Trends in Molecular Medicine

CellPress

Review

Role of intrinsic apoptosis in environmental exposure health outcomes

Lissah Johnson[1,2,3] and Kristopher A. Sarosiek[1,2,3,4,*]

Environmental exposures are linked to diseases of high public health concern, including cancer, neurodegenerative disorders, and autoimmunity. These diseases are caused by excessive or insufficient cell death, prompting investigation of mechanistic links between environmental toxicants and dysregulation of cell death pathways, including apoptosis. This review describes how legacy and emerging environmental exposures target the intrinsic apoptosis pathway to potentially drive pathogenesis. Recent discoveries reveal that dynamic regulation of apoptosis may heighten the vulnerability of healthy tissues to exposures in children, and that apoptotic signaling can guide immune responses, tissue repair, and tumorigenesis. Understanding how environmental toxicants dysregulate apoptosis will uncover opportunities to deploy apoptosis-modulating agents for the treatment or prevention of exposure-linked diseases.

Highlights

Legacy and emerging toxicants of concern can modulate B cell lymphoma 2 (BCL-2) family protein expression and function, leading to excessive or insufficient levels of apoptosis and consequent pathogenesis.

Apoptosis dysregulation undergirds a host of diseases of great public health concern, including cancer, autoimmune diseases, and neurodegenerative disorders.

Recent discoveries show that apoptosis is dynamically regulated in healthy tissues across the lifespan, creating periods of heightened vulnerability to exposure-induced apoptosis such as early childhood.

Understanding changes in BCL-2 family regulation and apoptotic sensitivity (priming) caused by environmental exposures can help us better understand potential health risks and mechanisms of disease.

Novel agents that can modulate the apoptosis pathway may suppress or promote cell death in response to environmental toxicants, and ultimately prevent disease.

Environmental stressors influence whether a cell lives or dies

The myriad substances that humans are exposed to in the built, natural, and social environment profoundly affect health and wellbeing [1]. These exposures alter the molecular and chemical milieu within cells and tissues and, in conjunction with underlying genetic variations between individuals, drive the onset or modulate the risk of many diseases of high public health concern, including cancer, neurodegenerative diseases, respiratory illnesses, and metabolic disorders [2–5]. Thus, elucidating the molecular mechanisms by which these exposures can create pathological changes is critical for not only understanding disease etiology, but also uncovering more effective disease treatment and prevention strategies.

Most human diseases are driven by the inappropriate loss of essential cells or accumulation of cells that are dysfunctional or superfluous (comprehensively reviewed in [6,7]). Both of these situations can stem from malfunctions in apoptosis, which is an evolutionarily conserved form of cell death that is normally responsible for ridding the body of potentially harmful cells; but, it can also be triggered in otherwise healthy cells in response to damage or stress. Apoptosis is highly regulated, orchestrated via genetically programmed steps, and has two distinct pathways: extrinsic and intrinsic [6,7]. The extrinsic apoptosis pathway is initiated when a class of cell-surface receptors known as death receptors are activated through binding of their cognate ligands. This induces a signaling cascade that ultimately leads to **caspase** (see Glossary) activation for dismantling and clearance of the cell [7]. The intrinsic (mitochondrial) apoptosis pathway is triggered by extra- and intracellular stressors causing the release of pro-apoptogenic factors from mitochondria, and also culminates in caspase activation. Both pathways are important for the maintenance of homeostasis in adult tissues and essential for normal embryonic and fetal development [6].

Across the life course, a careful balance between cell death and cell survival is critical for disease prevention (Figure 1). Highlighting the delicate nature of this balance, mice that are genetically

[1]John B. Little Center for Radiation Sciences, Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA
[2]Molecular and Integrative Physiological Sciences Program, Harvard T.H. Chan School of Public Health, Boston, MA, USA
[3]Laboratory for Systems Pharmacology, Harvard Medical School, Boston, MA 02115, USA
[4]Department of Medical Oncology, Dana-Farber Cancer Institute/Harvard Cancer Center, Boston, MA, USA

*Correspondence:
sarosiek@hsph.havard.edu (K.A. Sarosiek).

engineered with apoptotic defects suffer from autoimmune disorders [8,9], accelerated tumorigenesis [10], and impaired fertility [11,12]. Therefore, environmental exposures that imbalance apoptosis in either direction can potentially drive disease. However, despite the clear links between cell death and environmental exposure-linked diseases, the mechanisms that trigger apoptosis in these settings are poorly understood. This is partly due to the challenges in discriminating between different forms of cell death, and confirming the causative role of apoptosis. This review seeks to summarize our current understanding of the intrinsic apoptosis pathway and how environmental exposures can dysregulate apoptosis to potentially drive pathogenesis. Although extrinsic apoptosis signaling is implicated in response to specific environmental toxicant exposures, the intrinsic apoptosis pathway will be the focus of this review given its prominent and integrative role in determining cell fate in response to a wide variety of upstream stress signals.

Molecular mechanisms governing intrinsic apoptosis and disease risk

Physiological regulation

Initiation of the intrinsic apoptosis pathway (Figure 2) is controlled by the B cell lymphoma 2 (BCL-2) family of proteins, comprised of proapoptotic and pro-survival members, that interact via their BCL-2 homology (BH) domains to prevent or promote apoptosis. Consistent with the importance of apoptosis for many physiological processes, multiple upstream stress signals can trigger this cell death pathway, including nutrient or growth factor withdrawal, DNA damage, **endoplasmic reticulum (ER) stress**, **reactive oxygen species (ROS)**, cytotoxic chemicals, or excessive mitogenic stimulation [6]. These upstream signaling pathways typically induce apoptosis by either upregulating proapoptotic BCL-2 homology domain 3 (BH3)-only activator proteins, such as BCL-2-interacting mediator of cell death (BIM) and BH3-interacting domain death agonist (BID), or by downregulating pro-survival proteins such as B cell lymphoma–extra large (BCL-X_L) and myeloid cell leukemia 1 (MCL-1). Either of these mechanisms will shift the balance of proapoptotic and pro-survival proteins closer to apoptosis initiation. Operationally, apoptosis is initiated when the activator proteins BIM and BID activate the proapoptotic, pore-forming proteins BCL-2-associated X protein (BAX) or BCL-2 homologous antagonist/killer (BAK) [13]. Activation of BAX or BAK promotes their oligomerization and results in **mitochondrial outer membrane permeabilization (MOMP)**. Importantly, apoptosis initiation can be prevented by the pro-survival members of the BCL-2 family, which bind and sequester the activator or pore-forming proteins to prevent MOMP. However, another class of proapoptotic proteins, BH3-only sensitizer proteins, have specific affinity for pro-survival proteins. They can compete for binding and disrupt pro-survival protein interactions with activator and pore-forming proteins, thus allowing them to initiate MOMP. The interactions between these BCL-2 family proteins are what ultimately determines whether MOMP occurs to initiate apoptosis.

MOMP is known as the commitment point for intrinsic apoptosis and causes the release of **cytochrome c** into the cytosol, where it forms the **apoptosome** by binding with apoptotic peptidase activating factor 1 (APAF-1) and promotes activation of the initiator caspase, caspase-9, and then executioner caspases 3 and 7. Activated caspases 3 and 7 cleave a wide variety of intracellular proteins and package the dying cell into membrane-bound apoptotic bodies that are phagocytized and cleared by cells such as macrophages.

The rapid and efficient clearance of apoptotic bodies renders apoptosis immunogenically silent in most settings [6]. However, recent discoveries show that impaired caspase activation during apoptosis execution can promote instead of prevent inflammation due to the release of immunogenic factors from mitochondria via enlarged BAX/BAK pores [14,15]. For example, mitochondrial DNA (mtDNA) is normally present within the mitochondrial matrix, but can be released during prolonged, caspase-deficient MOMP [14,15], or when MOMP occurs in only a subset of

Glossary

Apoptosome: a protein complex – comprising cytoplasmic cytochrome c, APAF1, and dATP – that recruits and activates (via cleavage) the normally inactive pro-caspase 9, which then activates effector caspases 3 and 7 to dismantle the cell and prepare it for phagocytosis.
BH3 mimetics: newly-developed small molecules that mimic the activity of proapoptotic, BH3-only proteins from the BCL-2 family, such as BAD, Noxa, or HRK.
BH3 profiling: an assay developed to functionally measure the state of the apoptosis pathway in any given cell. This assay involves the exposure of mitochondria – where the BCL-2 family of proteins resides and controls the commitment to apoptosis – to proapoptotic BH3 peptides that mimic the activity of endogenous proapoptotic proteins. By using either activator or sensitizer peptides, this assay can quantify the level of mitochondrial apoptotic priming (proximity to the cell death threshold) as well as dependence on pro-survival BCL-2 family proteins.
Caspases (cysteine–aspartic proteases): a family of protease enzymes responsible for carrying out the execution stages of apoptotic cell death by cleaving target proteins at aspartic acid residues.
cGAS-STING: cyclic guanosine monophosphate (GMP)–adenosine monophosphate (AMP) synthase (cGAS) is a cytosolic DNA-sensing molecule that activates innate immune responses by producing the second messenger cyclic GMP–AMP (cGAMP), which activates the adaptor protein stimulator of IFN genes (STING). cGAS recognizes double-stranded DNA that is exogenous or self-DNA (from the mitochondrion or nucleus).
Cytochrome c: an essential component of the mitochondrial electron transport chain within mitochondria, where it carries electrons. When released from mitochondria as a result of BAX/BAK activation and mitochondrial outer membrane permeabilization, cytochrome c binds to APAF1 to form the apoptosome, which initially activates caspase 9 and then caspases 3 and 7 for execution of the cell.
Damage-associated molecular pattern (DAMP) molecules: signals produced by dying cells that initiate and perpetuate immune activation in

mitochondria, a process referred to as 'minority MOMP' (miMOMP) [16]. Cytoplasmic mtDNA can be recognized as a **damage-associated molecular pattern (DAMP)** by the double-stranded DNA (dsDNA) sensor cyclic GMP-AMP synthase-stimulator of interferon genes (**cGAS-STING**), ultimately triggering a type I interferon response and production of proinflammatory signaling [6,17]. Interestingly, mtDNA release and cGAS-STING activation resulting from miMOMP has been recently shown to drive senescence and the senescence-associated secretory phenotype (SASP), which promotes tissue dysfunction [16]. Based on these discoveries, environmental exposures that affect caspase activity or produce sublethal mitochondrial apoptotic stress could potentially switch apoptosis into a more inflammatory process, which is of particular interest given the well-established causal links between inflammation and disease.

Cellular sensitivity to apoptotic stimuli is highly variable and can modulate disease risk

To ensure that cells have proper lifespans, the apoptosis pathway is dynamically regulated in various cell types to match their function, differentiation state, and growth [18,19]. This variability in apoptosis sensitivity, referred to as 'apoptotic priming', helps to determine their fate in response to cellular stressors. The state of the apoptosis pathway can be functionally assessed with assays such as **BH3 profiling**, which measures apoptotic priming by detecting MOMP in response to proapoptotic signals. Cellular responses to these proapoptotic signals are ultimately determined by the expression levels and interactions between BCL-2 family proteins at the mitochondrial level. Based on their responses, cells are designated as being 'primed', 'unprimed', or 'apoptosis-refractory'. In primed cells, even mild cellular stresses produce enough proapoptotic proteins to overwhelm pro-survival proteins and activate BAX or BAK. Unprimed cells typically have lower expression of proapoptotic proteins at baseline coupled with excess pro-survival proteins and require more stress to trigger apoptosis. Finally, apoptosis-refractory cells lack sufficient expression of BAX or BAK to initiate MOMP, which renders these cells resistant to most apoptosis-inducing stimuli [18,20].

Studies of apoptotic priming in both human and mouse tissues revealed that apoptosis is dynamically regulated across lifespan and tissue type [18,21,22] (Figure 3). In general, as tissues mature and cells adopt a post-mitotic, fully differentiated state, parenchymal cells in many tissues transition from being highly primed to unprimed and eventually apoptosis-refractory. Cell lineage programming, proliferation state, and stemness were also found to influence apoptotic priming [18,19]. This has implications for the type of toxicities and pathologies that could result from environmental exposures since the age at exposure and tissues affected will determine the extent of apoptosis. For example, exposures occurring early in life – when tissues are remodeling and cells are more primed for apoptosis – will be more likely to trigger excessive apoptosis. Environmental exposures to unprimed or apoptosis-refractory tissues in adulthood will still stress cells but are less likely to induce apoptosis. This can instead result in the aberrant survival of damaged or dysfunctional cells that should have been cleared and, ideally, replaced by stem cells. This demonstrates the existence of critical windows of vulnerability to the detrimental effects of environmental exposures.

Environmental insults that alter apoptotic priming can disrupt the essential cell-death-versus-proliferation balance and drive pathogenesis. This has been demonstrated with both *ex vivo* and *in vivo* models of disease due to apoptotic disruption. For example, excessive cell death of irreplaceable cells promotes pathologies, including Alzheimer's disease [23], amyotrophic lateral sclerosis [24], and immunodeficiency [25,26]. Conversely, insufficient apoptosis permits the survival of dysfunctional cells and contributes to the development of hematological and solid cancers [10,27,28] and autoimmune disorders reminiscent of systemic lupus erythematosus in mouse models [29,30]. Interestingly, submaximal activation of intrinsic apoptosis and caspases

response to tissue damage, trauma, or ischemia, regardless of whether pathogens are present at the site of injury.

Endoplasmic reticulum (ER) stress: a response of the ER to excessive protein production or misfolding (and other proteotoxic stresses), which is aimed at clearing unfolded proteins and restoring ER homeostasis. In cases when this cannot be accomplished, cellular functions degenerate and apoptosis is triggered.

Mitochondrial outer membrane permeabilization (MOMP): the initiation step of intrinsic (mitochondrial) apoptosis caused by activation of the pore-forming proteins BAX and/or BAK and resulting in the release of cytochrome c into the cytosol.

Reactive oxygen species (ROS): highly reactive chemicals – including peroxides, superoxide, hydroxyl radicals, singlet oxygen, and alpha oxygen – that are formed from the reduction of diatomic oxygen (O_2). ROS are byproducts of oxygen metabolism and produced predominantly in mitochondria.

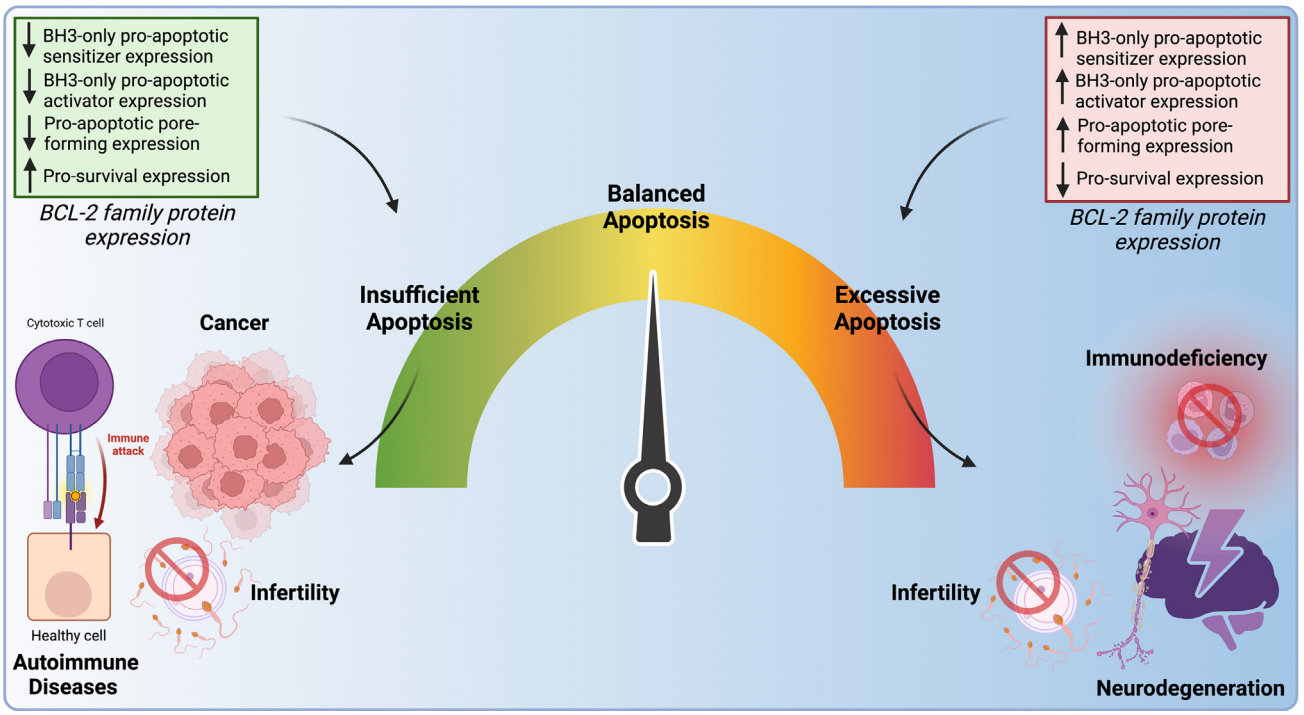

Figure 1. Maintaining optimal health requires maintenance of optimal rates of apoptotic cell death. Insufficient apoptosis can be mediated by increased expression of pro-survival proteins or decreased expression of proapoptotic proteins, including BCL-2 homology domain 3 (BH3)-only sensitizers and activators as well as the pore-forming proteins BCL-2-associated X protein (BAX) or BCL-2 homologous antagonist/killer (BAK). Deleterious health outcomes resulting from insufficient apoptosis include cancer, autoimmune disorders, and infertility. Conversely, excessive apoptosis can be mediated by decreased expression of pro-survival proteins or increased expression of proapoptotic proteins including BH3-only sensitizers and activators as well as pore-forming proteins BAX and BAK. Excessive apoptosis leads to loss of vital cell types and can result in neurodegeneration, immunodeficiency, and also infertility. Figure created with BioRender.

to levels that are insufficient to fully execute the cell can also drive genomic instability and tumorigenesis [31,32], thus blurring this ostensible dichotomy.

Signaling pathways targeted by environmental toxicants that are upstream of the intrinsic apoptosis pathway

The vast array of environmental toxicants that we are exposed to can elicit a vast array of mechanisms to drive their detrimental effects in either specific tissues or entire organ systems. Due to its prominent roles in development and homeostasis, the intrinsic apoptosis pathway is downstream of several signaling pathways that are normally activated by regulatory programs, but can also be aberrantly activated by environmental exposures. In the following sections, key molecular pathways that are engaged by environmental exposures to modulate apoptosis signaling are highlighted. In addition, Table 1 lists environmental toxicants that have been shown to either induce or suppress apoptotic cell death in mammalian cells. This list is not intended to be exhaustive but may provide examples to help guide and inform future studies examining links between exposures and disease.

p53 signaling

p53 is a transcription factor and tumor suppressor that is regarded as the 'guardian of the genome' for its prominent role in resolving DNA damage through cell cycle arrest, senescence, or mitochondrial apoptosis [33]. However, a wide range of cellular stresses that could be related to environmental exposures, and result in hypoxia, translation or replication stress, nutrient deprivation, or other metabolic changes, can activate p53 [33,34]. When a stressful stimulus is detected

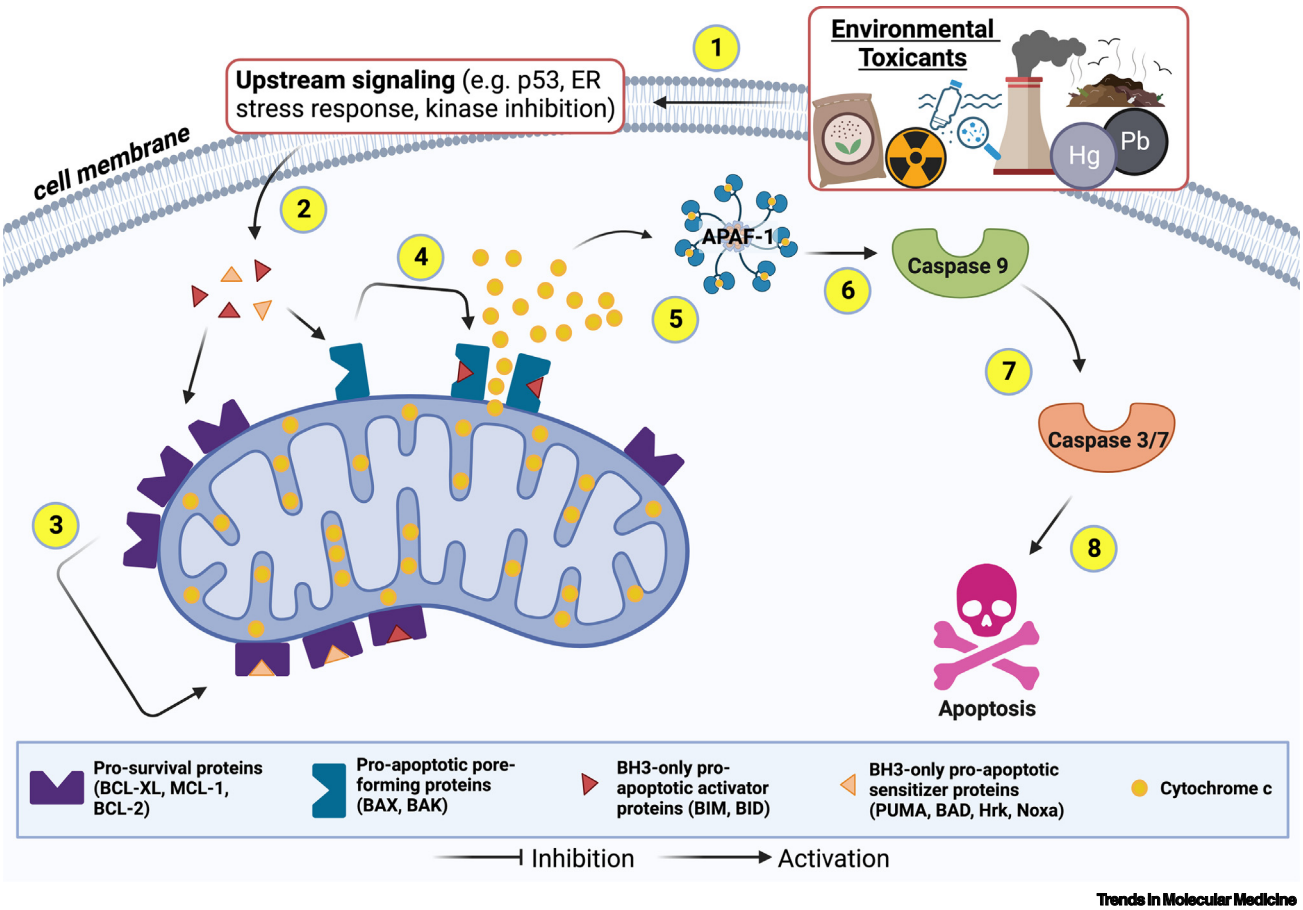

Figure 2. The intrinsic apoptosis pathway. ① Environmental toxicants can activate stress and damage-induced signaling pathways such as p53 and endoplasmic reticulum (ER) stress response. ② These signaling pathways upregulate BH3-only proapoptotic sensitizer or activator proteins. ③ Pro-survival proteins can bind and sequester proapoptotic proteins to inhibit apoptosis. ④ Proapoptotic activator proteins – for example, BCL-2-interacting mediator of cell death (BIM) and BH3-interacting domain death agonist (BID) – activate the pore-forming proteins BCL-2-associated X protein (BAX) or BCL-2 homologous antagonist/killer (BAK), leading to mitochondrial outer membrane permeabilization (MOMP) and cytochrome c release. ⑤ Released cytochrome c complexes with apoptotic peptidase activating factor 1 (APAF-1) to form the apoptosome. ⑥ Formation of the apoptosome leads to cleavage and activation of initiator caspase 9. ⑦ Activated caspase 9 cleaves and activates executioner caspases 3 and 7. ⑧ Executioner caspases cleave target proteins and package the cell for phagocytic clearance. Figure created with BioRender.

by upstream factors, p53 acetylation and phosphorylation promote its stabilization, tetramerization, and binding to DNA for activation of transcriptional activity [35]. p53 induces apoptosis by upregulating expression of proapoptotic proteins, including p53-upregulated modulator of apoptosis (PUMA), Noxa, BAX, APAF-1, and BIM [34–36]. Thus, environmental toxicants that cause p53 stabilization could increase apoptotic priming or induce apoptosis. For example, p53 activity was observed in response to different components of air pollution, including $PM_{2.5}$, diesel exhaust particles, and a benzo(a)pyrene metabolite (Table 1). These chemicals induced phosphorylation of p53, increased BAX expression, and decreased BCL-2 expression [37–39]. DNA damage resulting from ionizing radiation induces p53 stabilization and upregulation of canonical p53 transcriptional target PUMA [21,36,40]. Apart from these, several other environmental stressors listed in Table 1 induce p53 signaling and apoptosis, including but not limited to chemical mixtures, endocrine disrupting chemicals, and heavy metals. Conversely, mutagenic environmental exposures that cause loss-of-function mutations in p53 may abolish its ability to initiate apoptosis upon stress sensing, thus contributing to development of pathologies linked to insufficient apoptosis, especially cancer [41,42].

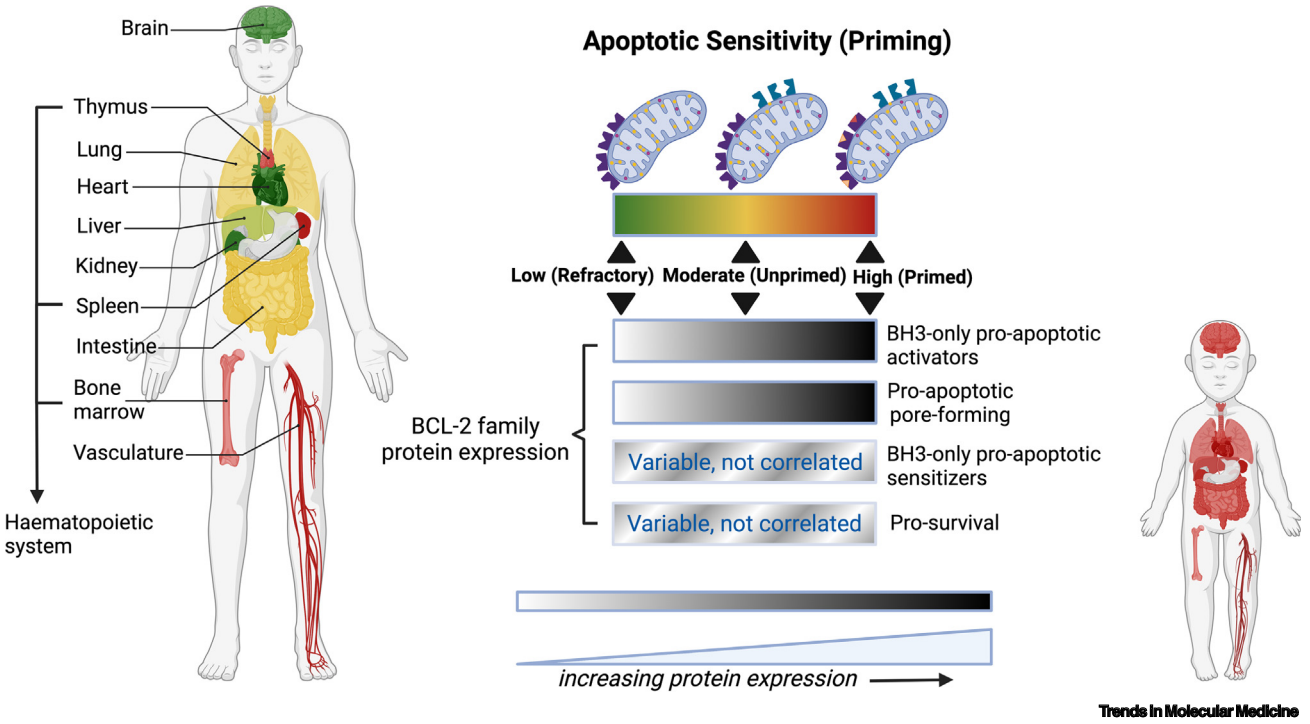

Figure 3. Intrinsic apoptosis is dynamically regulated across age and tissue type. Cells and tissues in neonatal/early life are more highly primed (denoted by red shading) across many organ systems. Cells and tissues of the adult body are overall less primed, though this varies between organ systems. Tissues that are terminally differentiated and are largely unable to re-enter mitosis – such as the brain, heart, and kidney – become apoptosis-refractory (denoted by green shading), while tissues composed of highly heterogeneous cell types that experience varied turnover – such as the lungs, liver, and intestines – become unprimed (denoted by yellow shading). Tissues that continually experience high turnover – including organs of the hematopoietic system and vascular endothelial cells that maintain an ability to rapidly proliferate to support neovascularization – remain primed across the lifespan. These changes in priming are reflected in the expression levels of some BCL-2 family proteins. Highly primed cells express higher levels of BH3-only proapoptotic activator and proapoptotic pore-forming proteins, while unprimed cells express moderate levels, and apoptosis-refractory cells express low levels. The expression levels of BH3-only sensitizer and pro-survival proteins vary and does not always correlate with apoptotic priming state. At the mitochondrial level, high priming is reflected by expression of pore-forming proteins and pro-survival proteins actively sequestering BH3-only proapoptotic proteins at the mitochondrial outer membrane (MOM). Unprimed mitochondria may have pro-survival proteins at the MOM that are unoccupied, while apoptosis-refractory mitochondria have no pore-forming proteins localized to the MOM and thus are resistant to undergoing MOM permeabilization (MOMP). Figure created with BioRender.

Estrogen receptor signaling

Estrogen receptors enact genomic and non-genomic programs in response to upstream signaling that are both ligand-dependent and independent [43,44]. The classical estrogen receptors, estrogen receptor α (ERα) and estrogen receptor β (ERβ), are nuclear receptors that activate transcription of estrogen-regulated genes either directly by acting as transcription factors and binding to DNA, or indirectly by activating other transcription factors [44]. The non-classical estrogen receptor, G-protein-coupled estrogen receptor 1 (GPER1), is a membrane receptor that can be activated by extracellular estrogen or other growth factors to then activate intracellular nuclear estrogen receptors [44]. Estrogen receptor signaling can suppress or activate apoptosis depending on the estrogen receptor isoform, the cell type, and the context [43,45–47]. Some BCL-2 family proteins are under the transcriptional control of estrogen-receptor-activated transcription factors like activator protein-1 (AP-1) and specificity protein 1 (SP1) [48,49]. Additionally, mitogen-activated protein kinase (MAPK) signaling (discussed later) and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signaling [50] are both downstream of GPER1 and suppress apoptosis via several mechanisms [44]. Also, ERβ can localize to mitochondria and inhibit the function of proapoptotic proteins, suppressing apoptosis [47]. Estrogen receptor signaling is activated not only by estrogen ligands but

Table 1. Environmental exposures that impact intrinsic apoptotic signaling

Chemical/stressor	Target organ/cell type used	Signaling pathway(s) implicated	Effect on intrinsic apoptosis pathway	Refs.
Endocrine disrupting chemicals				
2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)	Human trophoblast-like JAR cells	mtDNA damage induced p53 signaling	Induces apoptosis: increased AxV+/PI+ cells, CASP3 cleavage, enhanced p53 expression, increased cytosolic cytochrome c, decreased BCL-2:BAX ratio	[105]
	Human umbilical vein endothelial cells (HUVECs)	MAPK	Induces apoptosis: dose-dependent increase in AxV+/PI+ cells, increased CASP3 activity, increased p38 MAPK phosphorylation, p38 MAPK inhibition increased apoptosis and decreased BCL-2 expression	[106]
Bisphenol A	Immortalized and primary high-risk donor breast epithelial cell cultures, immortalized breast cancer cell lines	p53; estrogen receptor signaling	Suppresses apoptosis: decreased p53 and BAX expression, decreased fraction of apoptotic cells in BPA pretreated cells when challenged with tamoxifen in AxV FACS analysis	[53]
	Rat liver tissue	Mitochondrial damage	Induces apoptosis: mitochondrial swelling and mPTP formation, increased cytosolic cytochrome c, increased BAX expression, decreased BCL-2 expression, CASP9 and CASP3 cleavage	[107]
	INS-1 rat insulinoma cell line	Mitochondrial damage	Induces apoptosis: dose-dependent increase in fraction of AxV+/PI+ apoptotic cells, increased cytosolic cytochrome c, increased BAX expression, decreased BCL-2 expression, increased APAF-1 expression, CASP9 and CASP3 cleavage	[108]
4-Nonylphenol	TM4 murine Sertoli cells	MAPK	Induces apoptosis: ERK activation, increased BAX expression, decreased BCL-2 expression, increased CASP3 cleavage	[109]
Di(2-ethylhexyl) phthalate (DEHP)	INS-1 rat insulinoma cell line	ER stress/UPR	Induces apoptosis: dose-dependent increase in AxV+/PI- and AxV+/PI+ cells, induced PERK/eIF2a/ATF4/CHOP signaling cascade, increased CASP3 cleavage	[110]
Benzophenone-3	Murine primary neocortical and hippocampal neuronal cell cultures	Estrogen receptor signaling	Induces apoptosis: increased proapoptotic gene expression including ones encoding BIM, BAD, APAF-1, increased CASP9 and CASP3 activity, 24 h treatment decreased ERα expression and increased ERβ expression, CASP3 activity significantly increased with ERβ agonist and decreased with antagonist	[111]
Triclosan	BG-1 human ovarian cancer cell line	Estrogen receptor signaling	Suppresses apoptosis: decreased BAX expression which was abrogated by co-treatment with estrogen receptor antagonist	[54]
Persistent organic pollutants				
Perfluoro-octane sulfonate (PFOS)	Primary rat cerebellar granule cells	MAPK	Induces apoptosis: increased ERK phosphorylation, increased CASP3 activation which was abrogated by treatment with selective ERK inhibitor PD98059	[112]
Perfluorohexanesulfonate (PFHxS)	Primary rat cerebellar granule cell cultures	MAPK	Induces apoptosis: increased ERK phosphorylation, increased CASP3 activation at high concentrations which was abrogated by treatment with selective ERK inhibitor PD98059	[113]
Benzo(a) pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE)	Hct116 human colon carcinoma cell lines	p53	Induces apoptosis: p53 phosphorylation and stabilization, increased CASP3/7 activity in WT but not $p53^{-/-}$ cells, cells, increased CASP9 and CASP7 cleavage in WT but not *BAX* –/– cells	[39]

(continued on next page)

Table 1. (continued)

Chemical/stressor	Target organ/cell type used	Signaling pathway(s) implicated	Effect on intrinsic apoptosis pathway	Refs.
		Physical		
Heat stress	Human umbilical vein endothelial cells (HUVECs)	Mitochondrial damage; p53	Induces apoptosis: Ca^{2+} overload, MPTP opening, cytochrome c release, p53 stabilization, mitochondrial translocation, BAX activation	[88]
	Human umbilical vein endothelial cells (HUVECs)	ER stress/UPR; Ca^{2+} overload induced mitochondrial damage	Induces apoptosis: increased expression or activation of ER stress mediators, increased intracellular Ca^{2+}, induced APAF-1 expression, increased CASP9 and CASP3 activity	[89]
UVA radiation	Murine embryonic fibroblasts, AT, and GM847 human fibroblast cell lines	MAPK; p53	Induces apoptosis: JNK activation, ATM activation, p53 phosphorylation, CASP3 cleavage	[114]
Ionizing radiation	Murine intestinal stem cells	p53	Induces apoptosis: p53 phosphorylation, acetylation, and stabilization, increased PUMA expression, increased CASP3 activation	[40]
	Immature murine neural stem and progenitor cells	p53	Induces apoptosis: increased apoptotic priming (as measured by cytochrome c release), induced CASP3/7 cleavage and activity in WT but not *BAX* –/– animals, increased PUMA expression and BID cleavage	[36]
	Murine and human endothelial cells	p53	Induces apoptosis: increased AxV+ cells, increased CASP3 cleavage	[21]
		Heavy metals		
Cadmium	Primary murine neuronal cell cultures	p53; MAPK	Induces apoptosis: dose-dependent switch from autophagy to intrinsic apoptosis, JNK mediated Sirt1 degradation, increased p53 phosphorylation, increased BAX expression	[115]
	BEAS-2B human bronchial epithelial cell line	MAPK?	Induces apoptosis: dose-dependent increase in BAX protein expression, decreased BCL-2 expression, increased CASP3 cleavage	[116]
	Human prostate epithelial cell lines	p53	Induces apoptosis: increased p53 expression, dose-dependent increase in AxV+ cells abrogated by p53 knockdown or in p53-deficient cells	[117]
	CRL-2196 murine spermatocyte GC-2spd cell line, mouse testes tissue	ER stress/UPR; MAPK	Induces apoptosis: increased ATF6 and CHOP expression, increased intracellular Ca^{2+} and ROS levels, CASP3 cleavage, all decreased with ATF6 knockdown, p38 inhibition decreased ER stress and apoptosis markers	[118]
Methylmercury	Neuro-2a murine neuroblastoma cell line	ER stress/UPR	Induces apoptosis: dose-dependent increase in AxV+/PI– and AxV+/PI+ cells, increased GRP78 and CHOP expression, increased CASP9 and CASP3/7 cleavage attenuated by GRP78 and CHOP knockdown	[119]
Arsenic	MA-10 murine Leydig tumor cells	MAPK; mitochondrial damage	Induces apoptosis: JNK, ERK and p38 phosphorylation, increased BAX translocation to the mitochondria, increased cytosolic cytochrome c	[120]
	Rat hippocampus	Mitochondrial damage	Induces apoptosis: decreased MMP, increased cytochrome c release, increased BAX expression, decreased BCL:BAX ratio, CASP9 and CASP3 cleavage	[121]
	RIN-m5F rat insulinoma pancreatic β cell line	Mitochondrial damage; p53; ER	Induces apoptosis: dose dependent increase in AxV+ cells, decreased MMP, increased p53	[60]

Table 1. (continued)

Chemical/stressor	Target organ/cell type used	Signaling pathway(s) implicated	Effect on intrinsic apoptosis pathway	Refs.
		stress/UPR; MAPK	expression, decreased BCL-2 expression, increased cytosolic cytochrome c, CASP9 and CASP3/7 cleavage, JNK, p38, and ERK phosphorylation, increased expression of ER stress markers	
Nickel sulfate	Human umbilical vein endothelial cells (HUVECs)	MAPK	Induces apoptosis: increased AxV+/PI+ cells, decreased BCL-2 and BCL-X_L expression, increased BAX expression, increased CASP3 cleavage, mediated by p38 and JNK activation	[122]
Lead	Fetal rat brains	Unspecified	Induces apoptosis: increased BAX expression, decreased BCL-2 expression, non-significant increase inp53 expression, increased CASP3 expression but did not assay for cleaved CASP3	[123]
	PC12 rat pheochromocytoma cell line	p53	Induces apoptosis: increased CASP3 activity, increased p53 expression, increased BAX expression, increased BAX:BCL-2 ratio	[124]
	Rat bone-marrow-derived mesenchymal stem cells	p53	Induces apoptosis: increased BAX:BCL2 ratio, increased p53 expression, increased CASP9 expression (unclear if this is pro- or cleaved), decreased CASP3 expression but did not assay for cleaved CASP3	[125]
Pesticides				
Paraquat	SH-SY5Y human neuroblastoma cell line	ER stress/UPR	Induces apoptosis: activation of IRE1/ASK1/JNK signaling cascade, increased expression of ER stress markers, increased CASP3 activity	[126]
Methoxychlor	BG-1 human ovarian cancer cell line	Estrogen receptor signaling	Suppresses apoptosis: decreased BAX expression which was abrogated by co-treatment with estrogen receptor antagonist	[54]
Lindane	Primary rat hepatocytes	Unspecified	Suppresses apoptosis: increased BCL-X_L expression, increased BCL-X_L:BAX ratio, decreased cytochrome c release, decreased CASP9 and CASP3 activity in a time-dependent manner, increased necrotic cell death	[127]
Chlorpyrifos	SH-SY5Y human neuroblastoma cell line	MAPK	Induces apoptosis: phosphorylation of JNK, ERK1/2, and p38, dose-dependent increase in cytosolic fraction of cytochrome c, CASP9 and CASP3 cleavage	[128]
Air pollutants				
PM2.5	H9c2 rat cardiac cell line	MAPK	Induces apoptosis: dose-dependent increase in AxV+/PI+ cells, decreased BCL-2 expression, increased CASP3 cleavage, attenuated by p38 inhibition	[129]
	Human umbilical vein endothelial cells (HUVECs)	p53	Induces apoptosis: p53 phosphorylation, increased BAX expression, decreased BCL-2 expression, increased CASP9 and CASP3/7 cleavage, dose-dependent increase in AxV+/PI– and AxV+/PI+ cells	[37]
Diesel exhaust particles	J774A.1 murine macrophage cell line	p53	Induces apoptosis: p53 phosphorylation and activity, increased BAX expression, increased CASP3 cleavage	[38]
Cigarette smoke extract	BEAS-2B human bronchial epithelial cell line	ER stress/UPR	Induces apoptosis: PERK and eIF2a phosphorylation, increased CHOP expression, induced CASP3 cleavage	[62,63]

(continued on next page)

Table 1. (continued)

Chemical/stressor	Target organ/cell type used	Signaling pathway(s) implicated	Effect on intrinsic apoptosis pathway	Refs.
Nanoparticles/nanomaterials				
Silver nanoparticles	SH-SY5Y human neuroblastoma cell line	ER stress/UPR; Ca^{2+} overload induced mitochondrial damage	Induces apoptosis: induced CHOP expression, decreased BCL-2 expression, cytochrome c release, induced CASP9 and CASP3 activation	[130]
Titanium dioxide nanoparticles	Murine primary Sertoli cells	ER stress/UPR; mitochondrial damage	Induces apoptosis: dose-dependent increase in AxV+/PI+ cells, decreased MMP, increased BAX, GRP78, and CHOP expression, decreased BCL-2 expression	[131]
Zinc oxide nanoparticles	661 W murine photoreceptor cell line	Mitochondrial damage	Induces apoptosis: decreased MMP, increased BAX expression, decreased BCL-2 expression, cytochrome c release, increased CASP3 expression but did not assay for cleaved CASP3	[132]
Multi-walled carbon nanotubes	L02 human cell line	Unspecified	Induces apoptosis: increased AxV+/PI+ cells, decreased MMP, cytochrome c release, increased CASP9 and CASP3 activity	[133]
Silica nanoparticles	Human umbilical vein endothelial cells (HUVECs)	MAPK, mitochondrial damage	Induces apoptosis: p38 and JNK activation, MMP collapse, decreased BCL-2 expression, increased BAX expression	[134]
	Human umbilical vein endothelial cells (HUVECs)	ER stress/UPR; Ca^{2+} overload induced mitochondrial damage	Induces apoptosis: SiNP localization at ER and mitochondria, dose-dependent increase in AxV+/PI+ cells, MMP collapse, Ca^{2+} overload, increased expression of ER stress markers, increased BAX expression, decreased BCL-2 expression	[61]
Heterogeneous mixtures				
Arsenic and polystyrene nano-plastics	Murine liver tissue	p53	Induces apoptosis: increased p53 expression, increased BAX expression, decreased BCL-2 expression, increased CASP3 cleavage	[135]
Di-(2-ethylhexyl) phthalate and microplastics	Murine pancreatic tissue and MIN-6 insulinoma cell line	ER stress/UPR	Induces apoptosis: increased expression of ER stress markers, increased BAX expression, decreased BCL-2 expression, CASP9 and CASP3 cleavage, increased AxV+/PI+ cells	[76]

also by endocrine disrupting chemicals that have affinity for estrogen receptors, albeit to a lesser extent than the most potent estrogen, estradiol [51,52]. Thus, environmental endocrine disruptors – including bisphenol A, triclosan, and the pesticide methoxychlor – may lead to abnormal accumulation of cells by suppressing apoptosis [53,54] (Table 1).

ER stress

Mitochondrial apoptosis is also induced in response to severe ER stress, an accumulation of unfolded or misfolded proteins that is beyond resolution [55]. The ER, where proteins are assembled [56], is often in close proximity to or membrane-associated with mitochondria [57], and some BCL-2 family proteins, including BCL-X_L and BAX, are known to associate with the ER membrane [57]. Although there are multiple mechanisms linking ER stress and the unfolded protein response (UPR) to apoptosis, activation of the protein kinase RNA-like ER kinase (PERK)/ eukaryotic initiation factor 2 α (eIF2α)/activating transcription factor 4 (ATF4)/C/EBP homologous protein (CHOP) cascade leads to the upregulation of proapoptotic BH3-only proteins, including BIM, PUMA, and Noxa, and downregulation of pro-survival proteins, including MCL-1 and BCL-2 [55,56,58,59]. Inositol-requiring enzyme type 1 (IRE1) typically suppresses apoptosis during early periods of ER stress, but its prolonged and unresolved activation results in proapoptotic c-Jun N-terminal kinase (JNK) activity via phosphorylation and inactivation of pro-survival proteins, including BCL-X_L and

BCL-2 [55,56]. Several of the contaminants highlighted in Table 1 activate intrinsic apoptosis via ER stress signaling. For example, inorganic arsenic exposure has been reported to induce apoptosis in β-cells of the pancreas through MAPK signaling activated by the UPR [60]. Additionally, silica nanoparticles induce ER-stress-mediated intrinsic apoptosis via the IRE1 pathway in human umbilical vein endothelial cells (HUVECs) [61]. Finally, cigarette smoke extract induces apoptosis and caspase 3 activation in bronchial epithelial cells in an ER stress/CHOP-mediated fashion [62,63].

Mitochondrial damage and mitochondrial matrix remodeling

Mitochondria have function-associated features that may render them especially vulnerable to environmental toxicants. These include the difference in membrane potential between the inner and outer membranes and the cytosol, as well as the weakly basic pH of the matrix, which attracts electrophilic and lipophilic substances [64–66]. Additionally, calcium ions (Ca^{2+}) are an important factor in mitochondrial metabolism, leaving mitochondria particularly prone to accumulation of heavy metals that are similar to Ca^{2+}, including copper, cadmium, and lead [67]. Environmental insults that disrupt the mitochondrial membrane potential (MMP) or cytochrome c anchorage to the mitochondrial inner membrane can recruit proapoptotic proteins and modulate the amount of cytochrome c released into the cytosol to initiate apoptosis [68–71]. Additionally, mitochondrial matrix remodeling, following a decrease in MMP, positions cytochrome c for easier release upon challenge with a BH3-only activator protein [68,72]. Bisphenol A, heat stress, arsenic, and zinc oxide and silica nanoparticles can have this effect (Table 1), although it is not clear whether the change in MMP precedes or follows MOMP. Other toxicants such as bisphenol A (Table 1) may induce the mitochondrial permeability transition pore (mPTP) in the context of mitochondrial damage. If this opening of the inner mitochondrial membrane is prolonged, it can lead to a deleterious loss of MMP [69]. While this can cause release of mitochondrial cytochrome c and caspase activation, it is generally not regarded as canonical intrinsic apoptosis and may not be dependent on BAX/BAK [69,73].

Notably, normal mitochondrial function results in the production of ROS [74]. Cells experience oxidative stress when generation of ROS outpaces the processes that neutralize them; this results in ROS-mediated damage to cellular components, including lipids, proteins, and DNA [74]. Although oxidative stress has been linked to numerous environmental exposures, the many distinct ROS species that work in mechanistically diverse ways to induce damage and apoptosis complicate the establishment of definitive causal relationships [75]. Additionally, it is not always clear at what point in the signaling cascade ROS act to induce apoptosis. Cytosolic ROS levels may sometimes increase only after the induction of MOMP, which can release ROS that are normally contained within mitochondria. Despite these limitations, several studies have shown that ROS accumulation leads to upregulation of BH3-only proteins and initiation of apoptosis via upstream pathways including ER stress and the UPR as well as p53 activation (Table 1) [62,63,76,77].

MAPK signaling

MAPKs are a superfamily of kinases that induce intracellular signaling cascades in response to a diverse range of both intra- and extracellular stimuli [78]. The best-studied mammalian MAPK subgroups are extracellular signal-regulated kinase 1/2 (ERK1/2), JNK, and p38. ERK1/2 signaling is largely activated in response to growth factors, while JNK and p38 are associated with stressful or noxious stimuli, including environmental toxicants [78,79]. Environmental stressors are detected by various cell-surface receptors, or produce intracellular stimuli that activate GTP-binding proteins, which in turn activate the MAPK signaling cascade [78]. Expression and function of BCL-2 family proteins can be controlled by the action of this kinase family to promote or prevent apoptosis. For example, the transcription factors that JNK and p38 interact with, including AP-1 and p53, can transcriptionally alter the expression of both proapoptotic and pro-survival BCL-2 family members

[79]. These kinases also directly phosphorylate BCL-2 family proteins to modulate their interactions with scaffolding proteins such as 14-3-3, which can bind and inactivate them [79]. Phosphorylation of proapoptotic proteins –including BCL2-associated agonist of cell death (BAD) and BIM – by JNK and p38 induces their pro-death function, while phosphorylation of pro-survival proteins such as MCL-1 and BCL-2 inactivates them to also promote apoptosis [79]. Indeed, we see these proapoptotic effects in response to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure, which decreases BCL-2 expression in a p38 MAPK-mediated fashion (Table 1). Nickel sulfate also decreases BCL-2 and BCL-X_L expression while increasing BAX expression in a p38- and JNK-dependent manner (Table 1). MAPK signaling cascades also suppress apoptosis, but the effects of these modifications can be complex, context-dependent [79], and controversial [80].

Table 2. Apoptosis modulators

	Promote apoptosis							Inhibit apoptosis				Refs
	Inhibitors of pro-survival BCL-2 family proteins					Activators of BAX or BAK		Inhibitors of BAX or BAK (prevent apoptosis initiation)		Inhibitors of caspases (prevent apoptosis execution)		
Compounds	BCL-2	BCL-W	BCL-X_L	BFL-1/A1	MCL1	BAX	BAK	BAX	BAK	Caspases 3 & 7	Pan-caspase	
ABT-199	X											[6]
ABT-263	X	X	X									[6]
ABT-737	X	X	X									[6]
AZD4320	X		X									[6]
BM-1197	X		X									[6]
S44563	X		X									[6]
S55746	X		X									[6]
BCX2-32	X		X									[6]
A-1155463			X									[6]
A-1331852			X									[6]
DT2216												[136]
WEHI-539			X									[6]
A-1210477					X							[6]
AMG176					X							[6]
Compound 9					X							[6]
Compound 34					X							[6]
S63845					X							[6]
UMI-77					X							[6]
AIA1						X						[137]
BTSA1						X						[6]
BKA-073							X					[138]
SJ572946							X					[139]
BAI1								X				[140]
BAI2								X				[140]
MSN-125								X	X			[6]
IDN-6556 (emricasan)										X	X	[141]
DICA										X		[141]
FICA										X		[141]

Climate change will modulate the severity of environmental exposures

The ongoing accumulation of dangerous environmental toxicants poses major risks to human health, which may be further exacerbated by the global climate crisis [81]. Climate change is altering the release, fate, intensity, duration, and sources of physical and chemical environmental stressors via multiple routes, including the increased rates of extreme weather events such as wildfires and heatwaves [82]. Depending on fire temperature and substances being burned, wildfires increase the complexity and toxicity of air pollution [83]. As wildfires increasingly occur at urban/wildland interfaces, more people are exposed to smoke that contains environmental toxicants (listed in Table 1), including heavy metals, polycyclic aromatic hydrocarbons, and volatile organic compounds such as plasticizers, pesticides, and fire retardants [84,85]. Additionally, the increasing and rapidly shifting temperatures fueled by the climate crisis can exhaust the body's thermoregulatory systems and contribute to increased core body temperature, causing heat stress and heat-related illnesses that can dysregulate apoptotic signaling [86,87]. In fact, heightened temperatures can directly facilitate activation of mitochondrial apoptosis in various cell types, including HUVECs, via activation of p53, Ca^{2+} overload, and ER stress, inducing MOMP and caspase 3 activation [77,88,89]. Overall, climate change will likely exacerbate the risks posed by apoptosis dysregulation resulting from environmental exposures.

Modulation of apoptotic signaling by environmental exposures can contribute to health disparities

Sources of environmental toxicant exposures are not evenly distributed and tend to be more concentrated within groups/in areas with larger numbers of historically and currently excluded and marginalized groups. These include people of color, immigrants, members of the lesbian, gay, bisexual, transgender, queer or questioning, intersex, asexual, and more (LGBTQ+) community, and people of lower socioeconomic status [90–95]. This has been driven in significant part by historical disenfranchisement practices (i.e., redlining) and environmental injustices committed against these specific communities [96,97], placing a disproportionately high burden of exposure, and the associated pathologies, on them. Also, affected communities often lack access to positive structural and social determinants of health, including access to quality medical care and resources, or support networks to buffer against negative environmental stressors [90,91]. These factors, coupled with being part of an excluded/marginalized community, increase one's burden of psychosocial stress [91], which itself can alter apoptotic signaling. Stress hormones such as glucocorticoids and (nor)epinephrine induce apoptosis in immune cells via upregulation of the proapoptotic protein BIM and alternatively suppress apoptosis in other cell types by inactivating the proapoptotic protein BAD [98,99]. Taken together, this could create an increased risk of cancer, immunodeficiency, and autoimmune disease. Thus, while more research could better elucidate the role environmental exposures and disrupted intrinsic apoptotic signaling play in driving health disparities, more stringent policies targeting pollution sources and protecting environmental justice communities are also vitally important to pursue. Additionally, a strong commitment to efficacious interventions that mitigate exposures can increase health and well-being by protecting against environmental exposure-mediated apoptotic dysregulation.

Therapeutic interventions targeting the apoptosis pathway

Several novel agents that modulate mitochondrial apoptosis have recently been developed for potential therapeutic applications to restore optimal apoptosis regulation (Table 2). To initiate apoptosis, inhibitors of BCL-2 family pro-survival proteins – termed **BH3 mimetics** – work like BH3-only sensitizing proteins and inhibit the activity of pro-survival proteins. This strategy has proved to be remarkably successful clinically and has led to FDA approval for the BCL-2 inhibitor venetoclax (ABT-199) for use in cancers that are dependent on this protein for survival, including chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML). Conversely, compounds

Clinician's corner

The intrinsic apoptosis pathway is one of the main safeguards for ensuring tissue health and homeostasis. Cells that are irreparably damaged can be efficiently cleared via apoptosis, and impairment of this process by environmental toxicants can potentially lead to accumulation of dysfunctional cells. Insufficient cell death is a hallmark of diseases, including cancer and autoimmune disorders. Conversely, environmental toxicants can also damage or stress healthy cells and induce apoptosis, which may lead to diseases associated with excessive cell death, such as immunodeficiency and neurodegeneration.

The dynamic and varied regulation of apoptosis across tissues during our lifespan results in periods of heightened vulnerability to exposure-induced cell death. This is particularly evident in young children when tissue remodeling requires a highly responsive apoptosis pathway. Exposures to agents that induce apoptotic signaling during childhood leads to higher levels of apoptotic cell death in tissues, including the brain, heart, kidneys, and liver. The loss of irreplaceable cells within these tissues can cause lifelong impairments and further predisposition to disease.

Novel drugs targeting BCL-2 family proteins and caspases can potentially be used for treatment of diseases linked to insufficient or excessive apoptosis. However, additional research is needed to understand how tissue repair and immune responses are altered by these agents and to elucidate the potential risks associated with keeping damaged cells alive.

To uncover environmental factors that may be driving or contributing to a patient's disease, it is advisable for clinicians to do occupational and environmental history-taking during exams. Furthermore, apoptosis induced by environmental exposures may be exacerbated by other medicines that a patient is taking or have previously been administered. This may be particularly relevant for anticancer agents, which are also potent inducers of apoptosis.

that inhibit the activity of the pore-forming proteins BAX and BAK are also under development for apoptosis prevention [100,101]. These agents can block BAX/BAK activation, their recruitment to the mitochondrial outer membrane, or their oligomerization and pore formation [100,101]. In addition to their potential use in diseases linked to excessive cell death, such as neurodegeneration, BAX/BAK inhibitors could also be used to prevent apoptosis induced by environmental exposures.

Concluding remarks and future perspectives

The careful balance between mitochondrially mediated apoptosis suppression and activation allows our tissues to maintain proper function and overall optimal organismal health. When this becomes unbalanced, diseases of excess or insufficient apoptosis (e.g., neurodegenerative diseases or cancer, respectively) can result. Since apoptosis is dynamically and differently regulated in various cells and tissues, it is important to consider the exposure, which cells and tissues are targeted, and the life stage of the individual when evaluating health risks. As our understanding of apoptosis dysregulation by environmental exposures across the lifespan improves, we will uncover opportunities to modulate cell death for treatment and prevention of the toxicities and diseases that are environmentally mediated.

As highlighted in Table 1, many of the environmental contaminants of continuing and emerging concern have been shown to induce apoptosis by altering the expression of BCL-2 family proteins, inducing initiator and executioner caspase activation, and causing general metabolic distress. However, not many appear to suppress apoptosis. This could be due to the experimental systems and assays used; a single agent used at increasing doses is likely to eventually trigger observable cell death when a sufficiently high concentration is reached. Indeed, as Paracelsus once remarked, 'the dose makes the poison'. Detecting potential suppression of apoptosis by environmental toxicants requires a control stressor to be the primary death initiator, with the toxicant being administered while actively monitoring rates of cell death. As these more complex experimentation approaches are utilized, the apoptosis-suppressing activity of environmental exposures may come into focus.

Currently, there is a dearth of studies going beyond superficial measures of cell death that are not specific to apoptosis, such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assays (Box 1). Also, translationally relevant conclusions we can draw from current studies are often limited by the supraphysiological concentrations used that do not reflect environmentally relevant exposure levels. This obscures our ability to understand in a biologically relevant way how these chemicals and stressors could be contributing to the adverse health outcomes and health disparities that we see. We also see evidence for induction of multiple forms of cell death by the same exposures, including extrinsic apoptosis, necrosis, necroptosis, and ferroptosis [102,103]. This highlights the possibility of cross-talk between cell death pathways, yet it remains unclear what factors contribute to the activation of one pathway versus another, their temporal dynamics, and what conditions are conducive to cross-talk between cell death pathways. Non-apoptotic cell death pathways may also have a more prominent role in environmental exposures that cause acute toxicities as opposed to the chronic diseases that are highlighted here, although the two are inexorably linked.

Indeed, many outstanding questions remain (see Outstanding questions). Most of the historic and current studies do not capture how environmental exposures are altering sensitivity to apoptosis, or 'apoptotic priming'. This may be particularly important to environmental health research, in which environmentally relevant concentrations may not induce outright cell death but instead shift the apoptotic balance and predispose cells to undergo apoptosis when a secondary insult is experienced. The BH3 profiling assay can detect these increases in apoptotic priming as well as

Outstanding questions

What upstream signaling pathways are activated by various environmental exposures, and how does the duration and dose of exposure affect the outcome in terms of cell death and disease risk?

How do we rigorously model and measure low-level chronic exposures using *in vitro* and *in vivo* systems?

How do mixtures of exposures alter apoptotic signaling and cell fate?

How do we accurately model and measure these potentially complex interactions using *in vitro* and *in vivo* systems?

What factors are important for determining whether dysregulation of the apoptotic pathway will be transient and stimulate adaptation or permanent and stimulate disease processes?

How does potential cross-talk between cell death pathways alter cell fate decisions, and how do alterations in apoptosis signaling affect immune cells responses and tissue repair?

How can novel agents that modulate BCL-2 family protein activity be used to prevent diseases? What are the potential risks and benefits of keeping damaged cells alive?

Box 1. Reliably detecting apoptosis

There are more than 12 formally recognized forms of cell death [104], and distinguishing between them is critical for developing interventions to protect against disease. There are multiple assays that can reliably indicate whether observed cell death is mediated via mitochondrial apoptosis, utilizing a range of instrumentation that may be available, including examples listed as follows. Note that for each of the assays listed, caspase inhibitors such as quinoline-Val-Asp-difluorophenoxymethylketone (QVD-OPH) or carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone (Z-VAD-FMK), or genetic knockdowns of *BAX* and *BAK1* (encoding BAK) should be used to help confirm that cells are dying via mitochondrial apoptosis.

(i) Flow cytometry: staining cells with Annexin V (AxV) and a membrane-impermeable DNA dye such as propidium iodide (PI). Early in the execution stages of apoptosis, cells flip phosphatidylserine from their inner to outer membrane and Annexin V selectively binds to this protein. PI can only enter cells in late apoptosis when the plasma membrane is compromised. Therefore, cells that become positive for Annexin V prior to becoming positive for PI are likely undergoing apoptosis, which can be confirmed using caspase inhibitors and BAX/BAK knockout cells.

(ii) Fluorescence microscopy: immunostaining cells for cleaved caspase 3. Caspase 3 becomes activated during the execution stages of mitochondrial apoptosis. Therefore, dying cells that are stained positively for cleaved caspase 3 are likely undergoing apoptosis, which can be confirmed using caspase inhibitors and BAX/BAK knockout cells.

(iii) Microplate reader: staining cells with caspase-activated dyes such as CaspaseGlo (luminescence) or CellEvent Caspase 3/7 (fluorescence). Treatments that increase luminescence or fluorescence from these caspase-activated dyes while inducing cell death are likely inducing apoptosis, which can be confirmed using caspase inhibitors and BAX/BAK knockout cells.

(iv) Western blotting: blotting for cleaved caspase 3. Dying cells that contain cleaved caspase 3 as recognized by cleavage-specific antibodies and detected via Western blotting are likely undergoing apoptosis, which can be confirmed using caspase inhibitors and BAX/BAK knockout cells.

The assays listed are among those considered to detect apoptotic cell death most reliably. Note that often-used viability assays such as MTT (measures mitochondrial function), sodium 3′-[1- (phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzene sulfonic acid hydrate (XTT) (measures mitochondrial function), CellTiterGlo (measures ATP levels), Trypan Blue (measures membrane integrity), terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) (detects free 3' OH ends in DNA), or calcein-acetoxymethyl (AM) (measures esterase activity) cannot alone distinguish between apoptotic and other forms of cell death and should be used in combination with caspase inhibitors or BAX/BAK knockout cells to confirm apoptosis.

decreases, uncovering potential environmental exposure-driven apoptosis suppression. Furthermore, exposures rarely occur in isolation; future research should emphasize measuring the effects of chemical mixtures on apoptotic signaling. It is also unclear which exposures lead to apoptotic dysregulation that is transient, adaptive, and beneficial versus permanent, maladaptive, and predisposing to disease. Finally, ongoing efforts to develop small molecules that target intrinsic apoptosis are bringing us closer to agents that may effectively treat cancer or neurodegenerative diseases. However, particularly in the context of compounds used to inhibit apoptosis, more research is needed to understand the consequences of keeping a damaged cell alive after exposure. Environmental exposures will continue to be a critical determinant of human health, and our understanding of the ways in which they disrupt fundamental pathways in our body, such as apoptosis, will enhance our ability to prevent and treat the chronic diseases of greatest concern (see Clinician's corner).

Acknowledgments

We thank Kari Nadeau, Marc Weisskopf, Jin-Ah Park, Cheryl Rockwell, and Zintis Inde for their feedback and suggestions. We gratefully acknowledge funding support from the Alex's Lemonade Stand Foundation for Childhood Cancers, Charles Hood Foundation, Making Headway Foundation, St Baldrick's Foundation, Ovarian Cancer Research Alliance, HSPH NIEHS Center and NIH grants P30ES000002, R37CA248565, and R01DK125263.

Declaration of interests

The authors declare no conflicts of interest.

References

1. Rappaport, S.M. and Smith, M.T. (2010) Environment and disease risks. *Science* 330, 460–461
2. Prüss-Ustün, A. *et al.* (2019) Environmental risks and non-communicable diseases. *BMJ* 364, l265
3. Wilke, R.A. and Larson, E.A. (2021) Air, land, and sea: gene-environment interaction in chronic disease. *Am. J. Med.* 134, 1476–1482
4. Baccarelli, A. *et al.* (2023) A precision environmental health approach to prevention of human disease. *Nat. Commun.* 14, 2449
5. Lichtenstein, P. *et al.* (2000) Environmental and heritable factors in the causation of cancer — analyses of cohorts of twins from Sweden, Denmark, and Finland. *N. Engl. J. Med.* 343, 78–85
6. Singh, R. *et al.* (2019) Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. *Nat. Rev. Mol. Cell Biol.* 20, 175–193
7. Vitale, I. *et al.* (2023) Apoptotic cell death in disease – current understanding of the NCCD 2023. *Cell Death Differ.* 30, 1097–1154
8. Hughes, P. *et al.* (2006) Role of Bim and other Bcl-2 family members in autoimmune and degenerative diseases. *Curr. Dir. Autoimmun.* 9, 74–94
9. Bouillet, P. *et al.* (2002) BH3-only Bcl-2 family member Bim is required for apoptosis of autoreactive thymocytes. *Nature* 415, 922–926
10. Egle, A. *et al.* (2004) Bim is a suppressor of Myc-induced mouse B cell leukemia. *Proc. Natl. Acad. Sci.* 101, 6164–6169
11. Knudson, C.M. *et al.* (1995) Bax-deficient mice with lymphoid hyperplasia and male germ cell death. *Science* 270, 96
12. Lindsten, T. *et al.* (2000) The combined functions of proapoptotic Bcl-2 family members Bak and Bax are essential for normal development of multiple tissues. *Mol. Cell* 6, 1389–1399
13. Sarosiek, Kristopher A. *et al.* (2013) BID preferentially activates BAK while BIM preferentially activates BAX, affecting chemotherapy response. *Mol. Cell* 51, 751–765
14. White, Michael J. *et al.* (2014) Apoptotic caspases suppress mtDNA-induced STING-mediated type I IFN production. *Cell* 159, 1549–1562
15. McArthur, K. *et al.* (2018) BAK/BAX macropores facilitate mitochondrial herniation and mtDNA efflux during apoptosis. *Science* 359, eaao6047
16. Victorelli, S. *et al.* (2023) Apoptotic stress causes mtDNA release during senescence and drives the SASP. *Nature* 622, 627–636
17. West, A.P. and Shadel, G.S. (2017) Mitochondrial DNA in innate immune responses and inflammatory pathology. *Nat. Rev. Immunol.* 17, 363–375
18. Sarosiek, K.A. *et al.* (2017) Developmental regulation of mitochondrial apoptosis by c-Myc governs age- and tissue-specific sensitivity to cancer therapeutics. *Cancer Cell* 31, 142–156
19. Gutierrez-Martinez, P. *et al.* (2018) Diminished apoptotic priming and ATM signalling confer a survival advantage onto aged haematopoietic stem cells in response to DNA damage. *Nat. Cell Biol.* 20, 413–421
20. Ni Chonghaile, T. *et al.* (2011) Pretreatment mitochondrial priming correlates with clinical response to cytotoxic chemotherapy. *Science* 334, 1129–1133
21. Spetz, J.K.E. *et al.* (2022) Heightened apoptotic priming of vascular cells across tissues and life span predisposes them to cancer therapy-induced toxicities. *Sci. Adv.* 8, eabn6579
22. Madden, S. *et al.* (2007) Key apoptosis regulating proteins are down-regulated during postnatal tissue development. *Int. J. Dev. Biol.* 51, 415–424
23. Rohn, T.T. *et al.* (2008) Lack of pathology in a triple transgenic mouse model of Alzheimer's disease after overexpression of the anti-apoptotic protein Bcl-2. *J. Neurosci.* 28, 3051–3059
24. Reyes, N.A. *et al.* (2010) Blocking the mitochondrial apoptotic pathway preserves motor neuron viability and function in a mouse model of amyotrophic lateral sclerosis. *J. Clin. Invest.* 120, 3673–3679
25. Strack, P.R. *et al.* (1996) Apoptosis mediated by HIV protease is preceded by cleavage of Bcl-2. *Proc. Natl. Acad. Sci.* 93, 9571–9576
26. Boudet, F. *et al.* (1996) Apoptosis associated with *ex vivo* down-regulation of Bcl-2 and up-regulation of Fas in potential cytotoxic CD8+ T lymphocytes during HIV infection. *J. Immunol.* 156, 2282–2293
27. Strasser, A. *et al.* (1990) Novel primitive lymphoid tumours induced in transgenic mice by cooperation between myc and bcl-2. *Nature* 348, 331–333
28. Ramesh, P. *et al.* (2021) BCL-XL is crucial for progression through the adenoma-to-carcinoma sequence of colorectal cancer. *Cell Death Differ.* 28, 3282–3296
29. Strasser, A. *et al.* (1991) Enforced BCL2 expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. *Proc. Natl. Acad. Sci.* 88, 8661–8665
30. Bouillet, P. *et al.* (1999) Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. *Science* 286, 1735–1738
31. Ichim, G. *et al.* (2015) Limited mitochondrial permeabilization causes DNA damage and genomic instability in the absence of cell death. *Mol. Cell* 57, 860–872
32. Liu, X. *et al.* (2015) Caspase-3 promotes genetic instability and carcinogenesis. *Mol. Cell* 58, 284–296
33. Bieging, K.T. *et al.* (2014) Unravelling mechanisms of p53-mediated tumour suppression. *Nat. Rev. Cancer* 14, 359–370
34. Thomas, A.F. *et al.* (2022) Of the many cellular responses activated by TP53, which ones are critical for tumour suppression? *Cell Death Differ.* 29, 961–971
35. Aubrey, B.J. *et al.* (2018) How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression? *Cell Death Differ.* 25, 104–113
36. Singh, R. *et al.* (2023) Radiation therapy-induced neurocognitive impairment is driven by heightened apoptotic priming in early life and prevented by blocking BAX. *Cancer Res.* 83, 3442–3461
37. Wang, W. *et al.* (2017) PM2.5 induced apoptosis in endothelial cell through the activation of the p53-bax-caspase pathway. *Chemosphere* 177, 135–143
38. Yun, Y.-P. *et al.* (2009) Diesel exhaust particles induce apoptosis via p53 and Mdm2 in J774A.1 macrophage cell line. *Toxicol. in Vitro* 23, 21–28
39. Donauer, J. *et al.* (2012) Role and interaction of p53, BAX and the stress-activated protein kinases p38 and JNK in benzo(a)pyrene-diolepoxide induced apoptosis in human colon carcinoma cells. *Arch. Toxicol.* 86, 329–337
40. Wang, X. *et al.* (2015) Pharmacologically blocking p53-dependent apoptosis protects intestinal stem cells and mice from radiation. *Sci. Rep.* 5, 8566
41. Kucab, J.E. *et al.* (2010) Linking environmental carcinogen exposure to TP53 mutations in human tumours using the human TP53 knock-in (Hupki) mouse model. *FEBS J.* 277, 2567–2583
42. Su, Y. *et al.* (2022) Current insights into the regulation of programmed cell death by TP53 mutation in cancer. *Front. Oncol.* 12, 1023427
43. Chen, P. *et al.* (2022) Role of estrogen receptors in health and disease. *Front. Endocrinol.* 13, 839005
44. Fuentes, N. and Silveyra, P. (2019) Estrogen receptor signaling mechanisms. *Adv. Protein Chem. Struct. Biol.* 116, 135–170
45. Chimento, A. *et al.* (2022) Estrogen receptors-mediated apoptosis in hormone-dependent cancers. *Int. J. Mol. Sci.* 23, 1242
46. Han, S.J. *et al.* (2015) Estrogen receptor β modulates apoptosis complexes and the inflammasome to drive the pathogenesis of endometriosis. *Cell* 163, 960–974
47. Liang, J. *et al.* (2015) Mitochondrial estrogen receptor β inhibits cell apoptosis via interaction with Bad in a ligand-independent manner. *Mol. Cell. Biochem.* 401, 71–86
48. Schacter, J.L. *et al.* (2014) Estrogen regulation of anti-apoptotic Bcl-2 family member Mcl-1 expression in breast cancer cells. *PLoS One* 9, e100364
49. Kaloni, D. *et al.* (2022) BCL-2 protein family: attractive targets for cancer therapy. *Apoptosis* 28, 20–38
50. Hemmings, B.A. and Restuccia, D.F. (2012) PI3K-PKB/Akt pathway. *Cold Spring Harb. Perspect. Biol.* 4, a011189
51. Qie, Y. *et al.* (2021) Environmental estrogens and their biological effects through GPER mediated signal pathways. *Environ. Pollut.* 278, 116826

52. James-Todd, T. *et al.* (2021) Hormonal activity in commonly used Black hair care products: evaluating hormone disruption as a plausible contribution to health disparities. *J. Expo. Sci. Environ. Epidemiol.* 31, 476–486
53. Dairkee, S.H. *et al.* (2012) Bisphenol-A-induced inactivation of the p53 axis underlying deregulation of proliferation kinetics, and cell death in non-malignant human breast epithelial cells. *Carcinogenesis* 34, 703–712
54. Kim, J.-Y. *et al.* (2014) Methoxychlor and triclosan stimulates ovarian cancer growth by regulating cell cycle- and apoptosis-related genes via an estrogen receptor-dependent pathway. *Environ. Toxicol. Pharmacol.* 37, 1264–1274
55. Iurlaro, R. and Muñoz-Pinedo, C. (2016) Cell death induced by endoplasmic reticulum stress. *FEBS J.* 283, 2640–2652
56. Szegezdi, E. *et al.* (2006) Mediators of endoplasmic reticulum stress-induced apoptosis. *EMBO Rep.* 7, 880–885
57. Kale, J. *et al.* (2018) BCL-2 family proteins: changing partners in the dance towards death. *Cell Death Differ.* 25, 65–80
58. Fraser, C.S. *et al.* (2022) Exploiting endogenous and therapy-induced apoptotic vulnerabilities in immunoglobulin light chain amyloidosis with BH3 mimetics. *Nat. Commun.* 13, 5789
59. Inde, Z. *et al.* (2021) Age-dependent regulation of SARS-CoV-2 cell entry genes and cell death programs correlates with COVID-19 severity. *Sci. Adv.* 7, eabf8609
60. Lu, T.-H. *et al.* (2011) Arsenic induces pancreatic β-cell apoptosis via the oxidative stress-regulated mitochondria-dependent and endoplasmic reticulum stress-triggered signaling pathways. *Toxicol. Lett.* 201, 15–26
61. Guo, C. *et al.* (2018) Silica nanoparticles induced endothelial apoptosis via endoplasmic reticulum stress-mitochondrial apoptotic signaling pathway. *Chemosphere* 210, 183–192
62. Tagawa, Y. *et al.* (2008) Induction of apoptosis by cigarette smoke via ROS-dependent endoplasmic reticulum stress and CCAAT/enhancer-binding protein-homologous protein (CHOP). *Free Radic. Biol. Med.* 45, 50–59
63. Tagawa, Y. *et al.* (2011) Induction of CCAAT/enhancer-binding protein–homologous protein by cigarette smoke through the superoxide anion-triggered PERK–eIF2α pathway. *Toxicology* 287, 105–112
64. Wu, H. *et al.* (2023) Molecular mechanisms of environmental exposures and human disease. *Nat. Rev. Genet.* 24, 332–344
65. Meyer, J.N. *et al.* (2013) Mitochondria as a target of environmental toxicants. *Toxicol. Sci.* 134, 1–17
66. Duarte-Hospital, C. *et al.* (2022) Mitochondrial dysfunction as a hallmark of environmental injury. *Cells* 11, 110
67. Reddam, A. *et al.* (2022) Environmental chemical exposures and mitochondrial dysfunction: a review of recent literature. *Curr. Environ. Health Rep.* 9, 631–649
68. Gottlieb, E. *et al.* (2003) Mitochondrial membrane potential regulates matrix configuration and cytochrome c release during apoptosis. *Cell Death Differ.* 10, 709–717
69. Morciano, G. *et al.* (2021) The mitochondrial permeability transition pore: an evolving concept critical for cell life and death. *Biol. Rev.* 96, 2489–2521
70. Smaili, S.S. *et al.* (2001) Bax translocation to mitochondria subsequent to a rapid loss of mitochondrial membrane potential. *Cell Death Differ.* 8, 909–920
71. Fox, C.A. *et al.* (2021) Calcium-induced release of cytochrome c from cardiolipin nanodisks: implications for apoptosis. *Biochim. Biophys. Acta Biomembr.* 1863, 183722
72. Scorrano, L. *et al.* (2002) A distinct pathway remodels mitochondrial cristae and mobilizes cytochrome c during apoptosis. *Dev. Cell* 2, 55–67
73. Baines, C.P. *et al.* (2005) Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature* 434, 658–662
74. Redza-Dutordoir, M. and Averill-Bates, D.A. (2016) Activation of apoptosis signalling pathways by reactive oxygen species. *Biochim. Biophys. Acta, Mol. Cell Res.* 1863, 2977–2992
75. Dickinson, B.C. and Chang, C.J. (2011) Chemistry and biology of reactive oxygen species in signaling or stress responses. *Nat. Chem. Biol.* 7, 504–511
76. Wang, Y. *et al.* (2022) Microplastics and di(2-ethylhexyl)phthalate synergistically induce apoptosis in mouse pancreas through the GRP78/CHOP/Bcl-2 pathway activated by oxidative stress. *Food Chem. Toxicol.* 167, 113315
77. Gu, Z.T. *et al.* (2014) Heat stress induces apoptosis through transcription-independent p53-mediated mitochondrial pathways in human umbilical vein endothelial cell. *Sci. Rep.* 4, 4469
78. Cargnello, M. and Roux, P.P. (2011) Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. *Microbiol. Mol. Biol. Rev.* 75, 50–83
79. Yue, J. and López, J.M. (2020) Understanding MAPK signaling pathways in apoptosis. *Int. J. Mol. Sci.* 21, 21072346
80. Liu, J. and Lin, A. (2005) Role of JNK activation in apoptosis: a double-edged sword. *Cell Res.* 15, 36–42
81. Forster, P.M. *et al.* (2023) Indicators of Global Climate Change 2022: annual update of large-scale indicators of the state of the climate system and human influence. *Earth Syst. Sci. Data* 15, 2295–2327
82. LaKind, J.S. *et al.* (2016) Exposure science in an age of rapidly changing climate: challenges and opportunities. *J. Expo. Sci. Environ. Epidemiol.* 26, 529–538
83. Kim, Y.H. *et al.* (2023) Toxicity of fresh and aged anthropogenic smoke particles emitted from different burning conditions. *Sci. Total Environ.* 892, 164778
84. Radeloff, V.C. *et al.* (2018) Rapid growth of the US wildland-urban interface raises wildfire risk. *Proc. Natl. Acad. Sci.* 115, 3314–3319
85. Consensus study report (2022) *The Chemistry of Fires at the Wildland-Urban Interface*, National Academies of Sciences, Engineering, and Medicine
86. Sampath, V. *et al.* (2023) Acute and chronic impacts of heat stress on planetary health. *Allergy* 78, 2109–2120
87. Ahima, R.S. (2020) Global warming threatens human thermoregulation and survival. *J. Clin. Invest.* 130, 559–561
88. Gu, Z.T. *et al.* (2015) Heat stress induced apoptosis is triggered by transcription-independent p53, Ca^{2+} dyshomeostasis and the subsequent Bax mitochondrial translocation. *Sci. Rep.* 5, 11497
89. Li, L. *et al.* (2015) Heat stress induces apoptosis through a Ca^{2+}-mediated mitochondrial apoptotic pathway in human umbilical vein endothelial cells. *PLoS One* 9, e111083
90. Goldsmith, L. and Bell, M.L. (2022) Queering environmental justice: unequal environmental health burden on the LGBTQ+ community. *Am. J. Public Health* 112, 79–87
91. Gee, G.C. and Payne-Sturges, D.C. (2004) Environmental health disparities: a framework integrating psychosocial and environmental concepts. *Environ. Health Perspect.* 112, 1645–1653
92. Larsen, K. *et al.* (2023) Inequalities in environmental cancer risk and carcinogen exposures: a scoping review. *Int. J. Environ. Res. Public Health* 20, 5718
93. Kodros, J.K. *et al.* (2022) Unequal airborne exposure to toxic metals associated with race, ethnicity, and segregation in the USA. *Nat. Commun.* 13, 6329
94. James-Todd, T.M. *et al.* (2016) Racial/ethnic disparities in environmental endocrine disrupting chemicals and women's reproductive health outcomes: epidemiological examples across the life course. *Curr. Epidemiol. Rep.* 3, 161–180
95. Weiss, M.C. *et al.* (2023) Hormonal injustice: environmental toxicants as drivers of endocrine health disparities. *Endocrinol. Metab. Clin. N. Am.* 52, 719–736
96. Johnston, J. and Cushing, L. (2020) Chemical exposures, health, and environmental justice in communities living on the fenceline of industry. *Curr. Environ. Health Rep.* 7, 48–57
97. Banzhaf, S. *et al.* (2019) Environmental justice: the economics of race, place, and pollution. *J. Econ. Perspect.* 33, 185–208
98. Sastry, K.S.R. *et al.* (2007) Epinephrine protects cancer cells from apoptosis via activation of cAMP-dependent protein kinase and BAD phosphorylation. *J. Biol. Chem.* 282, 14094–14100
99. Zhang, L. and Insel, P.A. (2004) The pro-apoptotic protein Bim is a convergence point for cAMP/protein kinase A- and glucocorticoid-promoted apoptosis of lymphoid cells. *J. Biol. Chem.* 279, 20858–20865
100. Jensen, K. *et al.* (2019) Pharmacological inhibition of Bax-induced cell death: Bax-inhibiting peptides and small compounds inhibiting Bax. *Exp. Biol. Med.* 244, 621–629
101. Niu, X. *et al.* (2017) A small-molecule inhibitor of Bax and Bak oligomerization prevents genotoxic cell death and promotes neuroprotection. *Cell Chem. Biol.* 24, 493–506.e495

102. Sahoo, K. and Sharma, A. (2023) Understanding the mechanistic roles of environmental heavy metal stressors in regulating ferroptosis: adding new paradigms to the links with diseases. *Apoptosis* 28, 277–292
103. Sepand, M.-R. *et al.* (2020) Mechanisms and pathogenesis underlying environmental chemical-induced necroptosis. *Environ. Sci. Pollut. Res.* 27, 37488–37501
104. Galluzzi, L. *et al.* (2018) Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ.* 25, 486–541
105. Chen, S.-C. *et al.* (2010) Endocrine disruptor, dioxin (TCDD)-induced mitochondrial dysfunction and apoptosis in human trophoblast-like JAR cells. *Mol. Hum. Reprod.* 16, 361–372
106. Yu, Y. *et al.* (2017) 2,3,7,8-Tetrachlorodibenzo-p-dioxin promotes endothelial cell apoptosis through activation of EP3/p38MAPK/Bcl-2 pathway. *J. Cell. Mol. Med.* 21, 3540–3551
107. Xia, W. *et al.* (2014) Early-life exposure to bisphenol A induces liver injury in rats involvement of mitochondria-mediated apoptosis. *PLoS One* 9, e90443
108. Lin, Y. *et al.* (2013) Exposure to bisphenol A induces dysfunction of insulin secretion and apoptosis through the damage of mitochondria in rat insulinoma (INS-1) cells. *Cell Death Dis.* 4, e460
109. Choi, M.-S. *et al.* (2014) Nonylphenol-induced apoptotic cell death in mouse TM4 Sertoli cells via the generation of reactive oxygen species and activation of the ERK signaling pathway. *J. Appl. Toxicol.* 34, 628–636
110. Sun, X. *et al.* (2015) Di(2-ethylhexyl) phthalate-induced apoptosis in rat INS-1 cells is dependent on activation of endoplasmic reticulum stress and suppression of antioxidant protection. *J. Cell. Mol. Med.* 19, 581–594
111. Wnuk, A. *et al.* (2018) Apoptosis induced by the UV filter benzophenone-3 in mouse neuronal cells is mediated via attenuation of Erα/Pparγ and stimulation of Erβ/Gpr30 signaling. *Mol. Neurobiol.* 55, 2362–2383
112. Lee, Y.J. *et al.* (2013) Perfluorooctane sulfonate-induced apoptosis of cerebellar granule cells is mediated by ERK 1/2 pathway. *Chemosphere* 90, 1597–1602
113. Lee, Y.J. *et al.* (2014) PFHxS induces apoptosis of neuronal cells via ERK1/2-mediated pathway. *Chemosphere* 94, 121–127
114. Zhang, Y. *et al.* (2002) Requirement of ATM in UVA-induced signaling and apoptosis. *J. Biol. Chem.* 277, 3124–3131
115. Li, T. *et al.* (2022) Apoptosis, rather than neurogenesis, induces significant hippocampal-dependent learning and memory impairment in chronic low Cd^{2+} exposure. *Environ. Toxicol.* 37, 814–824
116. Cao, X. *et al.* (2021) Cadmium-induced BEAS-2B cells apoptosis and mitochondria damage via MAPK signaling pathway. *Chemosphere* 263, 128346
117. Aimola, P. *et al.* (2012) Cadmium induces p53-dependent apoptosis in human prostate epithelial cells. *PLoS One* 7, e33647
118. Lee, S.W. *et al.* (2023) ATF6 is a critical regulator of cadmium-mediated apoptosis in spermatocytes. *Toxicol. Sci.* 194, 167–177
119. Chung, Y.-P. *et al.* (2019) Methylmercury exposure induces ROS/Akt inactivation-triggered endoplasmic reticulum stress-regulated neuronal cell apoptosis. *Toxicology* 425, 152245
120. Juan, W.-S. *et al.* (2023) Arsenic compounds activate MAPK and inhibit Akt pathways to induce apoptosis in MA-10 mouse Leydig tumor cells. *Cancer Med.* 12, 3260–3275
121. Keshavarz-Bahaghighat, H. *et al.* (2018) Acetyl-L-carnitine attenuates arsenic-induced oxidative stress and hippocampal mitochondrial dysfunction. *Biol. Trace Elem. Res.* 184, 422–435
122. Liu, Y. *et al.* (2022) Investigation of nickel sulfate-induced cytotoxicity and underlying toxicological mechanisms in human umbilical vein endothelial cells through oxidative stress, inflammation, apoptosis, and MAPK signaling pathways. *Environ. Toxicol.* 37, 2058–2071
123. Mousa, A.M. *et al.* (2015) Gestational lead exposure induces developmental abnormalities and up-regulates apoptosis of fetal cerebellar cells in rats. *Drug Chem. Toxicol.* 38, 73–83
124. Xu, J. *et al.* (2006) Lead-induced apoptosis in PC 12 cells: involvement of p53, Bcl-2 family and caspase-3. *Toxicol. Lett.* 166, 160–167
125. Sharifi, A.M. *et al.* (2011) Investigating the effect of lead acetate on rat bone marrow-derived mesenchymal stem cells toxicity: role of apoptosis. *Toxicol. Mech. Methods* 21, 225–230
126. Yang, W. *et al.* (2009) Paraquat activates the IRE1/ASK1/JNK cascade associated with apoptosis in human neuroblastoma SH-SY5Y cells. *Toxicol. Lett.* 191, 203–210
127. Zucchini-Pascal, N. *et al.* (2009) Lindane and cell death: at the crossroads between apoptosis, necrosis and autophagy. *Toxicology* 256, 32–41
128. Ki, Y.-W. *et al.* (2013) JNK and p38 MAPK regulate oxidative stress and the inflammatory response in chlorpyrifos-induced apoptosis. *Toxicol. Lett.* 218, 235–245
129. Cao, J. *et al.* (2016) Overproduction of reactive oxygen species and activation of MAPKs are involved in apoptosis induced by PM2.5 in rat cardiac H9c2 cells. *J. Appl. Toxicol.* 36, 609–617
130. Li, L. *et al.* (2018) Silver nanoparticles induce SH-SY5Y cell apoptosis via endoplasmic reticulum and mitochondrial pathways that lengthen endoplasmic reticulum–mitochondria contact sites and alter inositol-3-phosphate receptor function. *Toxicol. Lett.* 285, 156–167
131. Hong, F. *et al.* (2016) TiO2 nanoparticles-induced apoptosis of primary cultured Sertoli cells of mice. *J. Biomed. Mater. Res. Part A* 104, 124–135
132. Wang, L. *et al.* (2018) Zinc oxide nanoparticles induce murine photoreceptor cell death via mitochondria-related signaling pathway. *Artif. Cells Nanomed. Biotechnol.* 46, 1102–1113
133. Liu, Z. *et al.* (2014) Carboxylation of multiwalled carbon nanotube enhanced its biocompatibility with L02 cells through decreased activation of mitochondrial apoptotic pathway. *J. Biomed. Mater. Res. Part A* 102, 665–673
134. Guo, C. *et al.* (2016) Amorphous silica nanoparticles trigger vascular endothelial cell injury through apoptosis and autophagy via reactive oxygen species-mediated MAPK/Bcl-2 and PI3K/Akt/mTOR signaling. *Int. J. Nanomedicine* 11, 5257–5276
135. Zhong, G. *et al.* (2022) Combined effect of arsenic and polystyrene-nanoplastics at environmentally relevant concentrations in mice liver: activation of apoptosis, pyroptosis and excessive autophagy. *Chemosphere* 300, 134566
136. Khan, S. *et al.* (2019) A selective BCL-XL PROTAC degrader achieves safe and potent antitumor activity. *Nat. Med.* 25, 1938–1947
137. Qin, X. *et al.* (2023) Apoptosis inducing agent 1 enhances cancer therapy-induced apoptosis by direct interaction with BAX and BAK. *Cancer Res.* 83, 5731 (Abstract 5731)
138. Park, D. *et al.* (2021) Discovery of small molecule Bak activator for lung cancer therapy. *Theranostics* 11, 8500–8516
139. Sekar, G. *et al.* (2022) Small molecule SJ572946 activates BAK to initiate apoptosis. *iScience* 25, 105064
140. Garner, T.P. *et al.* (2019) Small-molecule allosteric inhibitors of BAX. *Nat. Chem. Biol.* 15, 322–330
141. Dhani, S. *et al.* (2021) A long way to go: caspase inhibitors in clinical use. *Cell Death Dis.* 12, 949