

REVIEW

Parthanatos: mitochondrial-linked mechanisms and therapeutic opportunities

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Cells die by a variety of mechanisms. Terminally differentiated cells such as neurones die in a variety of disorders, in part, via parthanatos, a process dependent on the activity of poly (ADP-ribose)-polymerase (PARP). Parthanatos does not require the mediation of caspases for its execution, but is clearly mechanistically dependent on the nuclear translocation of the mitochondrial-associated apoptosis-inducing factor (AIF). The nuclear translocation of this otherwise beneficial mitochondrial protein, occasioned by poly (ADP-ribose) (PAR) produced through PARP overactivation, causes large-scale DNA fragmentation and chromatin condensation, leading to cell death. This review describes the multistep course of parthanatos and its dependence on PAR signalling and nuclear AIF translocation. The review also discusses potential targets in the parthanatos cascade as promising avenues for the development of novel, disease-modifying, therapeutic agents.

LINKED ARTICLES

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Abbreviations

AIF, apoptosis-inducing factor; ARH3, ADP-ribose-(arginine) protein hydrolase; CypA, cyclophilin A; endoG, endonuclease G; Hq, harlequin; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; mPT, mitochondrial permeability transition; NADH, reduced nicotinamide adenine dinucleotide; PAAN, parthanatos AIF-associated nuclease; PAR, poly (ADP-ribose); PARG, poly (ADP-ribose) glycohydrolase; ROS, reactive oxygen species

Cell death: introduction and current nomenclature

The phenomenon of cell death is a subject of intense investigation. Current research is focused on unravelling the sequence of induction of the molecular events and characteristic signatures that define a particular death type, as well as the interactions and molecular bridges linking the different death types. As a consequence, cell death nomenclature con-

tinues to grow, as more evidence comes to light regarding multiple and distinct ways by which a cell can die. To date, morphological, enzymological, functional (biochemical) and immunological features are criteria commonly used to classify cell death (Kroemer *et al.*, 2009; Galluzzi *et al.*, 2012). Based on morphological appearance, apoptosis and necrosis were described as the first two distinct routes of cell death. Apoptosis, a term now more than four decades old (Kerr *et al.*, 1972), is an active form of cell death. It is highly regulated or programmed, which, consistent with an exceptionally

choreographed nature, is highly conserved among organisms (Chew *et al.*, 2009) and required for proper development (Lorenzo and Susin, 2007). Necrosis usually is a passive process, once thought to be mostly unregulated or random, but now considered to involve programmed components in some cases (e.g. necroptosis) (Lorenzo and Susin, 2007).

There are now many other modalities of cell death, including anoikis (Frisch and Ruoslahti, 1997), autophagic cell death, caspase-dependent intrinsic apoptosis, caspase-independent intrinsic apoptosis, regulated necrosis (some investigators prefer to use this term to describe or include caspase-independent intrinsic apoptosis, given their observation that apoptosis necessarily features early caspase activation), cornification, entosis (Mormone *et al.*, 2006), extrinsic apoptosis by death receptors, extrinsic apoptosis by dependence receptors, mitotic catastrophe, netosis, pyroptosis and parthanatos (see Galluzzi *et al.* 2012). Other forms of cell death may exist as well, including ferroptosis, an iron-dependent form of non-apoptotic cell death (Dixon *et al.*, 2012). Some of these new cell-death modalities were defined initially by morphological criteria, but biochemical/functional definitions of cell-death modalities are replacing the morphological definitions to reflect accuracy and consistency of description. It should be emphasized that there are many instances when cell death in a particular setting displays features of more than a single form of cell death. This makes it rather challenging to employ morphological criteria to describe such mixed cell-death scenarios. Thus, descriptions based on biochemical or functional characteristics are ultimately more relevant. In 2009, the Nomenclature Committee on Cell Death issued recommendations in relation to the use of these concepts and definitions (Kroemer *et al.*, 2009), which were expanded in their more recent (2012) round of recommendations, in order to produce a systematic classification of cell death based on measurable biochemical features (Galluzzi *et al.*, 2012).

In this review, we discuss the entire course and sequence of events of parthanatos and the key role of apoptosis-inducing factor (AIF), a mitochondrial protein whose nuclear translocation from the mitochondria sets in motion the commitment-to-death fate of cells or tissues in which the

event has taken place. Parthanatos is a unique and highly choreographed form of cell death, which occurs through the overactivation of the nuclear enzyme, PARP-1, also known as poly (ADP-ribose) synthetase 1 or poly (ADP-ribose) transferase 1.

PARP-1 in cell survival and cell death

PARP-1 (EC 2.4.2.30) is the best known, most abundant and seemingly most important of an expanding family of about 17 proteins classified on the basis of protein domain homology and enzymatic function (some of them have not been fully characterized). PARP-1 helps in the regulation of cellular homeostasis and the preservation of genomic stability under physiological conditions (Smith, 2001; Hong *et al.*, 2004; Krietsch *et al.*, 2012). It is a 116 kDa protein with three major domains: an N-terminal domain (42 kDa) that has two zinc finger motifs and a nuclear localization sequence used for DNA-binding; a central automodification domain (16 kDa) and a C-terminal catalytic domain (55 kDa) harbouring the NAD-binding site and the poly (ADP-ribose) (PAR)-synthesizing domain (Kameshita *et al.*, 1984; Hong *et al.*, 2004; Sousa *et al.*, 2012; Virag and Szabo, 2002) (Figure 1). In terms of amino acid sequence, there is nearly 92% homology between the human and mouse enzyme, evidence that PARP-1 is highly conserved in eukaryotes (Virag and Szabo, 2002). Interestingly, homology is highest for the catalytic domain that contains the PARP signature sequence (Virag and Szabo, 2002). Among a number of other possible roles, PARP-1 is known to regulate gene expression and amplification, cell differentiation, cell division, malignant transformation, DNA replication, mitochondrial function and cell death (D'Amours *et al.*, 1999; Chiarugi, 2002; Kraus and Lis, 2003; Hong *et al.*, 2004; Gagne *et al.*, 2008).

Consistent with its function in maintaining nuclear homeostasis, PARP-1 constitutes a DNA base-excision repair system by sensing DNA strand nicks and breaks and facilitating their repair through the synthesis of PAR polymer. When there is mild DNA damage, the activity of PARP-1 increases up to 500-fold and the enzyme makes use of oxidized NAD

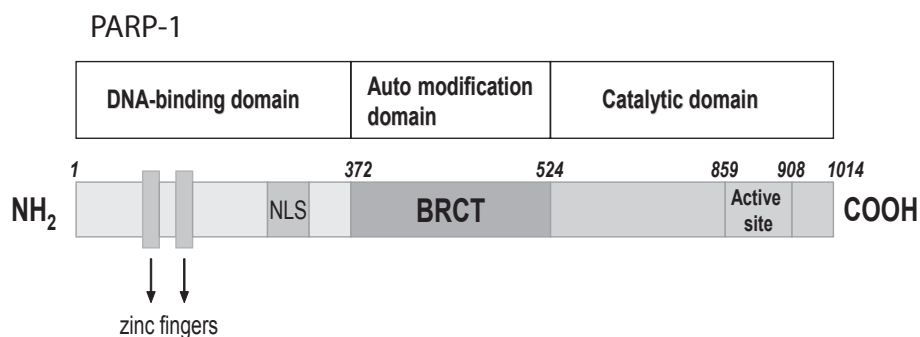


Figure 1

Primary structure of PARP-1. Primary structure of PARP-1 showing its three domains: An N-terminal DNA-binding domain containing two zinc-finger motifs and a nuclear localization sequence (NLS), a central automodification domain [with a breast cancer-associated gene 1 (BRCA1) C-terminal (BRCT) motif containing phosphorylation sites for regulating PARP-1 activity], and a C-terminal catalytic domain containing the nicotinamide adenine dinucleotide (NAD)-binding site and the poly (ADP-ribose) polymer (PAR)-synthesizing domain.

(NAD⁺), produced through the expenditure of ATP, to synthesize PAR polymer. More than 95% of the synthesized PAR polymer comes from PARP-1, in contrast to the very minimal contribution from other isoforms (Dawson and Dawson, 2004). About 50–200 PAR polymer residues are then attached to PARP itself (autoribosylation) and to other nuclear (acceptor) proteins (heteroribosylation) such as histones, topoisomerases I and II, DNA polymerases, DNA ligase-2, high-mobility group proteins, transcription factors, as well as many other proteins (Hong *et al.*, 2004; Shall and de Murcia, 2000; Smulson *et al.*, 2000). This biochemical pathway is a unique one and the synthesis and degradation of PAR has been found to occur in most mitotic and post-mitotic cells in mammalian organisms (Kanai *et al.*, 2000; Shall and de Murcia, 2000). However, when DNA damage is profound, PARP-1 becomes excessively activated and produces long-chained, branched polymers of PAR, leading to externalization of phosphatidylserine, dissipation of the mitochondrial membrane potential, translocation of AIF from the mitochondria to the nucleus, large-scale DNA fragmentation (≈ 50 kb) and chromatin condensation, followed by cell death (Andrabi *et al.*, 2008; David *et al.*, 2009).

In the context of the CNS, which is highlighted in this review to illustrate PARP-1-mediated cell death mechanisms that are shared in most cases by non-neuronal systems, stimuli that induce pathological activation of PARP-1 in *in vitro* and *in vivo* studies include oxidative stress by reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂) or hydroxyl radical, nitrosative stress from NO or peroxynitrite (ONOO⁻), inflammation, ischaemia (or ischaemic reperfusion), hypoxia, hypoglycaemia and DNA-alkylating agents, such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG). For example, ischaemic injury induces the release and accumulation of the excitatory neurotransmitter, glutamate, which causes excessive activation of its ionotropic receptors (NMDA, AMPA and kainate receptors; receptor nomenclature follows Alexander *et al.*, 2013). These can all cause calcium influx, but NMDA receptor activation plays a primary role, leading to the activation of neuronal NO synthase (Dawson *et al.*, 1991; 1993; 1996). Activation of endothelial NOS and inducible NOS is also important to NO production (and PARP-1 activation) in non-neuronal tissues (Virag and Szabo, 2002). ROS such as superoxide anion (O₂⁻) or H₂O₂ are also produced in tissue injury. NO is able to induce poly (ADP-ribosylation) (Zhang *et al.*, 1994) but, more importantly, O₂⁻ has the capacity to react with NO to form ONOO⁻, a potent inducer of DNA damage and consequently of PARP-1 activation (Zhang *et al.*, 1994; Xia *et al.*, 1996; Eliasson *et al.*, 1997; Gonzalez-Zulueta *et al.*, 1998; Wang *et al.*, 2004; Pacher and Szabo, 2008). Excessive stimulation of glutamate receptors also results from hypoxia, hypoglycaemia and inflammation, leading to DNA damage (Stone and Addae, 2002), while oxidative stress and DNA-alkylating agents may damage DNA through other means, directly or indirectly (Fatokun *et al.*, 2008) (Figure 2).

Parthanatos (PARP-1-mediated cell death)

Following a relatively recent coinage, the form of cell death that occurs as a result of the overactivation of PARP-1

is referred to as 'parthanatos,' a portmanteau term (parthanatos) derived from 'par' (for PAR polymer, synthesized following PARP-1 activation), and 'Thanatos,' the personification of death in Greek mythology (Andrabi *et al.*, 2008; Harraz *et al.*, 2008; David *et al.*, 2009; Wang *et al.*, 2009a). The name 'parthanatos' is now officially recognized and has thus entered the cell death pantheon (Galluzzi *et al.*, 2012). As a distinct death pathway, the time course of parthanatos is associated with the biochemical events of rapid activation of PARP-1, synthesis and accumulation of PAR polymer, mitochondrial depolarization, nuclear AIF translocation (which occurs early) and, perhaps at the late stage, caspase activation, although caspase activation is not obligatory (Yu *et al.*, 2002; 2006; Andrabi *et al.*, 2006). It should be emphasized that, while events such as rapid activation of PARP-1, PAR synthesis and accumulation and nuclear AIF translocation are unique to parthanatos, which therefore characterize this process biochemically, other events that accompany it, such as mitochondrial depolarization, are not exclusive occurrences but shared with other forms of cell death. Some further account of the nature and sequence of this array of events has been provided by recent reviews (Andrabi *et al.*, 2008; Wang *et al.*, 2009a). Of particular interest is that parthanatos does not obligatorily require caspases for its execution, as it cannot be rescued by pan-caspase inhibitors, for example boc-aspartyl-fmk or Z-VAD-fmk (Yu *et al.*, 2002). This feature, therefore, distinguishes parthanatos from caspase-dependent apoptosis that cannot proceed without the involvement of relevant caspases. Furthermore, unlike apoptosis, parthanatos does not induce the formation of apoptotic bodies, neither does it engender small-scale DNA fragmentation (Wang *et al.*, 2009a) – it causes large-scale DNA fragmentation. Like necrosis, parthanatic death involves loss of cell membrane integrity, but, unlike it, is not accompanied by cell swelling (Yu *et al.*, 2002; Wang *et al.*, 2004). However, it should be emphasized that some investigators consider parthanatos and necroptosis as two subsets or examples of regulated or programmed necrosis (see Nagley *et al.* 2010), but even in this context, the co-involvement of PARP-1, PAR and AIF, which is lacking in any (other) forms of programmed or regulated necrosis, still distinguishes parthanatos. Major similarities and differences in biochemical, structural and other changes that occur in apoptosis, necrosis, autophagy and parthanatos are shown in Table 1 (adapted from Bredesen *et al.*, 2006; Wang *et al.*, 2009a; Galluzzi *et al.*, 2012).

Extensive details of molecular mechanisms in parthanatos and the order in which they are presented in the cascade are only beginning to be unglued. It is established that factors such as the magnitude of the death-inducing stimulus, the length of exposure to such stimulus, the type and nature of cell used, and the purity of cultures are among those that can largely determine the death cascade that is activated in any experimental paradigm (Meli *et al.*, 2004; Wang *et al.*, 2009b). In its pure form, parthanatos has been described in several experimental cell culture and animal models that feature excessive activation of the PARP-1 enzyme, leading to severe, rather than mild-to-moderate, DNA damage. In most cases, the alkylating agent MNNG at concentrations between 50 and 500 μ M applied for usually 5 to 25 min has been used as the gold standard in cell-death research to induce parthanatos in cell lines, including

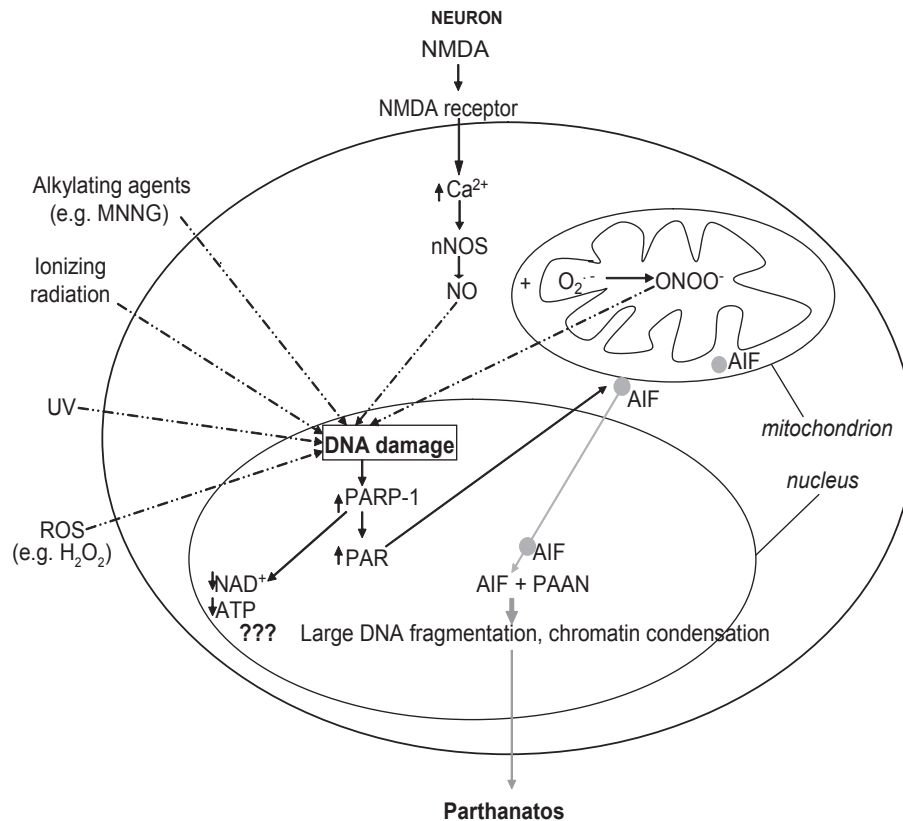


Figure 2

Cascade of events leading to parthanatos. Diagrammatic representation, in a typical (neuronal or non-neuronal) cell, of the multiple steps in parthanatos. In neurones, activation of the NMDA receptor leads to increased calcium influx, resulting in the activation of calcium-dependent neuronal nitric oxide synthase (nNOS) that produces NO. NO may induce DNA damage directly, but more commonly reacts with superoxide (O_2^-) in the mitochondria to generate peroxynitrite ($ONOO^-$), which is a very potent inducer of DNA damage. Production of NO through activation of endothelial NOS (eNOS) or inducible NOS (iNOS) may be more important in non-neuronal cells, leading to $ONOO^-$ generation (not shown). Some stimuli can induce DNA damage directly, including reactive oxygen species (ROS), for example hydrogen peroxide (H_2O_2), alkylating agents [e.g. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)], UV radiation and ionizing radiation. Stimuli inducing DNA damage are shown by broken arrows. DNA damage causes PARP-1 overactivation that leads to poly (ADP-ribose) (PAR) polymer synthesis and accumulation. PARP-1 overactivation depletes cellular pool of nicotinamide adenine dinucleotide (NAD^+) and ATP, but this does not seem to be the primary cause of cell death (indicated by ???). PAR polymer signals to the mitochondria and directly binds to the PAR polymer-binding site on apoptosis-inducing factor (AIF), inducing its mitochondrial release and translocation to the nucleus. Once in the nucleus, it causes large-scale (≈ 50 kb) DNA fragmentation and chromatin condensation through as yet unidentified parthanatos AIF-associated nuclease (PAAN). This is believed to be the cause of cell death. Events after AIF release from the mitochondria are depicted in grey, solid arrows.

human cervical carcinoma HeLa cells (mostly used), CHO cells and rarely MDCK cells and the lymphoblastoid cell line TK-6 (Kim *et al.*, 2005). This agent (MNNG) has also been used to study parthanatos in immortalized mouse embryonic fibroblasts and human fibroblasts (Yu *et al.*, 2002; Yeh *et al.*, 2005; David *et al.*, 2006; Keil *et al.*, 2006; Ethier *et al.*, 2007; Lee *et al.*, 2007b). Treatment of human pulmonary epithelial and vascular endothelial cells with MNNG or human umbilical vein endothelial cells with peroxide has also been found to cause activation of parthanatos (Geraets *et al.*, 2007; Mathews and Berk, 2008). In primary cortical neuronal cultures prepared from fetal mice, NMDA (500 μM) applied briefly (5 min) robustly activates parthanatos (Yu *et al.*, 2002; Wang *et al.*, 2009b; Fatokun *et al.*, 2013). Exposure to MNNG (10–300 μM , 30 min) also induced parthanatos in primary

astrocyte cultures (Suzuki *et al.*, 2010; Tang *et al.*, 2010). It should be noted that a number of other stimuli or toxic conditions, such as H_2O_2 , NO, $ONOO^-$ generation or oxygen-glucose deprivation, although relatively less commonly used, also induce parthanatos in a variety of cells (Moroni *et al.*, 2001; Yu *et al.*, 2002; Mathews and Berk, 2008; Son *et al.*, 2009). The *in vitro* experimental conditions as highlighted here are known to favour parthanatos over other forms of cell death. Depending on the type, length and strength of the toxic stimulus, other forms of cell death due to DNA damage can be induced in addition to parthanatos. Thus, cell death that involves primarily parthanatos should be completely blocked by inhibitors of PARP or knockout of PARP. This is important in the design of experiments to investigate this form of cell death.

Table 1

Similarities and differences between apoptosis, necrosis, autophagy and parthanatos

	Apoptosis	Necrosis	Autophagy	Parthanatos
Variations/Subsets in the literature	Caspase-dependent intrinsic ^a Caspase-independent intrinsic ^b Extrinsic apoptosis by death receptors ^c Extrinsic apoptosis by dependence receptors ^d	Random or unregulated Programmed or regulated, e.g. necroptosis (some think parthanatos could also be considered a case of regulated necrosis)	Macroautophagy Microautophagy Chaperone-mediated autophagy Mitophagy (but here we consider autophagy as macroautophagy)	
(Biochemical) Signatures				
Mitochondrial	Caspase activation (except in ^b) Mitochondrial depolarization MOMP ^{a,c} Irreversible $\Delta\psi_m$ dissipation ^a CYT c release Release of IMS proteins ^b Respiratory chain inhibition ^b BID cleavage ^c PP2A activation ^d DAPK1 activation ^d	Loss of ultrastructure Swelling	Degradation	Depolarization Irreversible $\Delta\psi_m$ dissipation ATP and NADH depletion AIF release CYT c release Caspase activation (late stage, non-obligatory)
Cytoplasmic	Shrinkage	Swelling (including of organelles) Vacuolation Organelle disintegration	Massive vacuolization Lysosomal degradation MAP1LC3 lipidation	PAR polymer accumulation PAR-AIF interactions (binding) Condensation AIF translocation to the nucleus
Nuclear	PARP cleavage Chromatin condensation DNA fragmentation (small-scale, DNA ladder)	Chromatin digestion DNA hydrolysis (smear)	SQSTM1 degradation	Rapid PARP-1 activation (not cleavage) PARP-1-mediated PAR synthesis Chromatin condensation PAAN activation (putative) DNA fragmentation (large-scale, ≈ 50 kb)
Structural (plasma membrane) changes	Membrane integrity preserved Formation of apoptotic bodies Membrane blebbing Phosphatidylserine externalization	Loss of integrity Blebbing Cell lysis	Double membrane-bound autophagosomes formed	Loss of integrity Phosphatidylserine externalization
Examples of trigger factors and/or conditions	Death receptor signalling ^c Dependence receptor signalling ^d DNA damage Trophic factor withdrawal Viral infections	Excitotoxicity Ischaemia Stroke Reactive oxygen/nitrogen species	Amino acid starvation Serum starvation Protein aggregates	Excitotoxicity Ischaemia DNA damage Stroke Reactive oxygen/nitrogen species
Energy (ATP) requirement	+	-	+	-
(Obligatory) Caspase-dependence	+ ^{a,c,d} - ^b	-	-	-
Inflammatory component	-	+	-	-
Major mediator(s)	Caspases (except in ^b)	Calpains, CYPD, RIP-1, RIP-3 (and PARP-1 and AIF, if parthanatos is considered regulated necrosis), etc.	ATG5, ATG6 (Beclin-1), ATG7, ATG12, VPS34, AMBRA-1	PARP-1 PAR AIF
Pharmacological inhibition	Caspase inhibitors, e.g. Z-VAD-fmk (except in ^b)	RIP-1 inhibitors, e.g. necrostatin-1, calpain inhibitors, etc.	VPS34 inhibitors, e.g. 3-methyladenine and wortmannin	PARP-1 inhibitors, e.g. DPQ
Genetic inhibition (knockout/mutation, RNAi targeting) or inhibition by protein overexpression	BCL2 overexpression ^{a, b} Inhibition of caspases (3, 8 and 9) ^{c,d} Inhibition of PP2A ^d Crma expression ^c	Inhibition of RIP-1 or RIP-3	Inhibition of AMBRA1, ATG5, ATG7, ATG12 or BECN1	PARP-1 knockout, AIF down-regulation (e.g. in Harlequin mouse)

Major similarities and differences in the biochemical, structural and other changes that occur in apoptosis, necrosis, autophagy and parthanatos as some types of cell death, as adapted from (Bredesen *et al.*, 2006; Wang *et al.*, 2009a; Galluzzi *et al.*, 2012). For apoptosis, superscripts have been used where necessary to link each of its different subsets or variations to features that distinguish it. It is worthy to note that, while parthanatos is generally considered to be separate and distinct from necrosis, some investigators consider it to be a specific case of regulated necrosis, just as is necroptosis. $\Delta\psi_m$ is mitochondrial transmembrane potential. AIF, apoptosis-inducing factor; AMBRA1, activating molecule in Beclin-1-regulated autophagy protein 1; ATG, autophagy; BCL2, B-cell lymphoma 2; BECN1, Beclin-1; CrmA, cytokine response modifier A; CYPD, cyclophilin D; CYT, cytochrome; DAPK1, death-associated protein kinase 1; DPQ, 3,4-Dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)-isoquinolinone; IMS, intermembrane space; MAP1LC3, microtubule-associated protein 1 light chain 3; MOMP, mitochondrial outer membrane permeabilization; PAAN, parthanatos AIF-associated nuclease; PAR, poly (ADP-ribose); PP2A, protein phosphatase 2A; RIP, receptor-interacting protein; SQSTM1, sequestosome 1; VPS, vacuolar protein sorting; Z-VAD-fmk, N-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone.

Degradation of PAR polymer by PARG

Poly (ADP-ribose) glycohydrolase (PARG) is the enzyme that regulates PAR levels by catalyzing its degradation after it is synthesized by PARP (Kameshita *et al.*, 1984; Whitacre *et al.*, 1995). PARG causes PAR hydrolysis to free ADP-ribose units through its endoglycosidic or exoglycosidic activity (Davidovic *et al.*, 2001). Although encoded by a single gene, now characterized in humans and in other species (Ame *et al.*, 1999; Winstall *et al.*, 1999; Meyer-Ficca *et al.*, 2004), alternatively spliced PARG isoforms have been reported (Bonicalzi *et al.*, 2003; Haince *et al.*, 2006; Meyer *et al.*, 2007). Full-length PARG (110 kDa) localizes to the nucleus, but the splice variants have diverse localization patterns (Bonicalzi *et al.*, 2003; Meyer-Ficca *et al.*, 2004; Haince *et al.*, 2006; Meyer *et al.*, 2007; Burns *et al.*, 2009). However, the relevance of each of the variants and its subcellular localization in relation to parthanatos has not been clarified. Evidence demonstrating the importance of PARG in parthanatos comes from studies involving overexpression, genetic deletion and pharmacological approaches in which PARG was found to protect against PAR-mediated cell death (Andrabi *et al.*, 2006), H₂O₂-induced cell death (Blenn *et al.*, 2006), excitotoxicity and stroke (Andrabi *et al.*, 2006; Cozzi *et al.*, 2006), while its deletion (PARG knockouts) increased toxicity, most probably through the accumulation of PAR (Andrabi *et al.*, 2006). In mice, the whole-body knockout of PARG is embryonically lethal at around day E3.5 (Koh *et al.*, 2004a), which may be due to excessive accumulation of the synthesized PAR polymer. Furthermore, trophoblasts from early PARG-knockout embryos do not survive without the PARP inhibitor, benzamide (Koh *et al.*, 2004a). The cells were found to be extremely sensitive to MNNG and menadione, agents that activate PARP-1 excessively, leading to PAR accumulation (Koh *et al.*, 2004a). These data show that regulation of PAR levels is critical to cell survival in parthanatos (Zhou *et al.*, 2011).

A different enzyme, ADP-ribose-(arginine) protein hydrolase (ARH3), also has some PARG-like activity, but it does not appear that this activity plays a significant role in cell death, as ARH3 does not rescue *Drosophila* or mouse genetic knockouts of PARG from cell death or PAR accumulation (Hanai *et al.*, 2004; Koh *et al.*, 2004a).

Molecular mechanisms in parthanatos: PAR polymer as a death mediator

Quite understandably, a leading area of investigation that has generated much attention in parthanatos is the identification of the underlying molecular mechanisms, as the nature of cell death is unique and separate from those of other forms of cell death such as apoptosis and necrosis. Although there is considerable success in this area, our knowledge of molecular events in parthanatos is still not complete, and efforts are ongoing, not only to completely identify all the major players, but also to clarify the spatial and temporal relationships between such mediators.

So, how do cells die in parthanatos? It was initially assumed that the massive depletion of cellular NAD⁺ as a result of excessive activation of PARP-1 caused cells to

commit suicide (Berger *et al.*, 1983; Berger, 1985; Berger *et al.*, 1985; Berger and Berger, 1986; Ha and Snyder, 1999). NAD⁺ is an important cofactor in glycolysis and the tricarboxylic acid cycle. The same NAD⁺ is required for the synthesis of PAR and, in turn, ATP is required for the synthesis of NAD⁺. In fact, each molecule of NAD⁺ requires four molecules of ATP for its synthesis. The suicide hypothesis, therefore, argues that following PARP-1 overactivation, there is excessive depletion of cytosolic and nuclear pools of NAD⁺, over time. Despite this observation, there is no convincing and direct evidence to date in favour of a role for energy depletion as a primary cause of parthanatos. Instead, although infarct volumes after focal ischaemic injury in PARP-1 knockout mice are smaller than in wild-type animals, cellular energy stores are not different between the two groups (Goto *et al.*, 2002), suggesting that energy depletion is not the underlying cause of cell death in parthanatos. Moreover, in cells lacking PARG, activation of PARP-1 leads to cell death through parthanatos, in the absence of NAD⁺ depletion (Zhou *et al.*, 2011).

The PAR polymer, generated when PARP-1 is overactivated, is now recognized as a key signalling molecule in the parthanatos cascade (Andrabi *et al.*, 2006). When cells are exposed to a toxic stimulus that induces significant DNA damage, PARP-1 becomes overactivated and produces toxic levels of PAR, which translocates to the cytosol, from where it signals to AIF to effect its nuclear translocation (Andrabi *et al.*, 2006). As AIF is a mitochondrial protein, its translocation from the mitochondria to the nucleus is a critical step that inextricably links parthanatos with the mitochondria.

PAR has been shown to interact with several proteins and its binding to these proteins might regulate (inhibit or activate) their physiological functions (Gagne *et al.*, 2008; Krietsch *et al.*, 2012). Evidence that PAR mediates parthanatos comes from observation of protection against cell death induced by exposure of mouse primary neurones to NMDA when cytosolic PAR was neutralized with PAR-specific antibodies (Andrabi *et al.*, 2006). It was then established that PAR was directly toxic to neurones, as their exposure to the exogenous polymer through BioPorter-mediated delivery induced death (Andrabi *et al.*, 2006). The BioPorter reagent (Gene Therapy Systems, San Diego, CA, USA) enables the transport of recombinant proteins, peptides or antibodies into viable cells (Zelphati *et al.*, 2001). Toxicity increased with the dose and complexity of PAR: more death resulted from highly complex and long-chain polymers than from simple and shorter ones. This study suggests that PAR can translocate as a free polymer to induce cell death, although translocation of protein-conjugated PAR, which similarly induces cell death, cannot be ruled out and is currently the subject of active investigation. Interestingly, in a separate study, exposure to exogenous PAR was demonstrated to induce AIF translocation (Yu *et al.*, 2006).

AIF has been identified as a PAR polymer-binding protein (Gagne *et al.*, 2008) and a physical interaction between PAR and AIF is required in parthanatos for inducing the release of AIF from the mitochondria (Wang *et al.*, 2011). It is interesting to note that, despite AIF being a bigger protein than cytochrome *c*, its translocation precedes cytochrome *c* release (and caspase activation) in parthanatos (Yu *et al.*, 2002), arousing great interest in the study of its release, mitochondrial localization and translocation kinetics. Importantly,

there is a small pool (30%) of AIF on the outer mitochondrial membrane (cytosolic side) (Yu *et al.*, 2009). Using multiple biochemical and immunogold electron microscopic analyses of mouse brain mitochondria, this study showed that the localization accounted for the rapid release of a small pool of AIF, as 20% of the uncleaved AIF rapidly translocated to the nucleus and caused death following NMDA treatment (Yu *et al.*, 2009). This implies that the release of the outer mitochondrial membrane pool of AIF is required to induce cell death in parthanatos. However, more studies are needed to clarify the different mechanisms involved in the release of the two AIF pools and their spatiotemporal regulation and interrelationship. In addition, studies are required to understand how PAR signalling affects mitochondrial (i.e. metabolic) function, integrity and maintenance.

Mitochondrial AIF is a key mediator of parthanatos

One mediator of parthanatos that has been unequivocally recognized is AIF, a mitochondrially localized flavoprotein discovered more than a decade ago (Susin *et al.*, 1999), now reported to have five isoforms in humans (Delettre *et al.*, 2006a,b; Loeffler *et al.*, 2001; Lorenzo and Susin, 2007). Most death factors released from mitochondria participate in caspase-dependent cell death, whereas AIF mediates caspase-independent cell death, although some crosstalk between AIF and caspases has been proposed (Daugas *et al.*, 2000; Arnoult *et al.*, 2002). In retrospect, the name 'apoptosis-inducing factor' is somewhat a misnomer, because this protein is now known to participate in a form of cell death (parthanatos) that is distinct from apoptosis and it is possible that the involvement of AIF in caspase-dependent cell death (classical apoptosis) may be an epiphenomenon that occurs to supply caspase-activated cells with an alternative (non-caspase) pathway to death (Cregan *et al.*, 2002; Wang *et al.*, 2009a).

The AIF gene comprises 16 exons, is found on chromosome X (Susin *et al.*, 1999) and is synthesized in the cytoplasm as a 67 kDa precursor, but imported into the mitochondria, where it undergoes processing to the mature 62 kDa form (Otera *et al.*, 2005; Cao *et al.*, 2007). AIF localizes mainly to the intermembrane space (Susin *et al.*, 1999), although, as already mentioned, about 30% of it may loosely associate with the outer mitochondrial membrane, on the cytosolic side (Yu *et al.*, 2009). The crystal structures of mouse and human AIFs have been solved at 2.0 (Mate *et al.*, 2002) and 1.8 Å (Ye *et al.*, 2002) resolutions respectively. There is a strong, positive electrostatic potential at its surface that might bind to DNA (Ye *et al.*, 2002). There is a PAR-binding domain that is distinct from the DNA-binding domain (Wang *et al.*, 2011). AIF has three putative functional domains: an N-terminal FAD-binding domain and a central reduced NAD (NADH)-binding domain, responsible for its oxidoreductase activity, and a C-terminal domain that is mainly responsible for its cell death (parthanatos)-mediating ability (Susin *et al.*, 1999; Wang *et al.*, 2009a). The oxidoreductase activity of AIF is not required for its death-inducing property (Ye *et al.*, 2002). Although initial identification of this protein was as a cell-death mediator, subsequent attempts to understand its

physiological role have revealed that, in normal conditions (when AIF is localized to the mitochondria), it supports cell survival. A very useful model to study AIF-mediated cell death is the harlequin (Hq) mouse, which has about an 80% down-regulation of AIF expression (Klein *et al.*, 2002). This mouse shows evidence of oxidative stress and progressive degeneration of terminally differentiated cerebellar and retinal neurones (Klein *et al.*, 2002), suggesting that AIF supports cell survival. In addition, AIF may support cortical development (Cheung *et al.*, 2005) and may be associated with cytoprotective mechanisms in diabetes and obesity (Pospisilik *et al.*, 2007), while deletion of AIF in muscle brings about mitochondrial dysfunction, muscle atrophy and dilated cardiomyopathy (Joza *et al.*, 2005). Recently, when the PAR-binding site on AIF was mutated, the AIF mutant retained its NADH oxidase activity, bound FAD or DNA, and induced nuclear condensation. However, the AIF mutant was not released from the mitochondria and failed to translocate to the nucleus or mediate cell death following PARP-1 activation. This observation therefore seems to separate the role of AIF in cell metabolism (promoting survival) from its effect as a mitochondrial death effector (Wang *et al.*, 2011). However, further studies such as the generation and use of knock-in mice are required to fully understand the effect of the AIF mutants that are incapable of binding PAR on mitochondrial (i.e. metabolic) function, integrity and maintenance.

Relating to cell death, numerous studies using diverse experimental paradigms have demonstrated convincingly that AIF translocates to the nucleus following PARP-1 over-activation caused by cellular injury (see Yu *et al.*, 2003; Cregan *et al.*, 2004; Virag, 2005). Exposure of cells to insults that cause DNA damage, such as NMDA excitotoxicity, oxidative or nitrosative stress and DNA alkylating agents, leads to nuclear translocation of AIF, resulting in large-scale DNA fragmentation (≈ 50 kb) and chromatin condensation and, eventually, cell death (Yu *et al.*, 2002). Both NMDA and purified PAR polymer were less toxic to cortical neurones from Hq mice (Yu *et al.*, 2006), and these mice, when exposed to excitotoxicity and stroke, had decreased lesions compared to wild-type animals (Cheung *et al.*, 2005; Culmsee *et al.*, 2005; Andrabi *et al.*, 2006; Zhu *et al.*, 2007b; Yuan *et al.*, 2009; Wang *et al.*, 2011). A converse experiment of virally mediated, forced AIF expression raised NMDA-induced cell death in Hq neurones to the same level as was found in wild-type neurones (Yu *et al.*, 2006). Notably, AIF translocation from the mitochondria seems to be the commitment point for cell death in several disease states, including neurodegenerative disorders (Wang *et al.*, 2003; Dawson and Dawson, 2004), a realization that places a premium on targeting this event in the process of translating the study of parthanatos to therapy. We need to determine whether the initial release of AIF from the mitochondria and concomitant deficits in mitochondrial function, or the large-scale fragmentation of genomic DNA that ensues after AIF enters the nucleus, is the main cause of cell death. In the nucleus, AIF binds to DNA and this step is thought to be required for the precipitation of cell death (Ye *et al.*, 2002), although one drawback to this view comes from the realization that mouse AIF does not have recognizable structural motifs for DNA binding (Mate *et al.*, 2002), in contrast to human AIF that does (Ye *et al.*, 2002), thus suggesting the possibility of other mechanisms.

So far, it is not clear if AIF itself has any apparent or intrinsic endonuclease activity (Mate *et al.*, 2002; Ye *et al.*, 2002), raising the question of how does it induce DNA fragmentation? It has been suggested that AIF may recruit endogenous proteases or nucleases to precipitate fragmentation or, on the other hand, promote DNA vulnerability to these molecules through its interaction with DNA. Cyclophilin A (CypA) interacts with AIF, possibly forming a pro-apoptotic DNA-degradation complex and it may be a co-factor for AIF nuclear translocation and AIF-dependent chromatinolysis under conditions of cerebral hypoxia-ischaemia (Cande *et al.*, 2004; Zhu *et al.*, 2007a). How CypA and AIF form a pro-apoptotic DNA-degradation complex is not known, as there is no nuclease domain in CypA or AIF. Another suggested interactor with AIF is the mammalian endonuclease G (endoG). Its orthologue in *Caenorhabditis elegans* is CPS-6 and WAH-1, the AIF orthologue in *C. elegans*, associates and cooperates with CPS-6, causing DNA degradation (Wang *et al.*, 2002). However, endoG does not seem to play a role in DNA fragmentation following parthanatos in mammals (David *et al.*, 2006; Xu *et al.*, 2010), suggesting that there is a parthanatos AIF-associated nuclease (PAAN) which, so far, remains to be identified in vertebrates.

Involvement of PARP in diseases affecting multiple organ systems

There is ample evidence to implicate PARP, and by extension, parthanatos, in the pathogenesis of several human diseases (see Pacher and Szabo 2008), including those that do not directly affect the nervous system. Evidence of PARP-1 activation and its pharmacological inhibition and/or genetic knockout resulting in cytoprotection has been used to demonstrate its possible or definite involvement in the pathophysiology of many diseases in a wide range of organ systems, including neurological (Endres *et al.*, 1997; Joashi *et al.*, 1999; LaPlaca *et al.*, 2001) and neurodegenerative conditions (Mandir *et al.*, 1999; Love *et al.*, 1999a), diabetes (Yamamoto and Okamoto, 1980), colitis (Zingarelli *et al.*, 1999), arthritis (Miesel *et al.*, 1995), liver toxicity (Stubberfield and Cohen, 1988) and uveitis (Mabley *et al.*, 2001) (see Virag and Szabo, 2002; Dawson and Dawson, 2004). It was in the CNS that PARP-1-dependent cell death was first detected (Zhang *et al.*, 1994), and involvement of PARP-1, with or without the activation of parthanatos (cell death mediated by PARP-1 overactivation), has now been demonstrated, or at least suggested, in experimental models of stroke (Eliasson *et al.*, 1997), trauma (LaPlaca *et al.*, 2001), Alzheimer's disease (Love *et al.*, 1999a), Parkinson's disease (Mandir *et al.*, 1999; Outeiro *et al.*, 2007), Huntington's disease (Vis *et al.*, 2005), amyotrophic lateral sclerosis (Hivert *et al.*, 1998) and spinal cord injury (Maier *et al.*, 2007). Contributions of PARP to various dysfunctions in non-neuronal systems, such as diabetic endothelial dysfunction of the vasculature, interstitial pulmonary fibrosis and acute respiratory distress syndrome of the lung, uveitis of the eye, reperfusion injury of skeletal muscles, acetaminophen toxicity of the liver and sulfur mustard-induced vesication of the skin, are not the focus of this review but have been highlighted elsewhere (see

Virag and Szabo, 2002; Szabo, 2005; de la Lastra *et al.*, 2007). PARP has also been implicated in the ischaemic-reperfusion injury of the retina and cochlea, as well as in both haemorrhagic and endotoxin shock, and has been associated with multi-organ failure (see Virag and Szabo, 2002). Circulatory shock results in multi-organ failure and eventually death, and many changes that occur in this condition are related to oxidative and nitrosative stress in which PARP plays a major role (see Gero and Szabo, 2008). Furthermore, a role for PARP has been demonstrated in the development of vascular contractile failure, endothelial dysfunction, myocardial dysfunction, intestinal and pulmonary epithelial hyperpermeability and ultimately multi-organ failure (see Gero and Szabo, 2008).

It is also worthy of mention that a role for PARP has been established in cancer, which is leading the way for the development of new chemotherapeutic agents that block PARP in cancer cells to promote, rather than prevent, their death, through inhibition of DNA repair (see Peralta-Leal *et al.*, 2008), especially when other survival mechanisms are deficient in those cells. Overall, this wide-ranging involvement of PARP has generated much research interest in PARP inhibitors, as promising therapeutic interventions in relevant conditions affecting diverse tissue types.

Therapeutic opportunities

As already highlighted, parthanatos occurs in a highly choreographed, multistep fashion and a number of steps in the cascade could therefore serve as promising therapeutic targets to develop novel compounds for use in the management of diseases associated with cell death. Current approaches to the identification of small-molecule compounds specific for inhibiting parthanatos, including high-throughput screening of relevant chemical libraries, would lead the way in rational drug design in this area. There is some progress already regarding such pharmacological interventions and PARP blockers prevent parthanatos in cell culture and animal models of diseases involving overactivation of PARP-1 (see Virag and Szabo, 2002; Gero and Szabo, 2008). It should be noted that PARP inhibitors are now being tested also in other areas of therapeutic intervention not related to cell death, although these are outside the purview of this review (Peralta-Leal *et al.*, 2009; Virag and Szabo, 2002). Historically, first-generation PARP blockers include nicotinamide, benzamide and substituted benzamides, such as 3-aminobenzamide. Many analogues of benzamide with greater potency were later developed as second-generation PARP inhibitors, while third-generation inhibitors with greatly improved properties belong to a diverse family of chemical structures, such as derivatives of imidazopyridine, imidazoquinolinone and isoquinolindione (Eltze *et al.*, 2008). Others being developed include benzimidazoles, pthalazinones, ideno[1,2-c]isoquinolinones and tricyclic indoles (Peralta-Leal *et al.*, 2009). Interestingly, some natural compounds, especially flavonoids, have also been shown to inhibit PARP (Geraets *et al.*, 2007; Weseler *et al.*, 2009) and, very recently, 4'-methoxyflavone and 3',4'-dimethoxyflavone, identified through a high-throughput

screening campaign, were demonstrated to be neuroprotective PARP-1 inhibitors (Fatokun *et al.*, 2013).

Some PARP inhibitors have entered clinical trials, either as single agents (e.g. KU59436, BSI-201, ABT-888, INO-1001), or in combination with other agents (e.g. INO-1001, GPI 21016) (Green and Kroemer, 2005; Jagtap and Szabo, 2005; Jagtap *et al.*, 2005; Lorenzo and Susin, 2007; Pacher and Szabo, 2007; Peralta-Leal *et al.*, 2009). Third-generation PARP inhibitors that also entered clinical trials include ABT-888, AG014699, AZD2281, BSI-201, CEP-8983/CEP-9722 and MK-4827, designed to compete with NAD⁺ at the enzyme-active site [see Rouleau *et al.* (2010)]. They are more potent and specific compared to earlier inhibitors (Rouleau *et al.*, 2010). However, many of these PARP inhibitors have mainly been useful for attaining the therapeutic objective of promoting cell death, especially in cancer (Bryant *et al.*, 2005; Farmer *et al.*, 2005; McCabe *et al.*, 2005; Peralta-Leal *et al.*, 2008; 2009; Turner *et al.*, 2008), rather than preventing cell death, as would be desirable in, for example, neurodegenerative diseases. The rationale for this use is that sustained inhibition of PARP in cancer cells promotes their death by blocking their DNA repair machinery needed for survival. In terms of efforts towards protection, INO-1001 (Inotek) entered clinical trials for myocardial ischaemia (Lorenzo and Susin, 2007). There are also ongoing efforts to develop isoform-specific PARP inhibitors (Iwashita *et al.*, 2005; Ishida *et al.*, 2006; Pellicciari *et al.*, 2008; Peralta-Leal *et al.*, 2008; Turner *et al.*, 2008). It is anticipated that, due to their unknown potential long-term side effects, the development of PARP inhibitors for chronic neurodegenerative diseases and neuroinflammation may be more challenging (Graziani and Szabo, 2005). Conceptually, it might be much less desirable to seek to prevent cell death in the long term by blocking PARP, as doing so will occlude its beneficial effects, which are related to the maintenance of genomic stability. Nevertheless, shown in Table 2 are outcomes of the assessment of some PARP inhibitors in animal models of human neurological and neurodegenerative conditions linked to parthanatos.

Apart from direct PARP inhibitors already mentioned, some of which entered clinical trials, most levels of potential intervention in the parthanatos cascade as discussed subsequently are still essentially at the speculative or the experimental stage, but with the hope of making it to the clinic in the future. First, identifying pharmacological or other interventions that can block the accumulation of PAR polymer or its ability to signal to the mitochondria will be a rational means of combating parthanatos, just as direct PARP inhibition, as PAR is a death signal (Andrabi *et al.*, 2006). A novel, NMDA-induced survival protein, 'Iduna,' also known as RNF146, was recently shown to be a PAR-dependent E3 ubiquitin ligase (Kang *et al.*, 2011) and, like PARG, is an endogenous inhibitor of parthanatos (Andrabi *et al.*, 2011). Iduna was shown to protect against glutamate NMDA receptor-mediated excitotoxicity, both *in vitro* and *in vivo*, and also against stroke induced by middle cerebral artery occlusion in mice. The mechanisms of its protection are due to its E3 ubiquitin ligase activity and its ability to bind PAR. Like PARG, it acts downstream of, and does not affect, PARP-1 activity (Andrabi *et al.*, 2011).

Manipulation of PARG, the enzyme that hydrolyses PAR polymer, also constitutes a promising means of protecting

against parthanatos. Studies suggest that PARG plays an important role in cell survival by degrading PAR polymer (Koh *et al.*, 2004a). Animals lacking PARG are dramatically sensitive to toxic insults, while mice overexpressing PARG are correspondingly resistant (Koh *et al.*, 2004a; Andrabi *et al.*, 2006). There is, therefore, a unique therapeutic promise from achieving an efficient and rapid clearance of synthesized PAR polymer by boosting levels of PARG.

Release and translocation of AIF, which occur downstream of PAR signalling, are another set of attractive therapeutic targets. Because the mitochondrial translocation of AIF to the nucleus seems to be the commitment point in parthanatos, this particular step appears to have a critical place among therapeutic targets in this cell-death paradigm. Developing a means of monitoring the extent of the translocation (response) and relating this response to the degree of insult applied (concentration) will allow the generation of quantitative, concentration-response style data that should furnish excellent pharmacology for development of therapeutic agents. A way to achieve this could be fluorescently tagging AIF for visualization of its nuclear translocation in live cells in real time, which has been demonstrated (Landshamer *et al.*, 2008), and then achieving the ability to quantify this translocation in a manner that makes the approach amenable to high-content screening platforms for drug discovery purposes to identify compounds that modulate the event.

A further step that could potentially be targeted is the interaction of AIF with the DNA. This interaction has been suggested to be obligatory for the characteristic large-scale fragmentation and chromatin condensation that occur in parthanatos. As such, blocking the AIF-DNA interaction may be a further means to inhibit parthanatos, particularly if the concomitant deficits in mitochondrial function after AIF release are not the main cause of cell death in parthanatos. Thus, identifying PAAN and developing inhibitors to PAAN may be another attractive target to block parthanatos. As noted above, studies are required to dissect the relative importance of AIF release from the mitochondria versus the large-scale DNA fragmentation in the death process. The fact that AIF interacts with several proteins (mitochondrial, cytosolic, nuclear) en route to, or in, the nucleus provides a clue that identification of such AIF-interacting proteins and the blockade (or activation, in case of death-attenuating interactions) of such interactions by therapeutic agents may, at least in part, ameliorate cell death in parthanatos. Knowing that human AIF has some distinct characteristics that distinguish it from mouse AIF (e.g. human AIF has a fold identical to that in murine AIF but a different crystal packing that underlies a continuous positively charged patch thought to guide interactions with DNA) (Sevrioukova, 2011), the availability of its crystal structure (Ye *et al.*, 2002), alongside that of the murine AIF (Mate *et al.*, 2002), will enable a faster translation of basic investigations to therapeutic applications for human diseases. These highlights suggest that, although much still has to be done in this area to translate bench work to the bedside, they will provide credible approaches to the identification of disease-modifying therapeutic agents and thus give at least a glimmer of hope to sufferers of debilitating conditions in which parthanatos contributes significantly to pathophysiology. Figure 3 summarizes the various levels in parthanatos

Table 2

Effects of PARP-1 inhibitors in animal models of human neurological and neurodegenerative conditions linked to parthanatos

Relevant neurological condition(s) or neurodegenerative disease (s)	Evidence of parthanatos involvement in human disease	Animal models (rodents)	Inhibitors	Outcome of PARP-1 or parthanatos pathway inhibition in animal models/observation
Stroke, brain ischaemia, brain trauma (Pacher and Szabo, 2008)	PARP activated in brain sections from patients dying from stroke, brain ischaemia (from cardiac arrest) and brain trauma (Love <i>et al.</i> , 1999b; 2000)	Focal cerebral ischaemia (rat) (Strosznajder <i>et al.</i> , 2010)	DPQ	Decrease in infarct volume (Takahashi <i>et al.</i> , 1997)
			3-AB	Decrease in infarct volume after MCAO, reduction in NMDA-induced glutamate elevation, improvement in neurological outcome and motor function, marked decrease in the volume of damaged tissue (Ding <i>et al.</i> , 2001; Lo <i>et al.</i> , 1998; Takahashi and Greenberg, 1999; Tokime <i>et al.</i> , 1998; Yap <i>et al.</i> , 2008)
		Cilostazol	Reduction in infarct size, nuclear AIF translocation and apoptosis after MCAO followed by reperfusion (Lee <i>et al.</i> , 2007a)	
		Focal cerebral ischaemia (mouse) (Strosznajder <i>et al.</i> , 2010)	3-AB	Neuroprotection, decrease in infarct volume, improvement of neurological score (Couturier <i>et al.</i> , 2003)
		Global cerebral ischaemia (rat) (Strosznajder <i>et al.</i> , 2010)	PJ34	Inhibition of microglia/macrophage activation, decrease in CA1 neuronal death after forebrain ischaemia (Hamby <i>et al.</i> , 2007)
Neurodegenerative diseases (Pacher and Szabo, 2008)	Evidence of poly (ADP-ribosyl)ation in brain sections from patients with Alzheimer's disease, Parkinson's disease and ALS (Love <i>et al.</i> , 1999a; Kim <i>et al.</i> , 2003; 2004; Soos <i>et al.</i> , 2004)	Middle cerebral artery occlusion, global brain ischaemia, ischaemic reperfusion, hypoxia-ischaemia, MPTP intoxication, hypothermia, cortical trauma (mouse, rat, gerbil, guinea pig) (Komjati <i>et al.</i> , 2005)	DPQ Benzamide	No improvement of CA1 neuronal survival after bilateral carotid artery occlusion (Moroni <i>et al.</i> , 2001)
			3-AB	Robust neuroprotection in CA1 neurones after 3-min ischaemia, reduced forebrain ischaemia (Strosznajder <i>et al.</i> , 2003)
			3-AB	Reduced infarct size, maintained or improved NAD, improved neurological function or status, attenuation in glutamate release, reduced neutrophil infiltration, reduced nitrosative stress, sustained ATP, decrease in water content, reduction in number of TUNEL-positive cells, decrease in immunoreactivity of PAR, CD11B, ICAM-1 and COX2, enhanced neuroprotection (in combination with melatonin), improved neuronal conductance, decrease in production of inflammatory mediator, improved survival and CNS function (Barc <i>et al.</i> , 2001; Chiang and Lam, 2000; Couturier <i>et al.</i> , 2003; Ding <i>et al.</i> , 2001; Ducrocq <i>et al.</i> , 2000; Eliasson <i>et al.</i> , 1997; Endres <i>et al.</i> , 1997; Goto <i>et al.</i> , 2002; Iwashita <i>et al.</i> , 2004; Koedel <i>et al.</i> , 2002; Koh <i>et al.</i> , 2004b; Lam, 1997; Lo <i>et al.</i> , 1998; Nagayama <i>et al.</i> , 2000; Plaschke <i>et al.</i> , 2000; Sun and Cheng, 1998; Tabuchi <i>et al.</i> , 2001; Tokime <i>et al.</i> , 1998)
			PJ34	Reduced infarct size, improved neurological status (Abdelkarim <i>et al.</i> , 2001; Iwashita <i>et al.</i> , 2004)
			DPQ	Reduced infarct size, reduced infarct size but not DNA damage, improved neurological status (high doses may cause protective effect to be lost) (Takahashi <i>et al.</i> , 1997; 1999; Giovannelli <i>et al.</i> , 2002)
Benzamide	Reduced neuronal death, reduced hyperalgesia and mechano-allodynia, reduced neuronal deficit, improved survival, maintained striatal NAD ⁺ and ATP, protection against dopamine loss (Cosi <i>et al.</i> , 1994; 1996; Mao <i>et al.</i> , 1997; Meier <i>et al.</i> , 1999)			
INO-1001	Reduced infarct volume, improved neurological outcome Reduced immunoreactivity of PAR, NT and APP, immunoreactivity changes of AIF restored. Entered clinical trial for myocardial ischaemia (Komjati <i>et al.</i> , 2004; Lorenzo and Susin, 2007)			

Animal models of neurological and neurodegenerative diseases in which parthanatos is implicated and the observed effects of selected PARP-1 inhibitors, most of which are currently at the experimental stage, except INO-1001 that entered clinical trial for myocardial ischaemia. Abbreviations: 3-AB, 3-aminobenzamide; AIF, apoptosis-inducing factor; ALS, amyotrophic lateral sclerosis; APP, amyloid precursor protein; DPQ, 3,4-Dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)-isoquinolinone; MCAO, middle cerebral artery occlusion; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NAD⁺, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; NT, nitrotyrosine; PAR, poly (ADP-ribose) polymer; PJ34, N-(4-oxo-5,6-dihydro-phenanthridin-2-yl)-N,N-dimethylacetamide. Table was adapted from references (Komjati *et al.*, 2005; Pacher and Szabo, 2008; Strosznajder *et al.*, 2010).

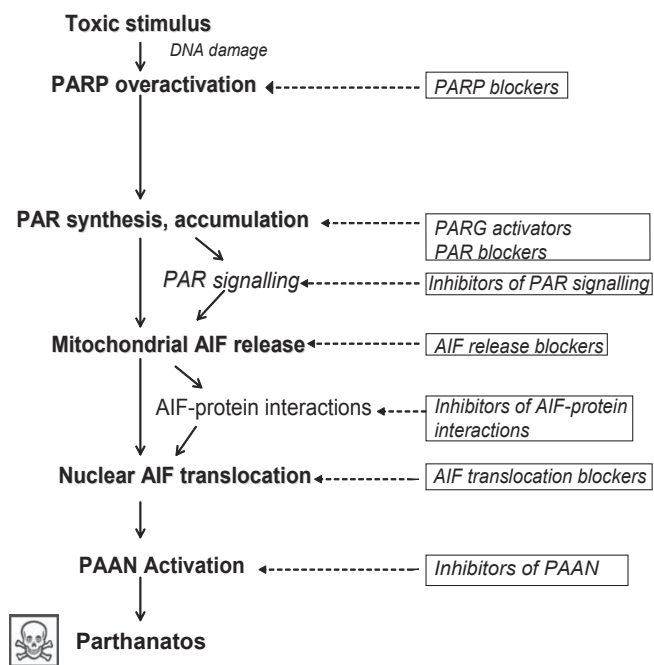


Figure 3

Potential therapeutic opportunities in the parthanatos cascade. Steps in the parthanatos cascade where therapeutic interventions are possible, from exposure of a cell to a toxic stimulus to the eventual cell death. Pathway progression is depicted using solid arrows. Proven or potential therapeutic interventions are in solid rectangular boxes, each linked with broken arrows to the step it modulates. To date, PARP blockers are the most advanced in development, with a number of them having entered clinical trials. The development of most other interventions is still at the experimental stage.

that could be targeted in the development of such therapeutic agents.

Summary

Parthanatos is here described as a unique, caspase-independent, cell-death pathway that is distinct from apoptosis, necrosis or other identified forms of cell death. Although largely discussed here in the context of cell death in the CNS, with relevance to neurological and neurodegenerative conditions, parthanatos is a multistep death pathway that may underlie many disease conditions, including diabetes and inflammatory disorders. Toxic stimuli that induce parthanatos activate nuclear PARP-1 excessively, causing it to synthesize a vast amount of PAR polymer. PAR polymer reaches a toxic level that translocates into the cytosol, where it constitutes a death signal through which mitochondrially localized AIF is forced out of its location and into the nucleus. Nuclear AIF translocation induced by mitochondriotoxic PAR establishes the mitochondrion as a central, indispensable player in parthanatos. AIF upon reaching the nucleus then interacts with DNA through an as-of-yet unidentified PAAN, resulting in large-scale DNA fragmentation and chromatin condensation. Parthanatic cell death is the eventual

outcome. There are checkpoints in the cascade that can be usefully exploited to create therapeutic interventions to manage pathologies associated with parthanatos. However, of all these potentially druggable molecules or steps of the death cascade, the event of AIF translocation from the mitochondria to the nucleus may currently be considered a very attractive target for drug design. Reassuringly, knowledge of parthanatos continues to grow rapidly, with the hope that the uncertainties in the pathway will soon become clarified and the existing controversies fully resolved.

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Conflict of interest

The authors declare no conflict of interest.

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