell receptor (Reghunathan *et al*., 2005). The authors speculated that the upregulation of lipocalin 2 is a host response to limit tissue damage and inflammation and the overexpression of lipocalin 2 could lead to lymphopenia in SARS patients.

Induction of cell death by SARS-CoV proteins

The SARS-CoV genome has the typical organization as other members of the *Coronaviridae* family (Marra *et al*., 2003; Rota *et al*., 2003). The first two-thirds of the SARS-CoV genome encodes the replicase polyproteins (pp1a and pp1ab) that are processed to yield 16 non-structural proteins, some of which are responsible for replicating the viral genome and/or generating a nested set of subgenomic mRNAs to express all the other ORFs in the genome (Ziebuhr, 2004). The ORFs for the main structural proteins, spike (S), envelope (E), membrane (M) and nucleocapsid (N), are encoded in the remaining portion of the genome, and interspaced between these are the ORFs for eight putative accessory proteins (i.e. ORFs 3a, 3b, 6, 7a, 7b, 8a, 8b and 9b). While the SARS-CoV replicase and structural proteins share some degree of sequence homology with those of other coronaviruses, the accessory proteins do not show significant homology to viral proteins of known coronaviruses (Tan *et al*., 2006). The overexpression of some of the SARS-CoV proteins could induce apoptosis and/or necrosis, and this is summarized in Table 1.

While the 3C-like protease (also known as $3CL^{pro}$ or M^{pro}) is the only replicase gene product that has been shown to induce apoptosis (Lin *et al*., 2006), all the four main structural proteins (S, E, N and M) could induce apoptosis (Surjit *et al*., 2004; Chow *et al*., 2005; Yang *et al*., 2005; Zhao *et al*., 2006). As shown in Table 1, the experiments were performed in one or two cell lines (Table 1), and it has not been demonstrated whether the induction of apoptosis by these structural proteins is cell line-specific. It is difficult to compare the apoptosis-inducing capabilities of the structural proteins, as different laboratories have used different cell lines for their investigations. For example, the apoptosis induction by the E protein was demonstrated in Jurkat T cells (Yang *et al*., 2005), while the apoptosis induction by the N protein was demonstrated in COS-1 cells (Surjit

Table 1. Summary of SARS-CoV proteins that induce cell death in primary cells or transformed cell lines and their effects on cellular functions.

Viral protein	Induction of cell death in primary cells or transformed cell lines	Effects on other cellular functions
3C-like protease	Human promonocytes (Lin *et al*., 2006)	Activates the transcription factor NF-κB (Lin *et al*., 2006).
Spike	Vero E6 cell line (Chow *et al*., 2005)	Upregulates the expression of COX-2 (Liu *et al*., 2006a).
Envelope	Jurkat T cell line (Yang *et al*., 2005)	Alters the membrane permeability of mammalian cells (Liao *et al*., 2006).
		Forms cation-selective ion channels in planar lipid bilayers (Wilson *et al*., 2004).
Membrane	Human pulmonary fibroblast (Zhao *et al*., 2006)	Not known.
Nucleocapsid	COS-1 cell line (Surjit *et al*., 2004);	Upregulates the JNK and p38 MAPK pathways (Surjit *et al*., 2004).
	Human pulmonary fibroblast (Zhao *et al*., 2006)	Downregulates ERK, phospho-Akt and Bcl-2 (Surjit *et al*., 2004).
		Inhibits the activity of cyclin–cyclin-dependent kinase complex and blocks S phase progression (Surjit *et al*., 2006).
		Activates the transcription factors, NF-κB and AP-1 (He *et al*., 2003; Liao *et al*., 2005).
ORF 3a	Vero E6 cell line (Law *et al*., 2005)	Upregulates the expression of COX-2 (Yan *et al*. 2006).
		Forms ion channel in *Xenopus* oocytes (Lu *et al*., 2006).
		Activates the transcription factor NF-κB (Kanzawa *et al*., 2006).
ORF 3b	COS-7 cell line (Yuan *et al*., 2005);	Induces cell cycle arrest at the G0/G1 phase (Yuan *et al*., 2005).
	Vero E6 cell line (Khan *et al*., 2006)	Localizes to the mitochondria (Yuan *et al*., 2006a).
ORF 7a	HeLa, HepG2, A549, 293T, COS-7 and	Inhibits cellular protein synthesis (Kopecky-Bromberg *et al*., 2006).
	Vero E6 cell lines (Tan *et al*., 2004);	Induces the phosphorylation and activation of p38 MAPK (Kopecky-Bromberg *et al*., 2006).
	A549 and 293T cell lines	Blocks cell cycle progression at G0/G1 phase via the cyclin D3/pRb pathway (Yuan *et al*., 2006b).
	(Kopecky-Bromberg *et al*., 2006)	Activates the transcription factor NF-κB (Kanzawa *et al*., 2006).

NF-κB, nuclear factor kappa B; COX-2, cyclooxygenase-2; JNK, c-Jun N-terminal kinase; p38 MAPK, p38 mitogen-activated protein kinase; ERK, extracellular-signal-regulated kinase; AP-1, activator protein 1.

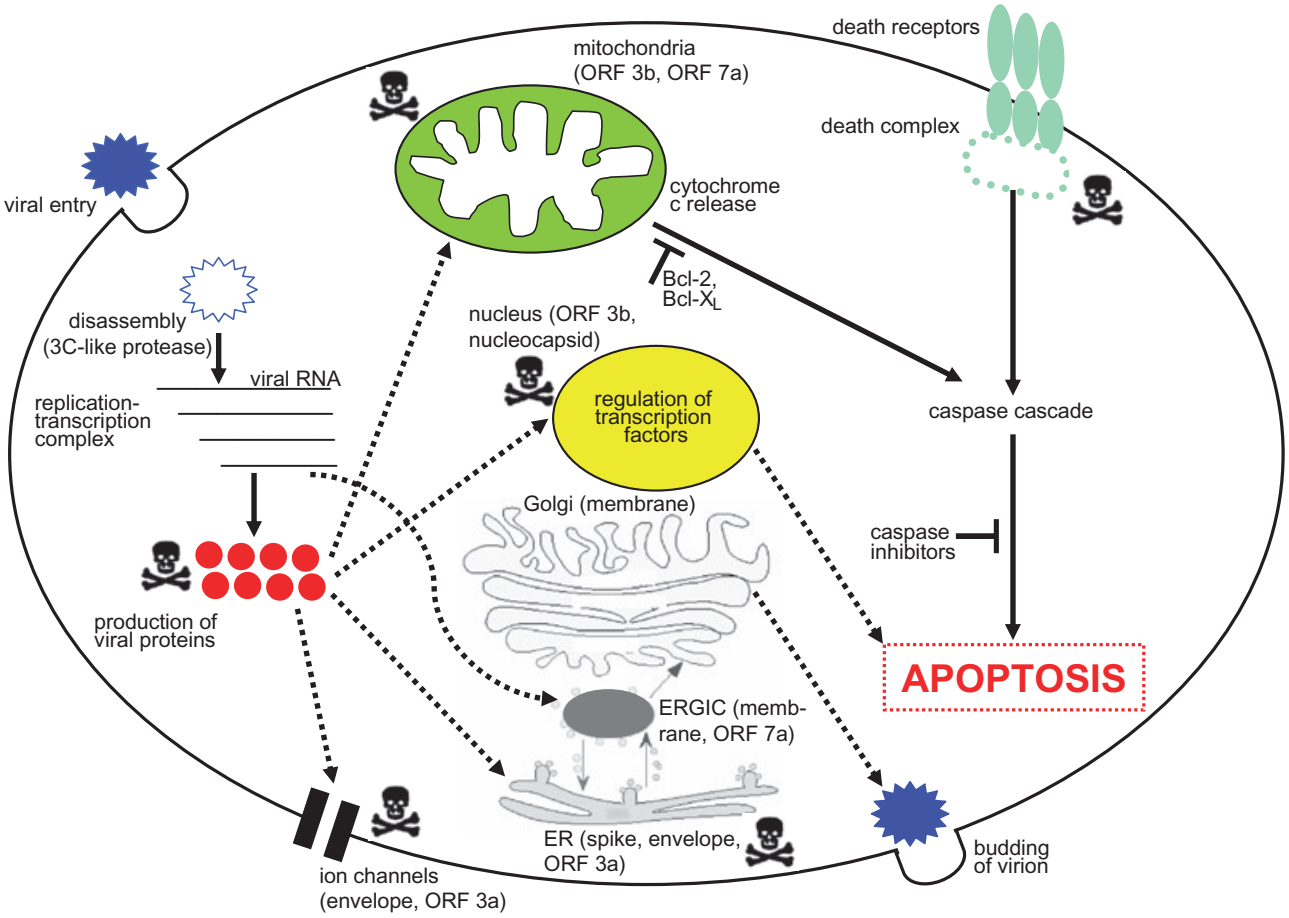

Fig. 1. A schematic diagram showing the complex network of viral–host interactions that can be formed after the SARS-CoV enters a cell. The SARS-CoV proteins can interfere with cellular functions at different compartments (cytoplasm, plasma membrane, ER, Golgi, ERGIC nucleus and mitochondria) and, eventually, cause apoptosis via the caspase cascade. The cellular localizations of SARS-CoV proteins are indicated in parentheses. ER, endoplasmic reticulum; ERGIC, endoplasmic reticulum to Golgi intermediate compartments. ☠ represents cellular compartments where viral infection can induce death stimuli.

et al., 2004) and human pulmonary fibroblast (Zhao *et al.*, 2006). It is also interesting to note that some of the structural viral proteins (E, N and M) induce apoptosis only in the absence of growth factors. This may imply that the induction of apoptosis by these proteins can only occur after the host cells become stressed at later stages of infection. Further studies are required to explore this possibility and define the precise mechanisms for cell-death induction.

Three of the accessory proteins, ORFs 3a, 3b and 7a, have also been shown to induce apoptosis (Tan *et al.*, 2004; Law *et al.*, 2005; Yuan *et al.*, 2005; Khan *et al.*, 2006). Again, for 3a and 3b, the studies were performed in only one or two cell lines. The overexpression of the 3a protein in Vero E6 (Law *et al.*, 2005) and the overexpression of the 3b protein in both COS-7 and Vero E6 cells induce apoptosis (Yuan *et al.*, 2005; Khan *et al.*, 2006). The overexpression of ORF 3b in Vero E6 cells also induces necrosis (Khan *et al.*, 2006). A more extensive range of cell lines, including HeLa, HepG2, A549, 293T, COS-7 and Vero E6, was used to demonstrate that the overexpression of 7a can induce apoptosis in cell lines derived from different organs, including lung, kidney and liver (Tan *et al.*, 2004).

The mechanisms for induction of apoptosis by these SARS-CoV proteins are unclear, although in some cases, it could be related to their abilities to interfere with cellular functions, such as blocking cell cycle progression, altering membrane permeability, activating signal transduction pathways, upregulating transcription factors and other regulatory genes (Table 1). These could lead to an imbalance in cellular homeostasis and, consequently, the induction of cell death. The cellular localizations of these SARS-CoV proteins have also been determined experimentally, and it is likely that they exert their pro-apoptotic effects by interacting with host proteins in these cellular compartments (Fig. 1). For example, the SARS-CoV E protein has the ability to modulate the membrane permeability of mammalian cells (Liao *et al.*, 2006) and form cation-selective

ion channels in planar lipid bilayers (Wilson *et al*., 2004). In addition, Yang *et al*. (2005) showed that the induction of apoptosis by E can be inhibited by the overexpression of Bcl-X_L, which is a pro-survival member of the Bcl-2 family. As Bcl-X_L is known to be a critical inhibitor of mitochondrial damage following apoptotic stimuli (Dejean *et al*., 2006), it is plausible that E induces apoptosis by perturbing the mitochondrial permeability. However, this has not been demonstrated experimentally. The E proteins of other three coronaviruses, mouse hepatitis virus (MHV), IBV and human coronavirus-229E (HCoV-229E), can also form ion channels in lipid bilayers (Wilson *et al*., 2006). Interestingly, the MHV E protein has also been shown to induce apoptosis (An *et al*., 1999) and alter membrane permeability (Madan *et al*., 2005).

The overexpression of the SARS-CoV N protein could upregulate the c-Jun N-terminal kinase (JNK) and mitogen-activated protein kinase (p38 MAPK) pathways and downregulate the expression levels of extracellular-signal-regulated kinase (ERK), phospho-Akt and Bcl-2 (Surjit *et al*., 2004). Further investigations revealed that it could inhibit the activity of cyclin–cyclin-dependent kinase complex and block S-phase progression (Surjit *et al*., 2006). The N protein could also activate the transcription factors, nuclear factor kappa B (NF-κB) and activator protein 1 (AP-1) (He *et al*., 2003; Liao *et al*., 2005). As these transcription factors regulate a wide variety of cellular processes, including cell proliferation, differentiation and apoptosis (Hess *et al*., 2004; Perkins and Gilmore, 2006; Tergaonkar, 2006), their activation may be linked to the apoptosis-inducing properties of N. For example, the N protein has been shown to upregulate the expression of cyclooxygenase-2 (COX-2), probably through the activation of NF-κB (Yan *et al*., 2006). The S protein can also upregulate COX-2 expression (Liu *et al*., 2006a).

Similar to the E protein, the SARS-CoV 3a protein has also been shown to form ion channel in *Xenopus* oocytes (Lu *et al*., 2006). The overexpression of the protein 3b, which was found in both the nucleus and mitochondria, induced cell cycle arrest at the G0/G1 phase (Yuan *et al*., 2005; 2006a). The 7a protein has been shown to block cell cycle progression at G0/G1 phase by reducing the expression of cyclin D3 and phosphorylation of retinoblastoma protein (Yuan *et al*., 2006b). Another study showed that the overexpression of 7a inhibited cellular protein synthesis and induced the phosphorylation and activation of p38 MAPK (Kopecky-Bromberg *et al*., 2006). The 3a and 7a proteins could also activate NF-κB and JNK, leading to the enhancement of IL-8 and RANTES production (Kanzawa *et al*., 2006). Recently, we also demonstrated that the 7a protein interacts with the pro-survival protein, Bcl-X_L, and the overexpression of Bcl-X_L prevents 7a-induced apoptosis (Tan *et al*., 2007). A good correlation between the abilities of 7a deletion mutants to induce apoptosis and to interact with Bcl-X_L was observed, suggesting that 7a triggers apoptosis by interfering directly with the pro-survival function of Bcl-X_L.

Comparison of SARS-CoV- and MHV-induced apoptosis in cell culture systems

Mouse hepatitis virus is the one of the most well-characterized coronaviruses in terms of its pathogenesis and molecular biology. In particular, a wealth of information is available for the neurotropic John Howard Mueller (JHM) and the dual hepato- and neurotropic A59 strains and their effects on the central nervous system (see recent reviews by Perlman and Dandekar, 2005; Bergmann *et al*., 2006). Unlike MHV, infection of young mice (4–8 weeks) with SARS-CoV did not result in morbidity or mortality associated with infections in human despite the high level of viral replication in the upper and lower respiratory tracts (Glass *et al*., 2004; Subbarao *et al*., 2004; Wentworth *et al*., 2004). As such, the regulation of cell death during SARS-CoV infection has been studied mainly in cell culture systems. Here, we shall compare the molecular aspects for the regulation of cell death during infection of immortal cell lines by MHV-JHM, MHV-A549 and SARS coronaviruses.

Rat oligodendrocytes, which were obtained from the CG-4 cell line after differentiation in the presence of a low concentration of serum, underwent caspase-dependent apoptosis following infection by MHV-JHM (Liu *et al*., 2003). Just as the induction of apoptosis by SARS-CoV could be inhibited by caspase inhibitors or the overexpression of the pro-survival protein, Bcl-2 (Bordi *et al*., 2006), the MHV-induced apoptosis could be inhibited by the caspase-9 inhibitor and the overexpression of Bcl-2 and Bcl-X_L, indicating that the mitochondrial pathway is involved further downstream (Liu and Zhang, 2005; Liu *et al*., 2006b). In 17Cl-1, a fibroblast cell line, caspase-dependent apoptosis was also observed after infection with both MHV-JHM and MHV-A59 (An *et al*., 1999). It was further demonstrated that MHV-A59 infection of 17Cl-1 cells activated caspase-8, which in turn cleaved Bid, a BH3-domain pro-apoptotic member of the Bcl-2 family (Chen and Makino, 2002). The resulting tBid p15 fragment was translocated to the mitochondria, where it induced mitochondrial damage and activation of the caspase cascades. In contrast to these two cell lines, no apoptosis was observed in MHV-A59- or MHV-JHM-infected DBT cells (a mouse astroytoma cell line) although the cells showed extensive cell fusion and detachment from the plates (An *et al*., 1999). Curiously, overexpression of the MHV E protein caused apoptosis in DBT cells but not in 17Cl-1 cells. The reason for the contrasting responses of these cell lines to MHV infection or expression of the E protein remains to be determined. Similarly,

the overexpression of the E protein of SARS-CoV can induce apoptosis, and this can be blocked by the Bcl-X_L protein (Yang *et al*., 2005).

As illustrated for SARS-CoV and MHV, the expression of a single viral protein in immortal cell lines could be sufficient to induce cell death. However, the viral protein may not be an important cell-death regulatory factor during infection. For example, the overexpression of the SARS-CoV 7a protein in Vero cells resulted in apoptosis, but a mutant virus without the 7a/7b gene still induced extensive cytopathic effects in Vero cells, suggesting that 7a does not contribute significantly to viral-induced cell death, at least in this cell culture system (Yount *et al*., 2005). Also, while the overexpression of the MHV E protein induced apoptosis in DBT cells, the expression of E during MHV infection of DBT cells was not sufficient to induce apoptosis (An *et al*., 1999). Other mechanisms may be more important for regulating apoptosis during infection and this is described in the next section.

Other mechanisms for the induction of apoptosis during coronaviral infection

Besides expressing viral proteins that have the abilities to induce apoptosis during infection, viruses can use many other intrinsic and extrinsic mechanisms to modulate cell death in the host cells (see reviews by Roulston *et al*., 1999; Barber, 2001; Hay and Kannourakis, 2002). Here, we further described two other mechanisms that have been documented to be involved in apoptosis induction during coronaviral infection, namely induction of apoptosis via the secretion of soluble factors from neighbouring infected cells (i.e. bystander effects) and fusion of the viral envelope with cellular membranes.

Numerous coronaviruses [feline infectious peritonitis virus (FIPV), OC43, 229E, transmissible porcine gastroenteritis virus (TGEV)] have been shown to induce apoptosis in non-infected cells indirectly via the release of soluble cell-death mediators from neighbouring infected cells. In these cases, although viral replication in the apoptotic cells is not required *per se*, viral replication has to take place in the nearby infected cells. For example, infection of cats with a highly virulent strain of FIPV also caused apoptosis in a large number of lymphocytes (Haagmans *et al*., 1996). However, apoptosis did not result directly from infection, as many of apoptotic cells were not FIPV-antigen positive. Thus, apoptosis in FIPV-infected cats is occurring via an indirect mechanism. Similarly for two human coronaviruses, it is believed that the high level of cytokine secreted from OC43-infected MRC-5 cells or 229E-infected monocytes/macrophages may be partially responsible for the induction of apoptosis (Collins, 2001; 2002). Caspase-dependent apoptosis was induced in different cell lines infected with TGEV (Eleouet *et al*., 1998; Sirinarumitr *et al*., 1998). Again, many of the apoptotic cells were bystander cells as they were not infected by TGEV (Eleouet *et al*., 1998). On the other hand, no evidence of apoptosis was observed in the intestinal tissues of TGEV-infected piglets, suggesting that there may be some host factors that can prevent TGEV-induced apoptosis (Kim *et al*., 2000).

For MHV infection, release of soluble factors has also been shown to be crucial for the induction of demyelination (see a recent review by Perlman and Dandekar, 2005). As for SARS-CoV-induced apoptosis in Vero cells, viral replication is required, but whether apoptosis induction is direct or not has not been established (Ren *et al*., 2005). However, there is clear evidence for increased production of certain cytokines and chemokines during SARS-CoV infection (see recent reviews by Cameron *et al*., 2007; Chen and Subbarao, 2007), and this could result in apoptosis via a bystander effect. Another mechanism that is used by MHV to induce apoptosis in rat oligodendrocytes is dependent on the fusion of the viral envelope with cellular membranes, which led to the activation of the Fas signalling pathway (Liu and Zhang, 2007). In this case, UV-inactivated MHV, which is no longer replicative but retains the ability to bind cell receptors and enter the cell, could trigger apoptosis. SARS-CoV does not appear to induce cell death in this manner, as UV-inactivated SARS-CoV does not induce apoptosis (Ren *et al*., 2005).

Concluding remarks and future directions

Since the identification of the SARS-CoV in the year 2003, extensive research on the SARS-CoV has yielded significant understanding of this newly emerged virus. In terms of cell-death regulation during SARS-CoV, the occurrence of cell death during infection both *in vitro* and *in vivo* has been established, and numerous viral factors have been suggested to contribute to the regulation of cell death in infected cells. Although apoptosis and necrosis have been observed in different tissues obtained from SARS patients, the ability of SARS-CoV to induce apoptosis was demonstrated in only one cell line, Vero E6, and was not extensively studied in animal models. Similarly, as summarized in Table 1, many of these studies have investigated the effects of the expression of individual viral proteins on cell death regulation (and other cellular pathways) in a limited number of cell lines; hence it is not clear whether each viral protein can induce cell death in all types of cells. It has also not been determined whether the expressions of these viral proteins are high enough during SARS-CoV infection, and whether they function in the presence of other viral factors present during infection. Given that analysis of host gene transcriptions has suggested that there are significant differences in cellular responses to

SARS-CoV infection in different cell typs, it is necessary to carry out future investigations in numerous cell lines that support SARS-CoV replication.

Clearly, these gaps in our knowledge will be addressed in future studies using infectious clones of SARS-CoV (Yount *et al*., 2003; Almazan *et al*., 2006) and animal models like aged Balb/c mice, which, unlike young mice, developed some histopathological damages upon SARS-CoV infection (Roberts *et al*., 2005). In order to understand the regulation of cell death during SARS-CoV infection, we urgently need to address whether induction of cell death occurs indirectly via bystander effects or directly via expression of viral proteins, or both. For the latter, there is currently no link between the effects of viral proteins on cellular pathways and the induction of apoptosis. To delineate the precise pathways involved, more experiments, like RNA interference and mutagenesis studies, are required to establish structure–function relationships. When compared with the other coronaviruses that have been studied for many years, future advancement in understanding the SARS-CoV may take considerable more time and effort to achieve because of the lack of a single animal model that reproduces all aspects of the human disease (Subbarao and Roberts, 2006) and requirement for infection studies to be performed in biosafety level 3 or 4 laboratories.

Infections of host cells by SARS-CoV and other coronaviruses have been reported to cause apoptosis. Necrosis has also been observed for some coronaviruses. The diverse responses to infection revealed the complex manner by which coronaviruses modulate cell death. Furthermore, infection of different cell types by the same virus could also activate distinct cell-death pathways, reflecting the intricate interaction between virus and cell host factors. In order to delineate the contributions of the different viral proteins and viral–host interactions to the regulation of cell death, the correlation between the expression of individual viral proteins and the extent of cell death during infection needs to be established. The availabilities of full-length infectious clones of several coronaviruses and robust animal models provide the essential tools for these future studies. When combined with the technologies to create transgenic or knockout mice and small interfering RNA methodologies for specific gene knockdown, such research endeavour will eventually lead to a better understanding of intricate interplay between virus and host.

Note added in proof

A recent study showed that SARS-CoV without gene 7a and 7b is not as efficient as wild-type virus in inducing DNA fragmentation, implying that 7a and/or 7b contribute to virus-induced apoptosis in cell culture. Schaecher, S.R., Touchette, E., Schriewer, J., Buller, R.M., and Pekosz, A. (2007) The Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) gene 7 products contribute to virus-induced apoptosis. *J Virol* (epub ahead of print). doi: 10.1128/JVI.01266-07

Acknowledgements

We thank the Agency for Science, Technology and Research (A*STAR), Singapore, for funding our research. We apologize to any investigators whose work we have inadvertently omitted.

References

Almazan, F., Dediego, M.L., Galan, C., Escors, D., Alvarez, E., Ortego, J., *et al.* (2006) Construction of a severe acute respiratory syndrome coronavirus infectious cDNA clone and a replicon to study coronavirus RNA synthesis. *J Virol* **80:** 10900–10906.

An, S., Chen, C.J., Yu, X., Leibowitz, J.L., and Makino, S. (1999) Induction of apoptosis in murine coronavirus-infected cultured cells and demonstration of E protein as an apoptosis inducer. *J Virol* **73:** 7853–7859.

Assuncao Guimaraes, C., and Linden, R. (2004) Programmed cell death: apoptosis and alternative deathstyles. *Eur J Biochem* **271:** 1638–1650.

Barber, G.N. (2001) Host defense, viruses and apoptosis. *Cell Death Differ* **8:** 113–126.

Bergmann, C.C., Lane, T.E., and Stohlman, S.A. (2006) Coronavirus infection of the central nervous system: host-virus stand-off. *Nat Rev Microbiol* **4:** 121–132.

Bordi, L., Castilletti, C., Falasca, L., Ciccosanti, F., Calcaterra, S., Rozera, G., *et al.* (2006) Bcl-2 inhibits the caspase-dependent apoptosis induced by SARS-CoV without affecting virus replication kinetics. *Arch Virol* **151:** 369–377.

Cameron, M.J., Bermejo-Martin, J.F., Danesh, A., Muller, M.P., and Kelvin, D.J. (2007) Human immunopathogenesis of severe acute respiratory syndrome (SARS). *Virus Res* (epub ahead of print). doi: 10.1016/j.virusres.2007.02.014

Chau, T.N., Lee, K.C., Yao, H., Tsang, T.Y., Chow, T.C., Yeung, Y.C., *et al.* (2004) SARS-associated viral hepatitis caused by a novel coronavirus: report of three cases. *Hepatology* **39:** 302–310.

Chen, C.J., and Makino, S. (2002) Murine coronavirus-induced apoptosis in 17Cl-1 cells involves a mitochondria-mediated pathway and its downstream caspase-8 activation and bid cleavage. *Virology* **302:** 321–332.

Chen, J., and Subbarao, K. (2007) The immunobiology of SARS. *Annu Rev Immunol* **25:** 443–472.

Chen, R.F., Chang, J.C., Yeh, W.T., Lee, C.H., Liu, J.W., Eng, H.L., and Yang, K.D. (2006) Role of vascular cell adhesion molecules and leukocyte apoptosis in the lymphopenia and thrombocytopenia of patients with severe acute respiratory syndrome (SARS). *Microbes Infect* **8:** 122–127.

Chng, W.J., Lai, H.C., Earnest, A., and Kuperan, P. (2005) Haematological parameters in severe acute respiratory syndrome. *Clin Lab Haematol* **27:** 15–20.

Chong, P.Y., Chui, P., Ling, A.E., Franks, T.J., Tai, D.Y., Leo, Y.S., *et al.* (2004) Analysis of deaths during the severe acute respiratory syndrome (SARS) epidemic in Singapore: challenges in determining a SARS diagnosis. *Arch Pathol Lab Med* **128:** 195–204.

Chow, K.Y., Yeung, Y.S., Hon, C.C., Zeng, F., Law, K.M., and Leung, F.C. (2005) Adenovirus-mediated expression of the C-terminal domain of SARS-CoV spike protein is sufficient to induce apoptosis in Vero E6 cells. *FEBS Lett* **579:** 6699–6704.

Cinatl, J. Jr, Hoever, G., Morgenstern, B., Preiser, W., Vogel, J.U., Hofmann, W.K., *et al.* (2004) Infection of cultured intestinal epithelial cells with severe acute respiratory syndrome coronavirus. *Cell Mol Life Sci* **61:** 2100–2012.

Collins, A.R. (2001) Induction of apoptosis in MRC-5, diploid human fetal lung cells after infection with human coronavirus OC43. *Adv Exp Med Biol* **494:** 677–682.

Collins, A.R. (2002) In vitro detection of apoptosis in monocytes/macrophages infected with human coronavirus. *Clin Diagn Lab Immunol* **9:** 1392–1395.

Dejean, L.M., Martinez-Caballero, S., Manon, S., and Kinnally, K.W. (2006) Regulation of the mitochondrial apoptosis-induced channel, MAC, by BCL-2 family proteins. *Biochim Biophys Acta* **1762:** 191–201.

Ding, Y., Wang, H., Shen, H., Li, Z., Geng, J., Han, H., *et al.* (2003) The clinical pathology of severe acute respiratory syndrome (SARS): a report from China. *J Pathol* **200:** 282–289.

Eleouet, J.F., Chilmonczyk, S., Besnardeau, L., and Laude, H. (1998) Transmissible gastroenteritis coronavirus induces programmed cell death in infected cells through a caspase-dependent pathway. *J Virol* **72:** 4918–4924.

Festjens, N., Vanden Berghe, T., and Vandenabeele, P. (2006) Necrosis, a well-orchestrated form of cell demise: signalling cascades, important mediators and concomitant immune response. *Biochim Biophys Acta* **1757:** 1371–1387.

Fouchier, R.A., Kuiken, T., Schutten, M., van Amerongen, G., van Doornum, G.J., van den Hoogen, B.G., *et al.* (2003) Aetiology: Koch's postulates fulfilled for SARS virus. *Nature* **423:** 240.

Gillim-Ross, L., Taylor, J., Scholl, D.R., Ridenour, J., Masters, P.S., and Wentworth, D.E. (2004) Discovery of novel human and animal cells infected by the severe acute respiratory syndrome coronavirus by replication-specific multiplex reverse transcription-PCR. *J Clin Microbiol* **42:** 3196–3206.

Glass, W.G., Subbarao, K., Murphy, B., and Murphy, P.M. (2004) Mechanisms of host defense following severe acute respiratory syndrome-coronavirus (SARS-CoV) pulmonary infection of mice. *J Immunol* **173:** 4030–4039.

Gu, J., Gong, E., Zhang, B., Zheng, J., Gao, Z., Zhong, Y., *et al.* (2005) Multiple organ infection and the pathogenesis of SARS. *J Exp Med* **202:** 415–424.

Haagmans, B.L., Egberink, H.F., and Horzinek, M.C. (1996) Apoptosis and T-cell depletion during feline infectious peritonitis. *J Virol* **70:** 8977–8983.

Hay, S., and Kannourakis, G. (2002) A time to kill: viral manipulation of the cell death program. *J Gen Virol* **83:** 1547–1564.

He, R., Leeson, A., Andonov, A., Li, Y., Bastien, N., Cao, J., *et al.* (2003) Activation of AP-1 signal transduction pathway by SARS coronavirus nucleocapsid protein. *Biochem Biophys Res Commun* **311:** 870–876.

Hess, J., Angel, P., and Schorpp-Kistner, M. (2004) AP-1 subunits: quarrel and harmony among siblings. *J Cell Sci* **117:** 5965–5973.

Jacomy, H., Fragoso, G., Almazan, G., Mushynski, W.E., and Talbot, P.J. (2006) Human coronavirus OC43 infection induces chronic encephalitis leading to disabilities in BALB/C mice. *Virology* **349:** 335–346.

Kanzawa, N., Nishigaki, K., Hayashi, T., Ishii, Y., Furukawa, S., Niiro, A., *et al.* (2006) Augmentation of chemokine production by severe acute respiratory syndrome coronavirus 3a/X1 and 7a/X4 proteins through NF-kappaB activation. *FEBS Lett* **580:** 6807–6812.

Kaye, M., Druce, J., Tran, T., Kostecki, R., Chibo, D., Morris, J., *et al.* (2006) SARS-associated coronavirus replication in cell lines. *Emerg Infect Dis* **12:** 128–133.

Khan, S., Fielding, B.C., Tan, T.H.P., Chou, C.-F., Shen, S., Lim, S.G., *et al.* (2006) Over-expression of severe acute respiratory syndrome coronavirus 3b protein induces both apoptosis and necrosis in Vero E6 cells. *Virus Res* **122:** 20–27.

Kim, B., Kim, O., Tai, J.H., and Chae, C. (2000) Transmissible gastroenteritis virus induces apoptosis in swine testicular cell lines but not in intestinal enterocytes. *J Comp Pathol* **123:** 64–66.

Kopecky-Bromberg, S.A., Martinez-Sobrido, L., and Palese, P. (2006) 7a protein of severe acute respiratory syndrome coronavirus inhibits cellular protein synthesis and activates p38 mitogen-activated protein kinase. *J Virol* **80:** 785–793.

Lang, Z.W., Zhang, L.J., Zhang, S.J., Meng, X., Li, J.Q., Song, C.Z., *et al.* (2003) A clinicopathological study of three cases of severe acute respiratory syndrome (SARS). *Pathology* **35:** 526–531.

Law, P.T., Wong, C.H., Au, T.C., Chuck, C.P., Kong, S.K., Chan, P.K., *et al.* (2005) The 3a protein of severe acute respiratory syndrome-associated coronavirus induces apoptosis in Vero E6 cells. *J Gen Virol* **86:** 1921–1930.

Leong, W.F., Tan, H.C., Ooi, E.E., Koh, D.R., and Chow, V.T.K. (2005) Microarray and real-time RT-PCR analyses of differential human gene expression patterns induced by severe acute respiratory syndrome (SARS) coronavirus infection of Vero cells. *Microbes Infect* **7:** 248–259.

Liao, Q.J., Ye, L.B., Timani, K.A., Zeng, Y.C., She, Y.L., Ye, L., and Wu, Z.H. (2005) Activation of NF-kappaB by the full-length nucleocapsid protein of the SARS coronavirus. *Acta Biochim Biophys Sin (Shanghai)* **37:** 607–612.

Liao, Y., Yuan, Q., Torres, J., Tam, J.P., and Liu, D.X. (2006) Biochemical and functional characterization of the membrane association and membrane permeabilizing activity of the severe acute respiratory syndrome coronavirus envelope protein. *Virology* **349:** 264–275.

Lin, C.W., Lin, K.H., Hsieh, T.H., Shiu, S.Y., and Li, J.Y. (2006) Severe acute respiratory syndrome coronavirus 3C-like protease-induced apoptosis. *FEMS Immunol Med Microbiol* **46:** 375–380.

Liu, C., Xu, H.Y., and Liu, D.X. (2001) Induction of caspase-dependent apoptosis in cultured cells by the avian coronavirus infectious bronchitis virus. *J Virol* **75:** 6402–6409.

Liu, Y., Cai, Y., and Zhang, X. (2003) Induction of caspase-dependent apoptosis in cultured rat oligodendrocytes by murine coronavirus is mediated during cell entry and does not require virus replication. *J Virol* **77:** 11952–11963.

Liu, Y., and Zhang, X. (2005) Expression of cellular oncogene Bcl-xL prevents coronavirus-induced cell death and converts acute infection to persistent infection in progenitor rat oligodendrocytes. *J Virol* **79:** 47–56.

Liu, M., Gu, C., Wu, J., and Zhu, Y. (2006a) Amino acids 1–422 of the spike protein of SARS associated coronavirus are required for induction of cyclooxygenase-2. *Virus Genes* **33:** 309–317.

Liu, Y., Pu, Y., and Zhang, X. (2006b) Role of the mitochondrial signaling pathway in murine coronavirus-induced oligodendrocyte apoptosis. *J Virol* **80:** 395–403.

Liu, Y., and Zhang, X. (2007) Murine coronavirus-induced oligodendrocyte apoptosis is mediated through the activation of the Fas signaling pathway. *Virology* **360:** 364–375.

Lu, W., Zheng, B.J., Xu, K., Du Schwarz, W.L., Wong, C.K., *et al.* (2006) Severe acute respiratory syndrome-associated coronavirus 3a protein forms an ion channel and modulates virus release. *Proc Natl Acad Sci USA* **103:** 12540–12545.

Madan, V., Garcia Mde, J., Sanz, M.A., and Carrasco, L. (2005) Viroporin activity of murine hepatitis virus E protein. *FEBS Lett* **579:** 3607–3612.

Marra, M.A., Jones, S.J., Astell, C.R., Holt, R.A., Brooks-Wilson, A., Butterfield, Y.S. *et al.* (2003) The Genome sequence of the SARS-associated coronavirus. *Science* **300:** 1399–1404.

Mizutani, T., Fukushi, S., Saijo, M., Kurane, I., and Morikawa, S. (2004) Phosphorylation of p38 MAPK and its downstream targets in SARS coronavirus-infected cells. *Biochem Biophys Res Commun* **319:** 1228–1234.

Nelson, D.A., and White, E. (2004) Exploiting different ways to die. *Genes Dev* **18:** 1223–1226.

Ng, L.F., Hibberd, M.L., Ooi, E.E., Tang, K.F., Neo, S.Y., Tan, J. *et al.* (2004) A human in vitro model system for investigating genome-wide host responses to SARS coronavirus infection. *BMC Infect Dis* **4:** 34.

Peiris, J.S., Yuen, K.Y., Osterhaus, A.D., and Stohr, K. (2003) The severe acute respiratory syndrome. *N Engl J Med* **349:** 2431–2441.

Perkins, N.D., and Gilmore, T.D. (2006) Good cop, bad cop: the different faces of NF-kappaB. *Cell Death Differ* **13:** 759–772.

Perlman, S., and Dandekar, A.A. (2005) Immunopathogenesis of coronavirus infections: implications for SARS. *Nat Rev Immunol* **5:** 917–927.

Reghunathan, R., Jayapal, M., Hsu, L.Y., Chng, H.H., Tai, D., Leung, B.P., and Melendez, A.J. (2005) Expression profile of immune response genes in patients with severe acute respiratory syndrome. *BMC Immunol* **6:** 2.

Ren, L., Yang, R., Guo, L., Qu, J., Wang, J., and Hung, T. (2005) Apoptosis induced by the SARS-associated coronavirus in Vero cells is replication-dependent and involves caspase. *DNA Cell Biol* **24:** 496–502.

Roberts, A., Paddock, C., Vogel, L., Butler, E., Zaki, S., and Subbarao, K. (2005) Aged BALB/c mice as a model for increased severity of severe acute respiratory syndrome in elderly humans. *J Virol* **79:** 5833–5838.

Rota, P.A., Oberste, M.S., Monroe, S.S., Nix, W.A., Campagnoli, R., Icenogle, J.P., *et al.* (2003) Characterization of a novel coronavirus associated with severe acute respiratory syndrome. *Science* **300:** 1394–1399.

Roulston, A., Marcellus, R.C., and Branton, P.E. (1999) Viruses and apoptosis. *Annu Rev Microbiol* **53**: 577–628.

Shao, H., Lan, D., Duan, Z., Liu, Z., Min, J., Zhang, L., *et al.* (2006) Upregulation of mitochondrial gene expression in PBMC from convalescent SARS patients. *J Clin Immunol* **26:** 546–554.

Shen, S., Wen, Z.L., and Liu, D.X. (2003) Emergence of a coronavirus infectious bronchitis virus mutant with a truncated 3b gene: functional characterization of the 3b protein in pathogenesis and repolication. *Virology* **311:** 16–27.

Siddell, S.G. (1995) The coronaviridae: An introduction. In *The Coronaviridae*. Siddell, SG, (ed.). New York: Plenum Press, pp. 1–9.

Sirinarumitr, T., Kluge, J.P., and Paul, P.S. (1998) Transmissible gastroenteritis virus induced apoptosis in swine testes cell cultures. *Arch Virol* **143:** 2471–2485.

Subbarao, K., and Roberts, A. (2006) Is there an ideal animal model for SARS? *Trends Microbiol* **14:** 299–303.

Subbarao, K., McAuliffe, J., Vogel, L., Fahle, G., Fischer, S., Tatti, K., *et al.* (2004) Prior infection and passive transfer of neutralizing antibody prevent replication of severe acute respiratory syndrome coronavirus in the respiratory tract of mice. *J Virol* **78:** 3572–3577.

Surjit, M., Liu, B., Jameel, S., Chow, V.T., and Lal, S.K. (2004) The SARS coronavirus nucleocapsid protein induces actin reorganization and apoptosis in COS-1 cells in the absence of growth factors. *Biochem J* **383:** 13–18.

Surjit, M., Liu, B., Chow, V.T., and Lal, S.K. (2006) The nucleocapsid protein of severe acute respiratory syndrome-coronavirus inhibits the activity of cyclin-cyclin-dependent kinase complex and blocks S phase progression in mammalian cells. *J Biol Chem* **281:** 10669–10681.

Tan, Y.-J., Fielding, B.C., Goh, P.Y., Shen, S., Tan, T.H.P., Lim, S.G., and Hong, W. (2004) Over-expression of 7a, a protein specifically encoded by the severe acute respiratory syndrome (SARS) –coronavirus, induces apoptosis via a caspase-dependent pathway. *J Virol* **78:** 14043–14047.

Tan, Y.-J., Lim, S.G., and Hong, W. (2006) Understanding the accessory viral proteins unique to the severe acute respiratory syndrome coronavirus. *Antiviral Res* **72:** 78–88.

Tan, Y.X., Tan, T.H., Lee, M.J., Tham, P.Y., Gunalan, V., Druce, J., *et al.* (2007) The induction of apoptosis by the severe acute respiratory syndrome (SARS) – coronavirus 7a protein is dependent on its interaction with the Bcl-XL protein. *J Virol* **81:** 6346–6355.

Tang, B.S., Chan, K.H., Cheng, V.C., Woo, P.C., Lau, S.K., Lam, C.C., *et al.* (2005) Comparative host gene transcription by microarray analysis early after infection of the Huh7 cell line by severe acute respiratory syndrome coronavirus and human coronavirus 229E. *J Virol* **79:** 6180–6193.

Tergaonkar, V. (2006) NFkappaB pathway: a good signaling paradigm and therapeutic target. *Int J Biochem Cell Biol* **38:** 1647–1653.

Wang, H., Mao, Y., Ju, L., Zhang, J., Liu, Z., Zhou, X., *et al.* (2004) Detection and monitoring of SARS coronavirus in the plasma and peripheral blood lymphocytes of patients with severe acute respiratory syndrome. *Clin Chem* **50:** 1237–1240.

Wei, L., Sun, S., Xu, C.H., Zhang, J., Xu, Y., Zhu, H., *et al.* (2007) Pathology of the thyroid in severe acute respiratory syndrome. *Hum Pathol* **38:** 95–102.

Wentworth, D.E., Gillim-Ross, L., Espina, N., and Bernard, K.A. (2004) Mice susceptible to SARS coronavirus. *Emerg Infect Dis* **10:** 1293–1296.

Wilson, L., McKinlay, C., Gage, P., and Ewart, G. (2004) SARS coronavirus E protein forms cation-selective ion channels. *Virology* **330:** 322–331.

Wilson, L., Gage, P., and Ewart, G. (2006) Hexamethylene amiloride blocks E protein ion channels and inhibits coronavirus replication. *Virology* **353:** 294–306.

Yan, H., Xiao, G., Zhang, J., Hu, Y., Yuan, F., Cole, D.K., *et al.* (2004) SARS coronavirus induces apoptosis in Vero E6 cells. *J Med Virol* **73:** 323–331.

Yan, X., Hao, Q., Mu, Y., Timani, K.A., Ye L., Zhu, Y., and Wu, J. (2006) Nucleocapsid protein of SARS-CoV activates the expression of cyclooxygenase-2 by binding directly to regulatory elements for nuclear factor-kappa B and CCAAT/enhancer binding protein. *Int J Biochem Cell Biol* **38:** 1417–1428.

Yang, Y., Xiong, Z., Zhang, S., Yan, Y., Nguyen, J., Ng, B., *et al.* (2005) Bcl-xL inhibits T cell apotosis induced by expression of SARS coronavirus E protein in the absence of growth factors. *Biochem J* **392:** 135–143.

Yount, B., Curtis, K.M., Fritz, E.A., Hensley, L.E., Jahrling, P.B., Prentice, E., *et al.* (2003) *R*everse genetics with a full-length infectious cDNA of severe acute respiratory syndrome coronavirus. *Proc Natl Acad Sci USA* **100:** 12995–13000.

Yount, B., Roberts, R.S., Sims, A.C., Deming, D., Frieman, M.B., Sparks, J., *et al.* (2005) Severe acute respiratory syndrome coronavirus group-specific open reading frames encode nonessential functions for replication in cell cultures and mice. *J Virol* **79:** 14909–14922.

Yu, S.Y., Hu, Y.W., Liu, X.Y., Xiong, W., Zhou, Z.T., and Yuan, Z.H. (2005) Gene expression profiles in peripheral blood mononuclear cells of SARS patients. *World J Gastroenterol* **11:** 5037–5043.

Yuan, X., Shan, Y., Zhao, Z., Chen, J., and Cong, Y. (2005) G0/G1 arrest and apoptosis induced by SARS-CoV 3b protein in transfected cells. *Virol J* **2:** 66.

Yuan, X., Shan, Y., Yao, Z., Li, J., Zhao, Z., Chen, J., and Cong, Y. (2006a) Mitochondrial location of severe acute respiratory syndrome coronavirus 3b protein. *Mol Cells* **21:** 186–191.

Yuan, X., Wu, J., Shan, Y., Yao, Z., Dong, B., Chen, B., *et al.* (2006b) SARS coronavirus 7a protein blocks cell cycle progression at G0/G1 phase via the cyclin D3/pRb pathway. *Virology* **346:** 74–85.

Zhao, G., Shi, S.Q., Yang, Y., and Peng, J.P. (2006) M and N proteins of SARS coronavirus induce apoptosis in HPF cells. *Cell Biol Toxicol* **22:** 313–322.

Ziebuhr, J. (2004) Molecular biology of severe acute respiratory syndrome coronavirus. *Curr Opin Microbiol* **7:** 412–419.

Zong, W.X., and Thompson, C.B. (2006) Necrotic death as a cell fate. *Genes Dev* **20:** 1–15.

