Review

Apoptosis in schistosomes: toward novel targets for the treatment of schistosomiasis

Erinna F. Lee[1,2], Neil D. Young[3], Nicholas T.Y. Lim[1], Robin B. Gasser[3], and W. Douglas Fairlie[1,2]

[1] The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria, Australia
[2] Department of Medical Biology, The University of Melbourne, Parkville, Victoria, Australia
[3] Faculty of Veterinary Science, The University of Melbourne, Parkville, Victoria, Australia

Schistosomiasis is one of the world's major neglected tropical diseases. Recent advances in schistosome genomics and transcriptomics have identified components of an intrinsic, B cell lymphoma-2 (Bcl-2)-regulated apoptotic cell death pathway. Molecular characterization of this pathway demonstrates its similarity to that in mammals. Gene expression and functional data indicate that apoptosis is active throughout the lifecycle. Moreover, drugs that activate apoptosis in human cells kill schistosome cells, raising the prospect of developing new treatments against schistosomiasis of humans. The development of new drugs is increasingly important in the face of the potential for resistance to currently available treatments, and the lack of an effective vaccine.

Schistosomiasis and the need for new treatments

Parasitic helminth infections afflict >1.5 billion people worldwide, particularly in developing countries, including regions of sub-Saharan Africa, Asia, and the Americas [1]. These worms belong to two phyla: the Nematoda (nematodes or roundworms) and Platyhelminthes, (platyhelminths or flatworms) [1]. Schistosomes are dioecious (see Glossary) platyhelminths with complex life cycles (Box 1) and cause disease in approximately 200 million people, with 300 000 deaths annually, mostly in sub-Saharan Africa [2]. There are three main species of schistosome infecting humans: *Schistosoma mansoni* and *Schistosoma japonicum*, which reside in the portal system of liver and mesenteric arteries, and *Schistosoma haematobium*, which dwells in the vessels of the bladder and/or genital tract [2]. Adult female schistosomes live *encopula* with males, within a gynaecophoric canal, and are capable of producing hundreds of eggs per day [2]. The eggs are the main contributor to the pathogenesis of schistosomiasis; this is termed 'egg-induced' disease [3]. They become entrapped in capillaries and induce the formation of granulomata within the liver, spleen, and/or intestinal wall when produced by *S. mansoni* and *S. japonicum*, or in the urogenital tract when produced by *S. haematobium* [3]. These pathological changes lead to morbidity associated with symptoms such as portal hypertension, splenomegaly, diarrhoea (*S. mansoni* and *S. japonicum*), increased frequency of urination, and haematuria (*S. haematobium*) [2–4]. *S. haematobium* also predisposes to HIV/AIDS and malignant bladder cancer [4].

Currently, there is no effective vaccine to prevent schistosomiasis [5], and treatment is entirely dependent on chemotherapy [6]. Although there are various drugs with efficacy against schistosomes, praziquantel (PZQ) has been the drug of choice since the 1980s because of its safety [6]. However, owing to the strong reliance on PZQ, there is growing concern about the development of resistance, which can be induced in schistosomes in experimentally infected mice [7]. In addition, there is some evidence of PZQ resistance in the field, with low cure-rates being observed following some schistosomiasis outbreaks [8]. Therefore, there has been an ongoing need to develop alternative drugs to combat schistosomiasis.

In the past 5 years complete genomes and transcriptomes of *S. mansoni, S. japonicum*, and *S. haematobium* have become publicly available [9–11], providing a wealth of information on the molecular biology of these worms and on potential drug targets. Among the newly identified genes are those involved in apoptosis (Box 2). Because potent small-molecule compounds, particularly in the context of cancer therapy, can successfully target apoptosis pathways in human cells [12–15], this raises the possibility that schistosomes might be similarly targeted. In this review we describe what is currently known about the molecular components of schistosome apoptosis pathways, provide recent insights into the role of apoptosis in schistosome physiology and responses to drug treatments, and indicate the potential of targeting apoptosis pathways in the treatment of schistosomiasis.

Apoptosis in schistosomes: an intrinsic cell-death pathway

Apoptosis is one of the most important forms of programmed cell death in humans. There are two major routes

Corresponding author: Fairlie, W.D. (fairlie@wehi.edu.au).
Keywords: schistosome; schistosomiasis; apoptosis; Bcl-2; cell death; drug targets.

1471-4922/$ – see front matter.

http://dx.doi.org/10.1016/j.pt.2013.12.005

Glossary

Ankyrin-repeat domain: a common protein–protein interaction platform assembled with multiple repeats of the ankyrin repeat, which is a 33 amino acid sequence that adopts a helix-loop-helix structure.
Apoptosis: a form of cell death executed in a regulated process by a genetically controlled program (Box 1).
Apoptosome: a ring-like platform formed through the assembly of multiple monomers of APAF-1 in humans, CED-4 in *Caenorhabditis elegans*, or ARK in *Drosophila*. This platform enables activation of caspases. Differences in platform symmetry and the requirement for cytochrome *c* in apoptosome assembly occur in mammals, flies, and worms.
Apoptotic protease-activating factor 1 (APAF-1): a large cytoplasmic adaptor protein that assembles into the apoptosome to enable caspase activation.
Baculovirus IAP repeat (BIR): a zinc-binding domain of approximately 70 amino acids that was first identified through sequence homology to inhibitor of apoptosis proteins (IAP).
Bax/Bak: proapoptotic proteins that can permeabilise the outer mitochondrial membrane following their activation and oligomerization. The presence of both Bax and Bak is essential for apoptosis signalling to occur in mammals.
B-cell lymphoma-2 (Bcl-2): the first-identified pro-survival protein of the intrinsic cell-death pathway.
Bcl-2 homology (BH) domains: regions of sequence homology that define all Bcl-2 proteins. There are four BH domains, known as BH1, BH2, BH3, and BH4, which are each characterized by unique sequence motifs.
BH3-mimetic: a drug that mimics the activity of a BH3-only protein by antagonizing pro-survival proteins to trigger apoptosis. Currently BH3-mimetics are in clinical trials for the treatment of cancer.
BH3-only protein: a proapoptotic protein that is related to other Bcl-2 proteins only by the presence of the BH3 domain, and which initiates the apoptotic cascade.
Blebbing: the formation of irregular bulges in the plasma membrane of a cell, usually brought about when the cytoskeleton separates from the plasma membrane. Blebbing is a hallmark of cells undergoing apoptosis.
Caspase: a cysteine-aspartyl protease that cleaves vital cellular substrates leading to the death of the cell. They can be classified as 'initiators' or 'effectors' caspases.
Caspase-recruitment domain (CARD): an homotypic interaction motif present in proteins typically involved in inflammation and apoptosis that belongs to the death-domain superfamily.
Cercariae: free-swimming forms of the schistosome parasite released from the snail intermediate host.
Death domain (DD): defines a large superfamily of proteins primarily involved in apoptosis and inflammation. There are four main subfamilies based on the homotypic interaction domains present including the death domain (DD), death-effector domain (DED), caspase recruitment domain (CARD), and the pyrin domain (PYD). These domains mediate interactions that allow formation of large signalling complexes.
Death-effector domain (DED): a homotypic interaction motif that belongs to the DD superfamily and is present in proteins found to regulate a variety of cell signalling pathways including apoptosis.
Death-inducing signalling complex (DISC): a ternary assembly comprising the FAS receptor, FADD, and caspase-8 or caspase-10, which is formed upon FasL binding. The DISC activates downstream caspases.
Dioecious: where a species has distinct male and female organisms.
Encopula: pairing of an adult female schistosome with an adult male within the gynaecophoric canal of the male.
Extrinsic apoptosis: apoptosis initiated by binding of extracellular ligands (e.g., TNFα, FasL) to cell-surface death receptors.
FAS receptor: a death receptor, belonging to the tumour necrosis factor receptor superfamily, which contains a cytoplasmic death domain. Binding of its ligand (FasL) induces the formation of the death-inducing signalling complex (DISC) and triggers the extrinsic apoptosis pathway.
Gene related to anergy in lymphocytes (GRAIL): a type I transmembrane E3 ligase identified as an early gene that promotes T cell anergy.
Granuloma: a lesion composed of collagen fibres and cells that include $CD4^+$ T cells, eosinophils, and macrophages, that is formed as a result of a $CD4^+$ T cell response to antigens presented by tissue-trapped eggs during chronic schistosome infection.
Homotypic interaction motif: a defined amino acid sequence in a protein that mediates preferential binding to the same motif present in a second protein molecule.
Inhibitor of apoptosis protein (IAP): a protein that can bind to caspases via its BIR domains and inhibit the activity of the caspases. The IAPs play an additional role in TNFR1 signalling.
Intrinsic apoptosis: apoptosis regulated and mediated by the Bcl-2 family of proteins in response to cellular stresses. It is otherwise known as the mitochondrial or stress-induced pathway to apoptosis.
Mitochondrial outer-membrane permeabilization (MOMP): activation and oligomerization of proapoptotic Bax/Bak leads to MOMP, which enables soluble proteins, such as cytochrome *c*, to diffuse from the intermembrane space of the mitochondria into the cytosol.
Percutaneous infection: occurs when the free-living water-borne stage of the schistosome life-cycle, known as cercariae, penetrate human skin and transform into schistosomulae.
Praziquantel (PZQ): an orally administered antihelminthic which is the most widely used drug for treatment of schistosomiasis. The mechanism-of-action of PZQ is not well-defined but likely involves the induction of calcium uptake and apoptosis in schistosomes.
Really interesting new gene (RING) finger domain: a type of zinc finger defined by a consensus sequence of approximately 40–60 amino acids that includes cysteines and histidines as the zinc-binding residues.
Schistosomula: an immature form of the schistosome parasite that appears once the cercariae penetrate through the skin and lose their 'tails'.
Second mitochondria-derived activator of caspases (SMAC)-mimetic: a drug that mimics the activity of natural antagonists of the IAP proteins, resulting in apoptosis and inactivation of the nuclear factor-κB (NF-κB) survival signalling pathway.
Th1/Th2 cell response: CD4+ T helper cell responses based on cytokine secretion profiles. Th1 cells produce interferon-γ, IL-2, and TNFα, whereas Th2 cells produce IL-4, IL-5, IL-10, and IL-13. The different cytokines affect different classes of blood cells, resulting in different immune system responses.
Tumour necrosis family receptor 1 (TNFR1): a member of the TNFR superfamily that contains a cytoplasmic death domain. It serves as the main receptor for TNF-α and plays roles in apoptosis and NF-κB activation.
Vitellarium: a dedicated organ in schistosomes that is required for the production of yolk cells that surround a fertilized egg.
WD40 domain: a protein interaction domain made up of WD40 repeats [~40 amino acid motifs often terminating in a Trp–Asp (W–D) dipeptide] that together form a β-propeller architecture, and acts as an interacting platform for binding partners.

to apoptosis signalling, namely the intrinsic and extrinsic pathways [16,17]. Recently, all of the major components of an intrinsic cell-death pathway have been characterized in schistosomes [18,19].

Bcl-2 family proteins in schistosomes

The intrinsic apoptosis pathway is activated by developmental cues, cytotoxic insults, or stresses (e.g., DNA damage, growth factor deprivation). Proteins of the Bcl-2 family are the key regulators of this pathway (Figure 1) [17]. They are defined by the presence of at least one of four Bcl-2 homology (BH) domains (BH1 to BH4) and comprise three subgroups of proteins that promote either cell survival or death. In mammals, there are five members of the pro-survival subgroup, including Bcl-2, Bcl-x_L, Bcl-w, Mcl-1, and Bfl-1, which prevent activation of the essential mediators of cell death, Bax and Bak. Both pro-survival proteins and Bax/Bak contain at least three BH domains. The pro-survival proteins inhibit Bax/Bak activation, either by binding to them directly [20–22] and/or by sequestering another subgroup of the family, the BH3-only proteins [23,24]. The BH3-only proteins are upregulated or activated when a cell receives a death stimulus, and initiate the apoptotic cascade. Once activated, Bax/Bak oligomerize, forming pore-like structures in the outer mitochondrial membrane, leading to its permeabilization. The puncturing of the mitochondria releases apoptogenic factors, such as cytochrome *c*, which interact with the adaptor protein, apoptotic protease-activating factor 1 (APAF-1). APAF-1 forms an oligomeric platform, termed the apoptosome, which enables the activation of caspases, the proteolytic enzymes responsible for dismantling the cell [25].

The recent identification and characterization of the Bcl-2 family and related proteins in schistosomes [18,19,26] provided the first molecular evidence of an intrinsic apoptosis pathway in parasitic flatworms. Bioinformatic analyses of genome databases representing schistosomes