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A179L, a viral Bcl-2 homologue, targets the core Bcl-2 apoptotic machinery and its upstream BH3 activators with selective binding restrictions for Bid and Noxa

Inmaculada Galindo [1], Bruno Hernaez [1], Gema Díaz-Gil [1,2], Jose M. Escribano, Covadonga Alonso *

Departamento de Biotecnología, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Autopista A6 Km 7, 28040 Madrid, Spain

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Abstract

Several large DNA viruses encode Bcl-2 protein homologues involved in the regulation of the cellular apoptosis cascade. This regulation often involves the interaction of these viral proteins with diverse cellular Bcl-2 family members. We have identified the specific interactions of A179L, an African swine fever virus (ASFV) Bcl-2 homologue, with the active forms of the porcine BH3-only Bid protein (truncated Bid p13 and p15). Transient expression of ASFV A179L gene in Vero cells prevented apoptosis induced by these active forms of Bid protein. Interestingly, A179L protein was able to interact, also with the main core Bcl-2 proapoptotic proteins Bax and Bak, and with several BH3-only proteins with selective binding restrictions for full length Bid and Noxa. These results suggest a fine regulation for A179L action in the suppression of apoptosis in infected cells which is essential for efficient virus replication.

Keywords: Apoptosis; Bid; Virus–cell interaction; African swine fever virus

Introduction

Some of the best studied cellular apoptosis regulators belong to the Bcl-2 family, which include both proapoptotic and antiapoptotic effectors (Korsmeyer, 1995; White, 1996). Members of Bcl-2 family have common conserved regions, designated Bcl-2 homology regions 1, 2, 3 and 4 (BH1, BH2, BH3 and BH4). Bcl-2 protein is the prototypical member that negatively regulates apoptosis and contains all the Bcl-2 domains. This protein preserves mitochondrial integrity by interacting with Bcl-2 family proapoptotic members (Petros et al., 2004). Apoptosis inducer members include BH3-only proteins, which are cellular damage sensors that initiate rapidly the death process, and Bax-like proteins that act downstream of BH3-only proteins to permeabilise the mitochondrial outer membrane (Bouillet and Strasser, 2002).

The apoptosis cascade may be initiated by pathogenic agents such as viruses and is considered as part of the cellular defensive mechanism. Viruses have adapted numerous ways of circumventing this host defensive response, including regulation of endogenous host death receptors and ligands, expression of caspase activation inhibitors, regulation of host Bcl-2 proteins, and expression of viral homologues of Bcl-2 (vBcl-2s) (Benedict et al., 2002; Polster et al., 2004).These viral genes encoding proteins with amino acid sequence similarity to cellular Bcl-2 apoptosis inhibitors have been identified in several viral models including Epstein–Barr virus (EBV) (Bellows et al., 2002; Henderson et al., 1993), human herpes virus 8 (HHV8) (Cheng et al., 1997) and African swine fever virus (ASFV) (Afonso et al., 1996) between others. The role of these vBcl-2s in diverse aspects of the viral cell-cycle and their mechanism of action has been gradually emerging (Everett and McFadden, 1999; Hardwick and Bellows, 2003). VBcl-2s mediate inhibition of apoptosis in infected cells and prevent premature death of the host cell which would impair virus replication and might have also a role in the development of persistent infection (Cuconati and White, 2002).

Abbreviations: vBcl-2, viral Bcl-2; cBcl-2, cellular Bcl-2; ORF, open reading frame; HA, hemagglutinin tag; Bid, BH3 interacting domain death agonist; tBid, truncated Bid; ASFV, African swine fever virus.

* Corresponding author. Fax: +34 91 3573107.
E-mail address: calonso@inia.es (C. Alonso).
[1] The first three authors contributed equally to this work.
[2] Present address: Facultad Ciencias de la Salud, Universidad Rey Juan Carlos, 28922, Madrid, Spain.

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African swine fever virus (ASFV) is a double stranded large DNA virus that induces an acute disease of swine in which apoptosis plays a central role in pathogenesis. Virus infection induces apoptosis in target and immune defence cells (Oura et al., 1998; Ramiro-Ibanez et al., 1996). This programmed cell death induction in the target cell has been recently tracked in vivo in living cells as infection progresses and the execution phase of apoptosis becomes evident at late infection times (Hernaez et al., 2006). In fact, ASFV encodes for various apoptosis inhibitor genes, one of these sharing high sequence similarity to cellular Bcl-2, the ASFV A179L gene (Revilla et al., 1997; Yanez et al., 1995). This gene encodes for a 21 kDa protein which is expressed at early and late times after infection and is essential for virus replication throughout the infection cycle (Brun et al., 1996; Neilan et al., 1993). A179L is highly conserved in most ASFV isolates, both in pathogenic and cell-cultured adapted isolates. In comparison to other viral Bcl-2s, its sequence is very similar to the cellular protein containing all the characteristic Bcl-2 homology domains (BH1, BH2, BH3 and BH4), but lacking the transmembrane region (Afonso et al., 1996). A179L is involved in the suppression of apoptosis in ASFV infected cells (Brun et al., 1996) and prolongs host cell survival until the replication of the large viral genome is completed. Prolonged cell survival could be relevant facilitating a persistent infection (Afonso et al., 1996; Brun et al., 1996). Moreover, given the fact that immune defence entails cytotoxic T lymphocytes attack against infected cells by TNFα, FasL or TRAIL, A179L could play a role in the infected cell escape to premature death due to cytokine signalling. Mutations in the BH1 domain of A179L abrogate its death-repressor activity (Revilla et al., 1997). Interestingly, this protein is functional and prevents virus induced apoptosis not only in mammalian cells but also in insect cells (Brun et al., 1998), indicating a very low degree of species-specificity, as would be required of a viral protein that should exert its function in both, mammals and the arthropod vector (White, 1996). The ASFV arthropod vectors are ticks of the *Ornithodorus* genus (Plowright et al., 1969).

However, the precise mechanism of action of A179L remains undefined. Some evidences suggest that most vBcl-2s might target the core cellular proapoptotic machinery for inhibition (Bellows et al., 2002; Nava et al., 1997), but also redundant actions on specific, short BH3 proapoptotic members have been described (Boyd et al., 1995; Han et al., 1996b) probably directed to secure apoptosis inhibition in the infected cell.

The aim of this work was to characterize the biochemical mechanisms by which the A179L protein suppresses apoptosis. Active forms of Bid protein from *Sus scrofa* were first identified as A179L interacting proteins. A179L blocked Bid-induced apoptosis when transfected in Vero cells, pointing out that A179L action may take place downstream of caspase 8 or granzyme B cleavage. In this work, we have shown that A179L protein interacted specifically with both BH3-only proapoptotic proteins and the core cellular proapoptotic machinery suggesting a central role for this protein in the inhibition of apoptosis induced by a wide variety of stimuli.

Results

Interaction of ASFV A179L protein with p13 truncated Bid protein

Although previous results demonstrated that ASVF A179L protects cells from programmed cell death, the molecular mechanisms supporting this biological effect remain to be determined. To elucidate the role of the virus Bcl-2 homologue, the yeast two hybrid system was used to screen a porcine macrophage cDNA library with full-length A179L as bait, searching for cellular interacting proteins. Two yeast clones were identified to induce the expression of the three reporter genes (*HIS3*, *LEU2*, *TRIP1*), as indicated by growth on SD medium and blue staining in the presence of X-α-Gal. These two cDNA clones were characterized by nucleotide sequence analysis. One of these clones was not included in these studies because the sequence revealed no significant homology to any known gene or translated product at the NCBI data base. Another cDNA clone was found to encode a porcine protein with high percentage homology (65%) to tBid-p13 protein from *Homo sapiens*. TBid-p13 protein corresponds to the carboxy-terminal fragment of Bid protein, named truncated Bid (tBid).

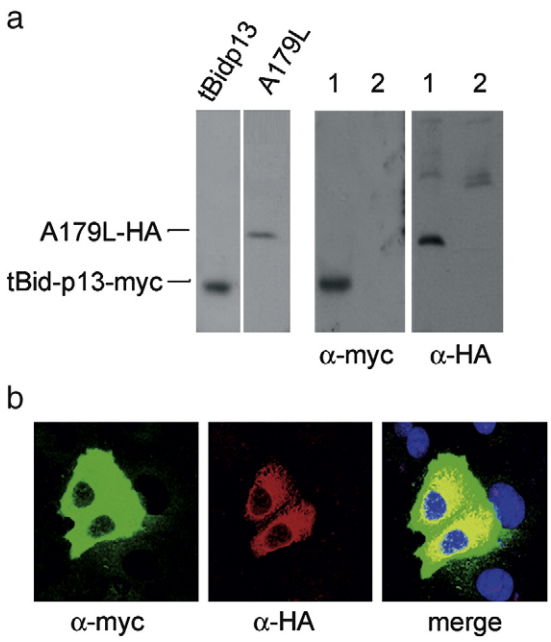

Fig. 1. ASFV A179L interaction with porcine tBid-p13 in mammalian cells by affinity chromatography and confocal microscopy. (a) Total cell lysates from single transfected cells with either tBid-p13–myc or A179L–HA were blotted for anti-myc or anti-HA (left panel). Interaction of A179L with tBid-p13 was confirmed by immunoprecipitation of HA-tagged A179L and myc-tagged tBid-p13, from Vero cells transfected with the corresponding expression constructs. The immunoprecipitates were analyzed by SDS-PAGE and subjected to immunoblotting with antibodies against myc or HA. Lane 1, immunoprecipitation with a mAb against HA. Lane 2, immunoprecipitation with normal mouse serum (right panel). (b) Vero cells were transiently transfected with pCMVA179L–HA and pCMVtBid-p13–myc plasmids. Colocalization of A179L and tBid-p13 was assayed by confocal microscopy. A179L was detected with anti-HA-Alexa 594 (red) and tBid-p13 with anti-myc-FITC (green). Nuclei were stained with Hoechst 33258 (blue). Colocalization areas are depicted in yellow.