[Cancer Biology & Therapy 4:2, e50-e74, EPUB Ahead of Print, http://www.landesbioscience.com/journals/cbt/abstract.php?id=1508, February 2005]; ©2005 Landes Bioscience

Review

Overview of Cell Death Signaling Pathways

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Received 12/23/04; Accepted 12/27/04

This manuscript has been published online, prior to printing, for *Cancer Biology & Therapy* Volume 4, Issue 2. Definitive page numbers have not been assigned. The current citation for this manuscript is: Cancer Biol Ther 2005; 4(2): http://www.landesbioscience.com/journals/cbt/abstract.php?id=1508
Once the issue is complete and page numbers have been assigned, the citation will change accordingly.

KEY WORDS

apoptosis, death receptor, mitochondria, caspase, cancer

ABSTRACT

Apoptosis plays an important role in development and maintenance of tissue homeostasis. Intensive efforts have been made to explore the molecular mechanisms of the apoptotic signaling pathways including the initiation, mediation, execution and regulation of apoptosis. Caspases are central effectors of apoptosis. Cells undergo apoptosis through two major pathways, namely the extrinsic pathway (death receptor pathway) or the intrinsic pathway (the mitochondrial pathway). Finally, the contents of dead cells are packaged into apoptotic bodies, which are recognized by neighboring cells or macrophages and cleared by phagocytosis. Cellular apoptosis is tightly controlled by a complex regulatory networks including balancing pro-survival signals. De-regulation of apoptosis may lead to pathological disorders such as developmental defects, autoimmune diseases, neurodegeneration or cancer. Increasing attention is being focused on alternative signaling pathways leading to cell death including necrosis, autophagy, and mitotic catastrophe. Understanding of cell death signaling pathways is relevant to understanding cancer and to developing more effective therapeutics.

OVERVIEW OF APOPTOSIS

The maintenance of cellular homeostasis is fundamental for tissue integrity in multicellular organisms. Apoptosis is a highly conserved mechanism that has evolved to maintain cell numbers and cellular positioning within tissues comprised of different cell compartments. Programmed cell death (apoptosis) was first described in 1972 by Currie and colleagues.[1] It is a common type of cell death associated morphological features that had been repeatedly observed in various tissues and cell types.

Apoptosis is an essential part of life for multicellular organisms that plays an important role in development and tissue homeostasis.[2] During development many cells are produced in excess which eventually undergo programmed cell death and thereby contribute to sculpturing organs and tissues.[3] Apoptosis is delicately regulated and balanced in a physiological context. Failure of this regulation results in pathological conditions such as developmental defects, autoimmune diseases, neurodegeneration or cancer.[4]

Characteristic apoptotic features include cell membrane blebbing, cell shrinkage, chromatin condensation and DNA fragmentation, finally ending with the engulfment by macrophages or neighboring cells, thereby avoiding an inflammatory response in surrounding tissues.[5] Apoptosis is distinct from necrosis in which the cells suffer a major insult, leading to a loss of membrane integrity, swelling and disruption of the cells. During necrosis, the cellular contents are released uncontrolled into the cell's environment which results in damage of surrounding cells and a strong inflammatory response in the corresponding tissue.[6]

Intensive effort has been made to explore the molecular mechanisms of the apoptotic signaling pathways including the initiation, mediation, execution, and regulation of apoptosis. The original understanding of cell death has come from genetic studies in the nematode C. elegans. One hundred thirty-one of the initial 1090 somatic cells generated during development are finally eliminated by apoptosis. Several genes have been identified that function in apoptotic killing in *C. elegans*. These cell deaths rely on the presence of CED-3 (caspase homologue), and CED-4 (Apaf-1 homologue) that binds to and activates CED-3. In healthy cells, CED-4 remains inactive by its association with CED-9 (anti-apoptotic Bcl-2 homologue). The protein EGL-1 (BH3 only member homologue) is a trigger of cell death and is expressed in response to certain developmental cues. EGL-1 binds to CED-9, displacing CED-4, which in turn activates CED-3 to induce apoptosis.[7] *C. elegans* as a model system contains the basic components of the cell death machinery. Activation and regulation of apoptosis in higher organisms depends on corresponding

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analogous components found in *C.elegans* with but more complexity (Fig. 1).

In mammals, a wide array of external signals may trigger two major apoptotic pathways, namely the extrinsic pathway (death receptor pathway) or the intrinsic pathway (the mitochondrial pathway) within a cell. The extrinsic pathway is activated by apoptotic stimuli comprising extrinsic signals such as the binding of death inducing ligands to cell surface receptors. In other cases, apoptosis is initiated following intrinsic signals including DNA damage induced by irradiation or chemicals, growth factor deprivation or oxidative stress. In general intrinsic signals initiate apoptosis via the involvement of the mitochondria.[8]

The extrinsic pathway is mediated by cell surface death receptors, such as Fas, tumor necrosis factor receptor, or TRAIL receptors. Death ligand stimulation results in oligomerization of the receptors and recruitment of the adaptor protein Fas-associated death domain (FADD) and caspase-8, forming a death-inducing signaling complex (DISC). Autoactivation of caspase-8 at the DISC is followed by activation of effector caspases, including caspase-3, -6 and -7, which function as downstream effectors of the cell death program.[9] The intrinsic pathway is mediated by diverse apoptotic stimuli, which converge at the mitochondria. Release of cytochrome c from the mitochondria to the cytoplasm initiates a caspase cascade. Cytosolic cytochrome c binds to apoptosis protease-activating factor 1 (Apaf-1) and procaspase-9, generating an intracellular DISC-like complex known as "apoptosome". Within the apoptosome, caspase-9 is activated, leading to processing of caspase-3.[10] The two pathways of apoptosis, extrinsic/death receptor and intrinsic/mitochondrial, converge on caspase-3 and subsequently on other proteases and nucleases that drive the terminal events of programmed cell death.

The last steps of apoptosis include packaging of cell content into apoptotic bodies and phagocytosis. The recognition of apoptotic cells by neighboring cells or macrophages has been intensively studied. Apoptotic cells give "come and eat me" signals in due time. One of these signals is Phosphatidylserine. Phosphatidylserine is normally present on the cytoplasmic side of the cell membrane, but exposure to the external surface of the cell membrane occurs when a cell undergoes apoptosis. This event triggers the phagocytosis reaction.[5]

Cellular apoptosis is tightly controlled by a complex regulatory network. Pro-survival signals enhance the expression and/or activity of anti-apoptotic regulatory molecules thereby keeping in check the activation of pro-apoptotic factors. A set of various anti-apoptotic molecules and mechanisms has been identified, such as NF-κB, AKT, Bcl-2, and the IAP family of proteins. Every step in the apoptotic cascade is monitored and controlled by certain pro-survival signals.[11] Pro-apoptotic factors can counteract those inhibitory molecules when apoptotic demise of a cell is timely and imperative.

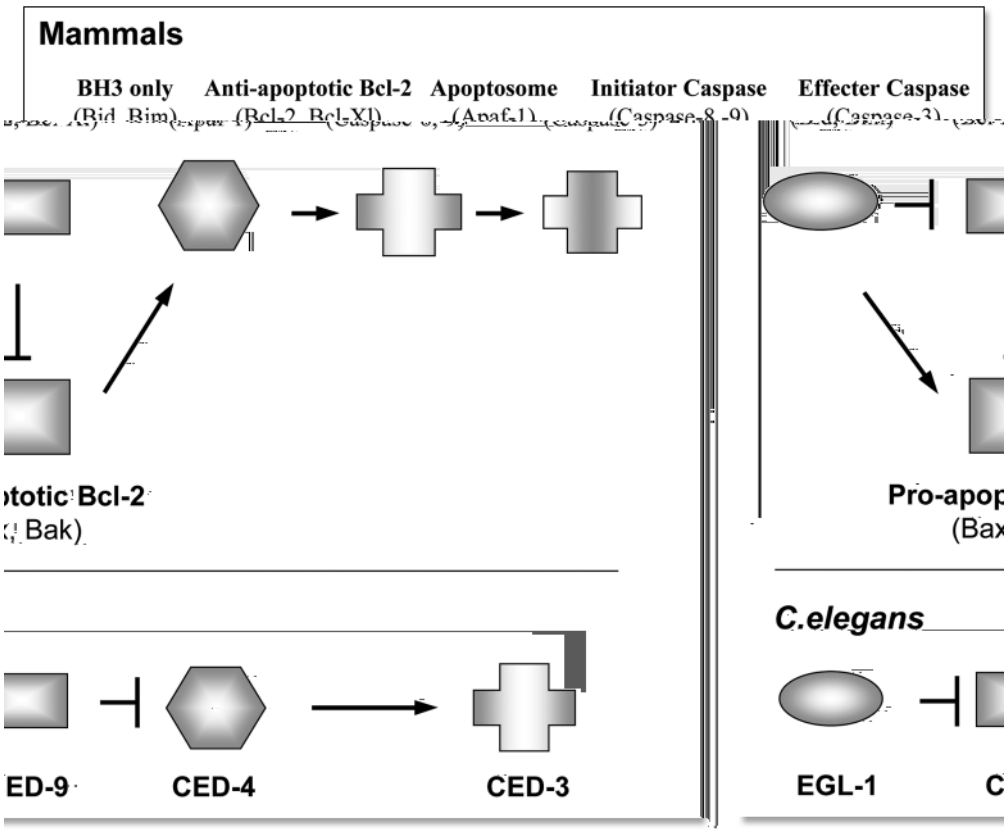


Figure 1. Comparison of mammals to the *C. Elegans* system. See text for details.

Because deregulated apoptosis lies at the heart of development of many diseases including cancer, it presents an attractive target for therapeutic intervention.

CASPASES ARE CENTRAL INITIATORS AND EXECUTIONERS OF APOPTOSIS

General features and classification of caspases

Caspases are a family of proteins that are one of the main effectors of apoptosis. Their activation is a hallmark of apoptosis. The study of caspases was originated from the discovery that the *C. elegans ced-3* gene encodes a homologue of the interleukin-1β processing enzyme (ICE).[12] This protein was then shown to be sufficient to induce apoptosis when overexpressed in mammalian cells.[13] A number of proteins possessing similar features were cloned subsequently, which were termed caspases family.

To date, about 14 mammalian caspases have been identified. All caspases share a number of common features. Caspases are synthesized as inactive zymogens containing a prodomain followed by p20 (large) and p10 (small) subunits. These zymogens can be cleaved to form active enzymes following the induction of apoptosis. All caspases can cleave substrates at Asp-Xxx bond, which is unique among mammalian proteases, except for the serine protease granzyme B. The caspases comprise the catalytic triad composed of Cys285, His237 and a carbonyl group at residue 177.[14]

Based on their function, the caspases can be classified into three groups. (1) Inflammatory caspases. This group includes caspase-1, -4, -5, -11, -12, -13 and -14, which are involved in inflammation instead of apoptosis. (2) Apoptotic initiator caspases. Initiator caspases possess long prodomains containing either a death effector domain

of caspase-8 and -10 mediate their homophilic interactions with CARD- or DED-containing adaptor proteins (e.g., Apaf-1 or FADD).[18] Genetic evidence from knockout mice indicates that caspase-8 is required for all known death receptor- mediated apoptotic pathways whereas caspase-9 is mostly involved in mitochondria-mediated apoptotic pathways.[19-21]

(DED) (caspase-8 and -10) or a caspase activation and recruitment domain (CARD) (caspase-2, -9), which mediate the interaction with upstream adaptor molecules. (3) apoptotic effector caspases. This executioner class (caspase-3, -6, -7) is characterized by the presence of a short prodomain (Fig. 2). They are typically processed and activated by upstream caspases and perform the downstream execution steps of apoptosis by cleaving multiple cellular substrates.[15]

Caspase activation

Initiator and effector caspases are activated by different mechanisms. The apoptotic signaling pathways that lead to caspase zymogens processing can be subdivided into two major categories: death receptor-mediated and mitochondria-mediated pathways. These apoptotic signals trigger oligomerization of death adaptor proteins (e.g., Apaf-1, or FADD); death adaptor oligomers, in turn, induce the aggregation of pro-caspases. It was previously believed that the initiator caspases are autoproteolytically activated when brought into close proximity of each other, which is called the 'induced proximity' model.[9] This model was further refined by the proximity-induced dimerization model. Based on this model, the homo-dimerization of caspase-9 is promoted by the apoptosome due to its increased local concentration. Similarly, the DISC induces dimerization and subsequent auto-activation of caspase-8.[16,17] The productive conformation of the active site in initiator caspases may be stabilized in these complexes. The adaptor-driven activation of the caspases depends on conserved motifs within their long prodomain. The caspase activation and recruitment domain (CARD) of caspase-9 and -2 and the death effector domain (DED)

than 100 substrates have been identified thus far. Based on the analyses of their cellular function, the caspase targets can be subdivided into six major categories: (1) mediators and regulators of apoptosis, (2) structural proteins, (3) cellular DNA repair proteins, (4) cell cycle-related proteins.[15]

Apoptotic proteins. Effector caspases are the most obvious examples of apoptotic factors targeted by initiator caspases (see above). In addition, the BH3-only protein, Bid is another notable substrate of an initiator caspase, namely caspase-8. Bid is cleaved by caspase-8 following death receptor signaling. Cleavage of Bid by caspase-8 facilitates its subsequent myristoylation, which results in its activation and mitochondrial translocation. The identification of Bid as a caspase-8 substrate established a link between the extrinsic pathway and the intrinsic pathway.[29,30]

Caspase-3 is a predominant effector to cleave the signaling components that effect morphologic changes associated with apoptosis. However, due to some similarity in their substrate specificity, caspase-6 and -7 can in part compensate for the loss of caspase-3 in term of apoptotic execution.[26] DNA fragmentation factor 45 kD subunit (DFF45/ICAD) is a major target of caspases. Caspase cleavage removes the N-terminal CIDE-N interaction domain, abrogating DFF45/ICAD-mediated suppression of the catalytic DFF40/CAD subunit, which plays a critical role in the internucleosomal DNA degradation characteristic of apoptosis.[31,32] NDUFS1, the 75 kD subunit of respiratory complex I, is another critical caspase substrate in the mitochondria. Caspase cleavage of NDUFS1 is related to several mitochondrial changes associated with apoptosis, such as loss of mitochondrial transmembrane potential, production of reactive oxygen species (ROS), and disruption of electron transport.[33]

Some anti-apoptotic proteins, such as Bcl-2 and Bcl-Xl, IAPs and $FLIP_L$, are also cleaved by caspases, but it is not currently clear whether this cleavage represents a ubiquitous positive feedback mechanism or a cell type or stimulus-specific event.[15]

Caspases also cleave some protein kinases, which play either pro-apoptotic or anti-apoptotic roles. The most notable anti-apoptotic kinase targets of caspases include AKT, FAK and RIP. AKT is cleaved following matrix detachment-induced apoptosis in epithelial cells.[34] RIP kinase, the cell signaling component of the DISC complex, is also cleaved by caspase-8, causing the suppression of anti-apoptotic RIP-mediated NF-κB induction.[35] FAK kinases transduce cell survival signals from the extracellular matrix through Raf-1. FAK is cleaved and inactivated during apoptosis induced by multiple stimuli, such as TRAIL, which contributes both to the loss of survival signals and matrix detachment.[36] The activation of transcription factor NF-κB is an important pro-survival mechanism. The p65RelA subunit of NF-κB and upstream activating kinase IKKβ may be inhibited through caspase-dependent cleavage, thereby facilitating apoptosis.[37,38]

A number of pro-apoptotic kinases were reported to be cleaved and activated by caspases, such as ROCK1 kinase, PAK2 (p21-activated kinase 2 and MEKK1). ROCK1 kinase was recently reported to be cleaved and activated in response to TNF and this event was shown to be both necessary and sufficient for apoptotic membrane blebbing.[39] A constitutively active form of PAK2 is generated by caspase-3-mediated cleavage, which contributes to the formation of apoptotic bodies.[40] MEKK1 can be activated during genotoxic stress and Fas-induced apoptosis. Apoptotic MEKK1 signaling involves JNK activation.[41]

Structural proteins. Caspase-mediated cleavage of specific substrates also explains several other characteristic features of apoptosis. Cleavage of fodrin and gelsolin leads to disruption of the actin filament network, which may specifically contribute to loss of overall cell shape and detaching from the matrix during apoptosis.[42] Cleavage of the nuclear lamins is required for nuclear shrinkage and budding.[43] A number of intermediate filament proteins (keratins 18 and 19, vimentin) are also cleaved by caspases.[44] Caspase cleavage of the adherence junction proteins β-catenin and plakoglobin γ-catenin results in the disruption of cell-cell interactions, which may attenuate their pro-survival signaling.[45] Cellular architecture is dismantled due to these cleavages by caspases. This process is critical for the completion of apoptosis.

Cellular DNA repair proteins. DNA repair is an energy-demanding process and may compete for valuable cellular assets, such as ATP, which is also an indispensable resource for the execution of apoptosis. The shutdown of cellular repair may facilitate the execution of apoptosis. In addition, DNA repair typically triggers cell cycle arrest, whereas apoptosis is normally not very active under this condition. DNA repair proteins including DNA- dependent protein kinase (DNA-PK), Rad51 and ATM proteins are substrates of caspases during apoptosis.[46] The significance of cellular energy balance for apoptosis execution is well illustrated by the studies of another DNA repair enzyme: poly(ADP-ribose) polymerase (PARP). PARP is activated by DNA breaks and catalyzes the attachment of ADP-ribose polymers to multiple nuclear factors, facilitating repair. When activated, this DNA repair process consumes large amounts of NAD^+, thereby indirectly depleting the cellular ATP store. Since the loss of cellular ATP was demonstrated to abrogate apoptosis, resulting in the induction of necrosis,[47] this may explain the observed caspase-mediated cleavage and inactivation of PARP during apoptosis.

Cell cycle proteins. A number of cell cycle inhibitory factors are cleaved during apoptosis. Those include Cdc27, Wee1, and two Cdk inhibitors, $p21^{CIP1}$ and $p27^{KIP1}$.[48,49] In addition, death receptor signaling was found to cause caspase-dependent inactivation of another proliferation inhibitor, Rb.[50] Induction of apoptosis appears to activate multiple cell cycle-associated factors through cleavage and inactivation of their inhibitors.

Cleavage of cell cycle regulators may promote apoptotic signaling by multiple means as illustrated by recent studies. Loss of Rb was found to promote accumulation of procaspases.[51] In another example, Cdc2 was shown to phosphorylate the pro-apoptotic Bcl-2 family member Bad at a site which inhibits its interaction with AKT and thereby activates its proapoptotic activity.[52] The cleavage of cell cycle regulators may have evolved as a specific tumor suppressor mechanism aimed at ensuring induction of cell death in response to improper cell cycle signaling by oncogenes.

Regulation of caspases

Although proteolytic activation of caspases is a major way to regulate caspase activity, other regulatory pathways also exist. Apoptosis is generally not dependent on protein synthesis, but regulation of gene expression of caspases can modulate the sensitivity of cells to apoptosis. Deregulation of E2F by adenovirus E1A, loss of Rb or enforced E2F-1 expression lead to the accumulation of caspase proenzymes, which potentiates p53-mediated apoptosis.[51] Caspase-6 and caspase-10 also have been identified as a transcriptional target of p53.[53,54] Some post-translational modification, such as nitrosylation, oxidation, ubiquitination and phosphorylation, also play roles in regulation of caspases activity. For example, mouse caspase-9 can be inactivated by AKT through phosphorylation.[55] A family of apoptotic inhibitors, CARPs, was recently isolated that bind to and

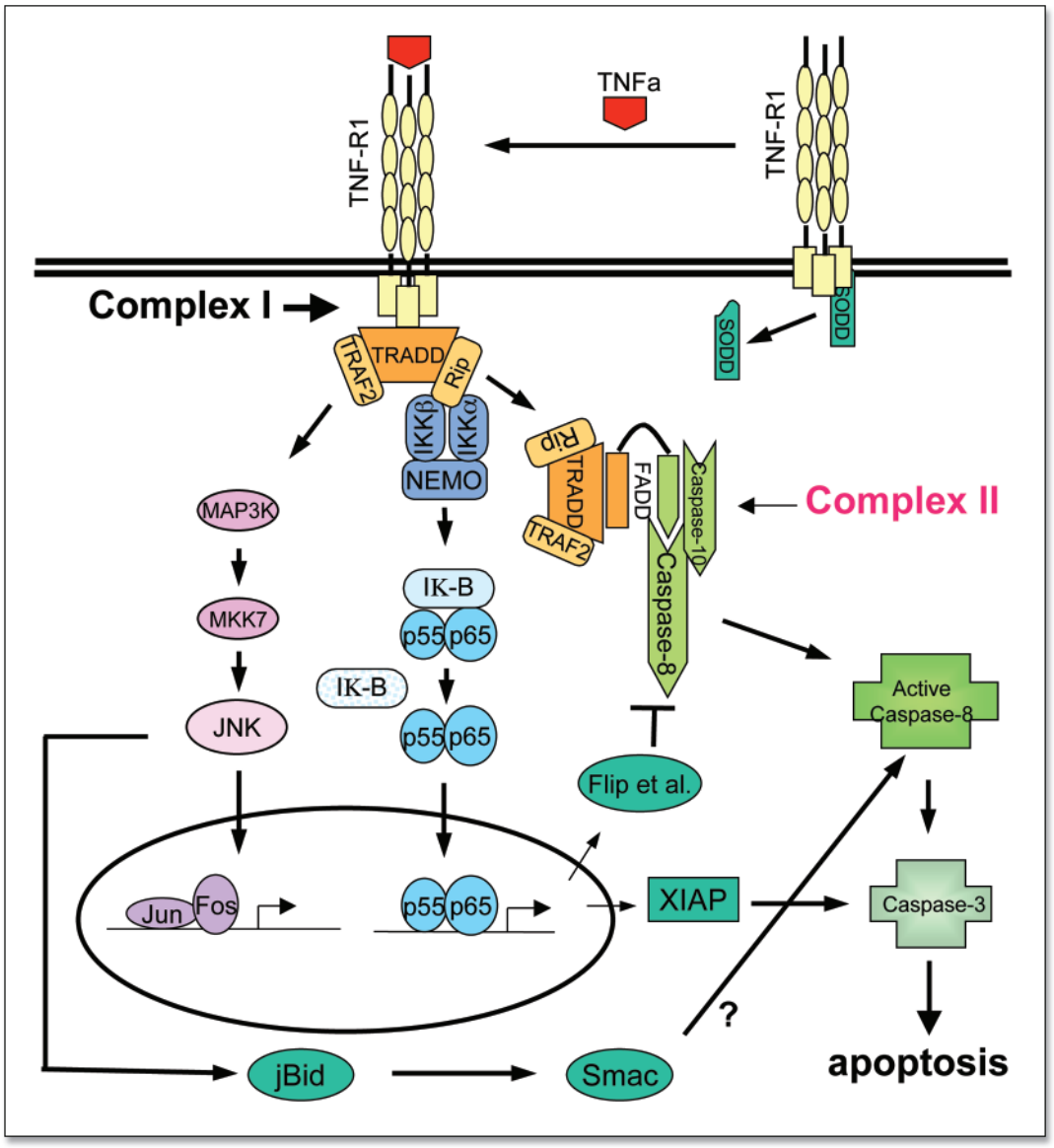


Figure 3. TNF signaling pathway (extrinsic pathway). See text for details.

negatively regulate DED caspases through the ubiquitin-mediated proteolysis of DED caspases.[56]

The potent proapoptotic activity of caspases has to be kept in check in order for healthy cells to survive. Several important negative regulators of caspases have been identified in virus and human. Some of them are exploited by virus to escape from host defense through inhibiting apoptosis, such as crmA, P35, v-FLIP and IAPs.[57]

IAPs may be the most important negative regulators of caspases. To date, eight human IAPs have been identified: including XIAP, c-IAP1, c-IAP2 and Survivin. Most human IAP family members share common features including the Baculovirus IAP repeat (BIR) domain located at the amino-terminus and a RING finger domain at the carboxy-terminus. IAP family proteins are able to inhibit apoptosis induced by a variety of stimuli including death ligands via direct binding and inhibition of certain caspases.[58] Among the human IAPs, XIAP is the most powerful caspase inhibitor and is also the best characterized. XIAP contains three BIR domains.[59] Regions closely related to the third BIR domain (BIR3) specifically act on caspase 9, while the linker region close to BIR 2 was shown to target caspases 3 and 7.[60,61] In addition, although most IAPs bind and directly suppress caspase catalytic activity, some of them function to downregulate caspase expression by acting as E3 ligases for their ubiquitination and degradation.[62,63]

The activity of IAPs is finely regulated during apoptosis. XIAP and cIAP-1 have been shown to undergo specific and functional cleavage by caspases to ensure the induction of apoptosis.[64] The second mitochondria-derived activator of caspases (Smac) and Omi/Htra2 are released from the mitochondria upon apoptotic stimuli, and promote caspase activation by inhibition of IAPs.[62,65] Recently, Smac was also identified as a substrate for the E3-ligase activity of XIAP and Smac/Diablo potentiates apoptosis by repressing IAP ubiquitin ligase activities besides antagonizing caspase-IAP interactions.[66]

EXTRINSIC APOPTOTIC PATHWAYS

General features of the extrinsic pathway

The extrinsic pathway is activated by ligand-bound death receptors mainly including TNF-TNFR1, FasL-Fas and TRAIL-DR4 or -DR5. Members of the TNF ligand family are primarily produced as type II transmembrane proteins arranged in stable homotrimers. They exert their biological functions via interaction with their cognate membrane receptors. Death receptors belong to the tumor necrosis factor receptor (TNFR) gene superfamily and generally can have several functions including initiating apoptosis.[67] The TNF receptor (TNFR) superfamily is characterized by the presence of cysteine rich domains (CRDs) that mediate binding between ligands and these type I transmembrane domain receptors. Among them, the death receptors including TNFR1 (TNF receptor-1), CD95 (or Fas) and the TRAIL receptors DR4 and DR5 are best characterized for induction of apoptosis. A soluble Fas decoy receptor, DcR3, and three decoys for TRAIL, DcR1, DcR2 and OPG were also identified. Due to lack of a functional death domain, these decoy receptors are unable to elicit the activation of the downstream apoptotic signaling pathway. However they can compete for the binding of death ligands to block the apoptosis triggered by death receptors.[9] Preassembly or self-association of death receptors through a distinct functional domain in

the death receptors extracellular domain, termed the preligand assembly domain (PLAD), is critical for ligand binding.[68] Ligand binding to the preformed TNF-R complex either induces an activating conformational change or allows the formation of higher-order receptor complexes to acquire signal competence. Ligation of death receptors with ligands results in the formation of a DISC that recruits the corresponding adaptors through the death domain. Death receptors and adaptors become activated in the DISC so that they can bind the effector molecules such as procaspase-8 to induce the activation of the downstream apoptotic signaling pathway.[69] The aggregation of the receptors is also followed by endocytosis into an endosomal pathway. Inhibition of this internalization either chemically or by mutation may abrogate the biological response.[70]

TNF pathway

Tumor necrosis factor is a multifunctional pro-inflammatory cytokine mainly produced by macrophages. TNF can elicit a broad spectrum of biologic responses. TNF is involved in the progression of many diseases including autoimmune disease, cancer and neurodegenerative disease. TNF-induced activation of NF-κB, JNK and apoptosis has been intensively studied. There are two major TNF receptors, TNF-R1 and TNF-R2. TNF-R1 is ubiquitously expressed in most tissues and is the major mediator of TNF signaling, whereas TNF-R2 is mainly found in the immune system and only can be fully activated by membrane bound TNF, but not by soluble TNF.[71]

The ability of preassembled TNF-R1 complexes to signal is masked by binding of the silencer of death domain (SODD) in the un-induced condition.[72] After TNF binding, SODD is released from TNF-R1 complexes and the death domain-containing adaptor protein TRADD is recruited to the death domain of TNF-R1 by homophilic interactions of the death domains.[73] It is generally believed that TNF-R1-bound TRADD then serves as a common assembly platform for binding of TNF receptor-associated factor (TRAF) 2 and the death domain-containing serine-threonine kinase RIP (receptor-interacting kinase). TRAF2 is a member of the phylogenetically conserved TRAF protein family. The association of TRAF2 to TNF-R1-bound TRADD is mediated by the interaction of its carboxy-terminal TRAF domain with the amino-terminal death domain of TRADD. In contrast, RIP is recruited to the DD of TNF-R1-bound TRADD via its carboxy-terminal death domain. RIP is also able to interact with TRAF2 via its amino-terminal kinase domain and its central intermediate domain.[74] However, studies with TRAF2- and RIP-deficient mouse embryonic fibroblasts have shown that both molecules can be competitively recruited into the TNF-R1 signaling complex.[75]

TNF activates NF-κB through ubiquitin-mediated degradation of its inhibitor, IκB. This process is triggered by phosphorylation of IκB by the IκB kinase (IKK) complex. TRAF2 can recruit the IKK complex to the TNF-R1 signaling complex, whereas RIP seems to stabilize the IKK complex.[75] The IKK complex comprises a heteromer of two related I-κB kinases, called IKK1 and IKK2 (IKKα and IKKβ), the regulatory protein NEMO (Fip-3, IKKγ, IKKAP) and a homodimer of the heat shock protein-90 (Hsp90), as well as two or three molecules of the Hsp90-asscociated cdc37 protein. In an un-induced state, cellular I-κB proteins interact with NF-κB dimers to retain it in the cytoplasm. The IKK complex is activated by binding to the TNF-R1 signaling complex. The activated IKK complex then phosphorylates and targets I-κB for proteolytic degradation by the proteasome. This finally results in the liberation of NF-κB and allows it to translocate to the nucleus where it can activate the genes involved in different cell functions.[76]

TNF also induces the activation of the stress-activated protein kinase (SAPK)/c-Jun N-terminal kinase (JNK) pathway. TNF-induced activation of the JNK pathway occurs through a TRADD-TRAF2 axis. TRAF2 knockout mice showed a significantly impaired JNK activation after TNF treatment.[77] Analyses of MKK7- and MKK4-deficient mouse embryonic fibroblasts suggest that MKK7 is essentially involved in TNF-induced JNK activation.[78] Upon activation, JNK kinases translocate into the nucleus and enhance the transcriptional activity of transcription factors, for example, c-Jun and ATF2, by phosphorylation of their amino-terminal activation domains. c-Jun belongs to a group of basic region-leucine zipper proteins that dimerize to form transcription factors commonly designated as activator protein-1 (AP-1). The AP-1 proteins have an important role in a variety of cellular processes including proliferation, differentiation and induction, as well as prevention of apoptosis.[79]

Unlike Fas and TRAIL signaling, TNF does not induce cell death spontaneously. Activation of NF-κB by TNF mediates a strong pro-survival signaling pathway. The NF-κB pathway targets several anti-apoptotic factors, including cFLIP, IEX-1L, Bfl-1/A1, XIAP, cIAP1, cIAP2, and Bcl-Xl.[71,80] The cytotoxic effect of TNF is only manifested when NF-κB activation is blocked, either by inhibition of protein synthesis or overexpression of dominant negative mutant IκB.[81] Mice deficient in p65 or other components involved in TNF-induced NF-κB activation are embryonically lethal or die early after birth because of massive TNF-dependent liver failure.[82] Thus, the death-inducing capability of TNF is masked in vivo by concomitant activation of NF-κB. Although other death receptors (TRAIL-R or FAS) are also able to activate the NF-κB pathway, they show prominent apoptotic functions in vivo.[76] Genetic evidence from knock-out mice and mutagenized cell lines supports the notion that all death receptors investigated so far critically depend on the death domain-containing adaptor protein FADD and caspase-8 to induce cell death.[83,84] However, in contrast to Fas and TRAIL death receptors, TNF-R1 is indirectly linked to FADD, namely by TRADD which is also responsible for bridging TNF-R1 to TRAF2 and the IKK complex. The TNF-R1-IKK signaling complex could be immunoprecipitated in the DISC of TNF-treated cells, but a TNF-R1-DISC containing FADD and caspase-8 could not be isolated under same conditions.[85] Recent studies have shown that TNFR1-induced apoptosis involves 2 sequential signaling complexes. Complex I, the initial plasma membrane-bound complex, consists of TNFR1, the adaptor TRADD, the kinase RIP1, and TRAF2 and rapidly signals activation of NF-κB or JNK. In a second step, TRADD and RIP1 associate with FADD and caspase-8, forming a cytoplasmic complex, complex II. When NF-κB is activated by complex I, complex II harbors the caspase-8 inhibitor FLIP-L and the cell survives. Otherwise, cells undergo apoptosis through a complex II-mediated signaling pathway. Thus, TNFR1-mediated signal transduction includes a checkpoint, resulting in cell death (via complex II) in instances where the initial signal (via complex I and NF-κB) fails to be activated (Fig. 3).[86]

TNFR1 is also able to mediate apoptosis through the recruitment of an adaptor molecule called RAIDD (RIP-associated ICH-1/CED-3 homologous protein with a death domain). RAIDD associates with RIP through interactions between death domains and can recruit caspase 2 through an interaction with a motif, similar to the death effector domain, known as the CARD (caspase recruitment domain). Recruitment of caspase 2 leads to induction of apoptosis.[87] However, very few follow-up studies indicate an essential role for RAIDD or caspase-2 in TNF-induced apoptosis. The C-terminal

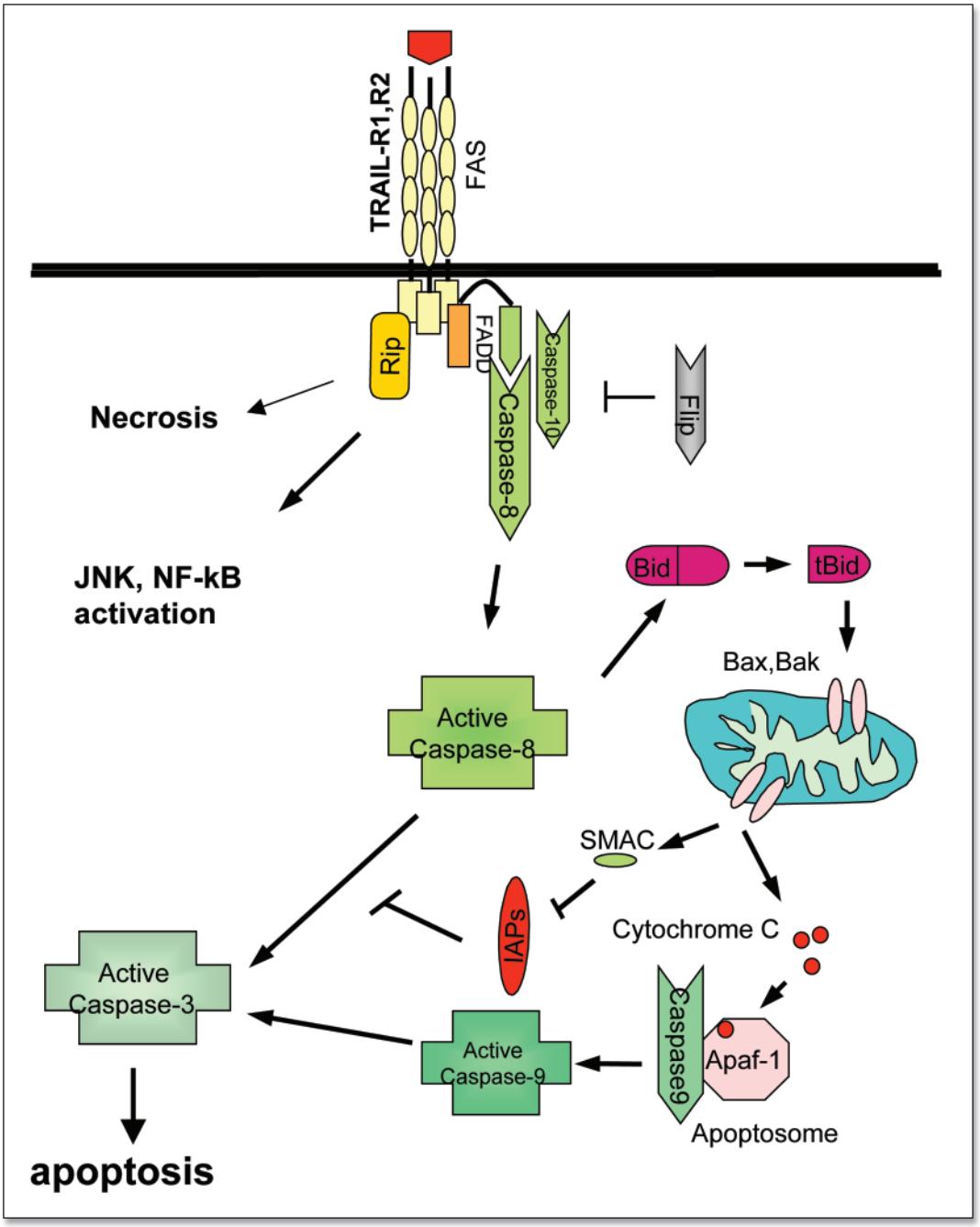


Figure 4. FAS and TRAIL signaling pathway (extrinsic pathway). See text for details.

fragment of RIP also enhances the association between TNFR1 and death domain proteins including TNFR1-associated death domain (TRADD) and Fas-associated death domain (FADD), resulting in the activation of caspase-8 and stimulation of apoptosis.[88] Necrosis can be induced by death receptors including TNF-R1 by a RIP-dependent pathway. In contrast to its role in the NF-κB pathway, the role of RIP in induction of necrosis is dependent on the kinase activity of the molecule.[89]

The role of JNK in TNF function, especially TNF-mediated apoptosis, is still poorly understood. While mouse embryonic fibroblasts of JNK1$^{-/-}$ and JNK2$^{-/-}$ mice show increased sensitivity against TNF-induced apoptosis,[90] mouse embryonic fibroblasts of mice deficient for ASK1 that is implicated in TNF-R1-mediated JNK activation are significantly protected against TNF-induced cell death.[78] This phenotype may be due to a reduction in apoptosis-related delayed JNK activation in ASK1-deficient MEFs. Moreover, studies in other cell types using inhibitors of the JNK pathway also revealed a pro-apoptotic function of the JNK pathway in TNF-induced cell death.[91] A recent study suggests that JNK may play an essential positive role in TNF-induced apoptosis.[92] In these studies, activation of the MKK7/JNK pathway by TNF results in the unique processing product of Bid (termed jBid), which selectively triggers the release of Smac to induce activation of caspase-8. Knockdown of MKK7, Bid or Smac prevents TNF-induced caspase-8 processing and cell death. However, it was recently demonstrated that JNK plays opposite roles in TNF-induced apoptosis and necrosis with Mkk4$^{-/-}$ Mkk7$^{-/-}$, and Jnk1$^{-/-}$ Jnk2$^{-/-}$ MEFs. Although JNK inhibits TNF-stimulated apoptosis, it can actually prompt TNF-induced necrosis by increasing the production of reactive oxygen species (ROS).[93] Different roles of JNK in TNF-induced apoptosis could be partly related to cell type specific effects, but may also be dependent on the status of other pathways, such as NF-κB pathway.

ROS have also been implicated as mediators of TNF-induced cell death. Cytotoxicity caused by ROS is mediated in part by the JNK pathway. ROS accumulation in response to TNF is regulated in an NF-κB-dependent manner.[94] Recently ferritin heavy chain (FHC) has been identified as a pivotal mediator of the NF-κB protective activity against TNF -induced toxicity. It can block apoptosis in NF-κB/RelA null cells. The anti-apoptotic activity of FHC depends on its functions to inhibit induction of ROS and, thereby, activation of JNK by TNFα.[95]

Although the TNF apoptotic signaling pathway has been extensively studied, some questions are still unclear. For example, many

molecules seem involved in TNF-induced cell death, but how much do they contribute to TNF-induced cell death in certain conditions? How do they interact with each other? In addition, TNF plays important roles in tumorgenesis, but it is unclear how the TNF apoptotic signaling pathway is modified during tumorgenesis. Answers to these questions will help us better understand the TNF pathway and it's role in tumorgenesis.

FAS pathway

Fas has a central role in the physiological regulation of apoptosis and has been implicated in the pathogenesis of various malignancies as well as in diseases of the immune system. Fas is involved in cytotoxic T-cell mediated killing of cells (for example, CTL-mediated killing of virus-infected cells), destruction of inflammatory and immune cells in immune-privileged sites, deletion of self-reacting B cells and activated T-cells at the end of an immune response. Some types of tumors have been reported to express FAS-L, which may be a mechanism they have developed to evade attacking lymphocytes.[96]

In mice, mutations at the *lpr* (lymphoproliferation) locus produce a defect in the FAS antigen. A point mutation near the extracellular COOH-terminal domain of FAS-L gives rise to the *gld* phenotype.[97] Both mice fail to delete excess lymphocytes and display lymphoproliferative phenotype including lymphadenopathy and splenomegaly. This dysfunction also results in increased production of auto-antibodies, such as antibodies against DNA and rheumatoid factor. In human disease, mutations in FAS-L and FAS have been found in children with autoimmune lymphoproliferative syndrome (ALPS) or Canale–Smith syndrome.[98] In the *lpr* and *gld* mice, dysfunction of the FAS pathway also results in the accumulation and selection for cancerous cells. So death ligands and their receptors are regarded as candidate tumor suppressor genes.[99]

The death-inducing signaling complex (DISC) was first described in the FasL-Fas apoptotic signaling pathway.[100] Binding to Fas ligand promotes receptor trimerization that in turns results in intracellular clustering of death domains (DD) followed by its internalization into an endosomal pathway. This allows an adaptor protein called FADD (Fas-associated death domain) to associate with the receptor through an interaction between homologous death domains on both molecules. FADD also contains a death effector domain (DED), which allows binding of pro-caspase 8 to the CD95-FADD complex.[101] Pro-caspase 8 (also known as FLICE) associates with FADD through its own death effector domain. Caspase-8, the main initiator caspase in CD95 signaling, is expressed as two isoforms, caspase-8/a and -8/b, which are both recruited to the activated CD95 receptor.[102] These two molecules, FADD and caspase-8 are the key components of the CD95 DISC. FasL-induced clustering of Fas, FADD, and caspase-8 within the DISC leads to autoproteolytic processing of caspase-8 by induced proximity and dimerization, followed by the release of the processed active proteases. Following the autoproteolytic cleavage of the enzyme, caspase-8 is released from the DISC as an active heterotetramer containing two p18 and two p10 subunits (Fig. 4).[103] Recently one study showed that all cleavage products of procaspase-8 actually remain bound to the DISC, including the p10 and p18 subunits of caspase-8.[104] Studies in FADD and caspase-8 deficient mice indicated that both are required for FAS-mediated apoptosis.[19,83,105] Cells can be divided into two types according to their requirement for mitochondrial pathway in FAS-induced apoptosis. In type I cells, processed caspase-8 is sufficient to directly activate other members of the caspase family, whose action on defined substrates leads to the execution phase of apoptosis. In type II cells, efficient activation of effector caspases by Fas depends on an amplification loop that relies on caspase-8-mediated cleavage of Bid and subsequent release of mitochondrial pro-apoptotic factors such as SMAC/Diablo or cytochrome c to drive the formation of the caspase-9-activating apoptosome[106] Active caspase-9 activates the executioner caspase-3, which in turn activates caspase-8, thereby completing a positive feedback loop.[14]

Another DED-containing protein caspase-10 has also been shown to be recruited to the DISC.[107] Although in vitro study showed that caspase-10 shares similar features with caspase-8 in many aspects, in vivo study demonstrated that it can not substitute for caspase-8 in DISC to trigger apoptosis.[108] The reason why caspase-10 is associated with the DISC while not being essential for cell death signaling remains unclear. Another study has implicated the lack of functional caspase-10 in one form of the autoimmune disease ALPS type II.[109] This finding suggests that caspase-10 may indeed have a role in elimination of excessive lymphocytes.

FLIP, acting as the important regulator of death receptor-mediated apoptosis, has also been identified in the DISC. Two forms of FLIP were subsequently cloned,[110,111] termed c-FLIP$_S$ (short) and c-FLIP$_L$ (long). c-FLIP$_S$ contains tandem DEDs and is highly homologous to the N-terminus of caspase-8. c-FLIP$_L$ contains not only the tandem DEDs, but also a protease-like domain, homologous to caspase-8 although lacking enzymatic activity. Several amino acids of c-FLIP$_L$ important for protease activity including the cysteine at the active site are altered. The role of FLIP in death receptor-mediated apoptosis is still controversial. FLIP can be recruited to the DISC of death receptors, thereby disabling DISC-mediated processing and release of active caspase-8.[112] FLIP may also signal the death receptor-mediated NF-κB activation to inhibit cell death.[113] Knockdown of FLIP sensitizes tumor cells to the apoptotic effect of the extrinsic pathway. Embryonic fibroblasts (MEFs) derived from c-FLIP knockout mice were shown to be more sensitive to CD95-induced apoptosis as compared to the wild-type MEFs. These data suggest FLIP acts as an inhibitor of death receptor-mediated apoptosis. However, c-FLIP$^{-/-}$ mice showed developmental defects that strikingly resembled those of caspase-8$^{-/-}$ or FADD$^{-/-}$ mice.[114] Transfection of c-FLIP(L) at levels comparable to physiological levels enhances apoptosis by promoting procaspase-8 processing in the CD95 DISC, while a decrease of c-FLIP(L) expression results in inhibition of apoptosis.[115,116] These other lines of evidence imply that c-FLIP may be an activator of caspase-8.

There are several other proteins that have been shown to bind to the FAS DISC, including Daxx, FAP-1, RIP and FLASH. Daxx has been shown to link FAS to the JNK-signaling pathway, which may mediate apoptosis through an alternate pathway.[117] FAP-1 is a phosphatase that binds to the c-terminus of FAS to negatively regulate its signaling.[118] Rip contains a death domain and may mediate FAS-induced necrosis through its kinase domain.[89,119]

Activation of FAS can also result in a nonapoptotic response like cell proliferation and differentiation in normal human diploid fibroblasts and T cells. FAS-mediated proliferation appears to involve activation of FADD, caspase-8 or FLIP because mice deficient in these molecules have a defect in T cell proliferation.[71]

TRAIL pathway

The biological role of Apo2L/TRAIL is not fully understood. Like Fas, TRAIL may also be involved in the immune response and in tumor surveillance. Evidence has been shown for involvement of Apo2L/TRAIL in target-killing by cytotoxic CD4$^+$ T lymphocytes.[120]

Further data suggest that Apo2L/TRAIL plays a role in tumor cell killing by natural killer (NK) cells and macrophages.[120,121] Studies with Apo2L/TRAIL gene knockout mice confirm a role for Apo2L/TRAIL in anti-tumor immune surveillance by NK cells, specifically in host defense against tumor initiation and metastasis.[122,123] Roles for TRAIL-induced apoptotic signaling have been proposed for primary human blood, human monocytes, stem cell- or monocyte-derived dendritic cells.[120] The TRAIL effect may be tightly related to interferon. The expression of TRAIL on liver NK cells and their anti-metastatic potential was dependent on the presence of IFN-γ and IL-12, since these effects could not be observed in mice deficient for IFN-γ.[123,124] Additionally microarray study showed that gene profile regulated by TRAIL or interferon are similar.[124]

Five distinct TRAIL receptors have been identified: death receptor 4 (TRAIL-R1),[125] KILLER/DR5 (TRAIL-R2, TRICK2),[126,127] DcR1 (TRID, TRAIL-R3),[128] DcR2 (TRUNDD OR TRAIL-R4)[129] and osteoprotegerin.[130] All these receptors have high sequence homology in their extracellular domains. They can be classified into two groups, death-inducing receptors (TRAIL-R1 and -R2) and death-inhibitory receptors (TRAIL-R3, TRAIL-R4, and osteoprotegerin). Both TRAIL-R1 and TRAIL-R2 contain a C-terminal death domain that signals downstream caspase activation to mediate TRAIL-induced apoptosis in a variety of tumor cell types. In contrast to these death-inducing receptors, TRAIL-R3 and TRAIL-R4 lack a functional cytoplasmic death domain. These two receptors serve as "decoys" and protect cells from TRAIL-induced apoptosis by competing with the death-inducing TRAIL-receptors for TRAIL binding. Osteoprotegerin (OPG) binds TRAIL, but has lower affinity at physiological temperature.[131]

The apoptotic signaling induced by TRAIL is similar to that induced by FAS. Binding of TRAIL to its receptors DR4 or DR5 triggers the formation of a DISC by recruiting FADD, caspase-8, -10 and FLIP, followed by activation of caspase-8 and -3 and rapid apoptosis in many cancer cell types (Fig. 4). TRAIL-induced apoptosis also involves the mitochondrial pathway, like Fas-induced apoptosis, in type II cells.[131] Release of Smac/Diablo from mitochondria to block the caspase-3 inhibitory effect of XIAP is required for cells to undergo apoptosis in response to TRAIL.[132] TRAIL is also a weak activator of the NF-κB pathway, which is masked by its pro-apoptotic function. Like TNF, the activation of NF-κB by TRAIL is also dependent on the adaptor Rip and TRAF2.[133]

Death ligands have been widely investigated in part due to their potential in cancer therapy. Unlike many conventional chemotherapeutic drugs, they stimulate cell death in tumor cells independently of the p53 tumor suppressor gene, which is often found inactivated in human cancers. However, clinical application of the prototypic death ligands TNF and CD95L has been hampered by toxicity to normal tissues. Intravenous TNF administration causes hypotension and a systemic inflammatory syndrome that resembles septic shock due to strong activation of pro-inflammatory NF-κB. Injection of agonist anti-Fas (CD95) antibodies induces hepatocyte apoptosis and lethal hepatic failure in mice.[99] The ability of Apo2L/TRAIL to induce apoptosis in a wide variety of cancer cell lines, while having little toxicity toward many types of normal cells, suggests that this molecule may be an ideal agent for cancer therapy.[134]

Because TRAIL is a promising cancer therapeutic agent, the regulation of TRAIL sensitivity and resistance in tumor and normal cells become particularly of interest. Several factors have been implicated in TRAIL sensitivity, such as method of preparation of TRAIL, expression levels of death receptors and decoy receptors on the cell surface, expression level of caspase-8, -10, FLIP or IAPs, the status of other signaling pathways such as the AKT pathway, NF-κB pathway or c-Myc pathway.

Various versions of TRAIL may have different ability to induce apoptosis in normal or tumor cells. The toxicity of His-tagged ligand toward normal hepatocytes and keratinocytes may be associated with recombinant variants of the protein that possess aberrant structural and biochemical properties.[135] Studies on human cells or primate model indicated that nontagged TRAIL is not toxic to normal cells. The difference may lie in the internal zinc atom bound by cysteine residues in each subunit of nontagged TRAIL, but not tagged TRAIL, which is crucial for trimer stability and biological function.[136] The toxicity of TRAIL toward normal cells also can be avoided by the combinatorial use of caspase-9 inhibitor[137] or specific monoclonal antibody against either DR4 or DR5.[138] Hyper-oligomerized TRAILs, including hexamers and nonomers exhibited an extremely high potency in apoptosis induction.[139]

The high expression of decoy receptors in normal cells, but not tumor cells was originally proposed as a major mechanism resulting in differential sensitivity to TRAIL.[9] However, in most cancer cell lines, DR4 and/or DR5 are expressed, whereas DcR expression is less frequent and does not correlate with resistance to TRAIL. Thus, there are probably additional determinants of sensitivity besides decoy receptor expression. Either DR4 or DR5 must be present on the cell surface to transduce the TRAIL signal. Deficiency or mutation in death receptors confer tumor cell resistance to TRAIL-induced apoptosis.[140] Transport of TRAIL receptors to the cell membrane seems also very important to determine TRAIL sensitivity. Tumor cells may become resistant to TRAIL through regulation of the death receptor cell surface transport.[141] In the screen of small interfering RNA (siRNA) library for genes regulating TRAIL sensitivity, the signal recognition particle (SRP) complex was identified and related to cell surface levels of DR4 as well as DR4-mediated apoptosis.[142] The sensitivity of tumor cells to TRAIL-induced apoptosis has been correlated with surface expression of death receptors in melanomas.[143]

Despite the central role of caspases in cell death execution, screening for mutations in initiator or executioner caspases in a variety of human tumors suggests caspase mutations as a rare event in human tumors.[144] MCF-7 breast carcinoma cells completely lack caspase-3 expression due to a frameshift mutation within exon 3 of the caspase-3 gene.[145] These cells can be sensitized by transfection of procaspase-3 towards treatment with cytotoxic drugs.[146] Alternative splicing has been identified as another level of transcriptional regulation of caspase expression. The genes encoding procaspase-2 or -9 can generate short isoforms that prevent apoptosis in a dominant-negative manner.[147] Caspase expression and function appears to be frequently impaired by epigenetic mechanisms in cancer cells.[148] Caspase-8 expression was found to be inactivated by hypermethylation of regulatory sequences of the caspase-8 gene in a number of different tumor cells derived from neuroblastoma, malignant brain tumors, Ewing tumor and small lung cell carcinoma and also in primary tumor samples. Importantly, restoration of caspase-8 expression by gene transfer or by de-methylation treatment sensitized resistant tumor cells for death receptor- or drug-induced apoptosis.[149]

The caspase activation inhibitor FLIP acts as important negative regulator of TRAIL-induced apoptosis. Overexpression of FLIP protects cells from TRAIL-induced apoptosis,[150] and suppression of FLIP by RNAi sensitizes cells to TRAIL-induced apoptosis.[151] A genetic screen in human placental cDNA retroviral library isolated c-FLIP(S) as a suppressor of TRAIL signaling in TRAIL-resistant

clones.[152] Correlations between FLIP levels and TRAIL resistance have also been observed.[153] Degradation of FLIP following exposure to a PPAR selective ligand apparently sensitizes tumor cells to Apo2L/TRAIL-induced apoptosis.[154] However, other studies failed to find such a correlation.[143,155] Moreover, recent work suggests that FLIP can actually promote caspase-8 activation in response to death ligand.[115,143] Thus more work is needed to further analyze the role of FLIP in the TRAIL resistance.

IAPs can inhibit TRAIL-induced apoptosis by modulating caspase activity.[156] Transfection of Smac/DIABLO, an inhibitor of IAPs, overcomes the resistance of BAX-null HCT116 cells to Apo2L/TRAIL-induced apoptosis of.[132] A comparison of Apo2L/TRAIL-sensitive and -resistant melanoma cell lines showed a strong correlation between binding of XIAP to cleaved caspase-3 and TRAIL sensitivity. More Smac/DIABLO is released to the cytosol in TRAIL-sensitive cell lines.[157] Smac/DIABLO agonists sensitize human acute leukemia Jurkat T cells for apoptosis induction by Apo2L/TRAIL and induces regression of malignant glioma in vivo.[158] A small molecule mimic of Smac strongly sensitizes both TNF and TRAIL-induced caspase activation and apoptosis.[159]

Although TRAIL can activate the mitochondrial pathway, the importance of the mitochondrial pathway in TRAIL-induced apoptosis is still controversial. Overexpression of Bcl-2 or Bcl-Xl does not block TRAIL-induced apoptosis in lymphoid cells.[160] In contrast, overexprssion of Bcl-2 or Bcl-Xl inhibits TRAIL-induced apoptosis in human lung or prostate cancer cells.[161] Thus, involvement of the mitochondria in TRAIL-induced apoptosis may depend on cell type, like type I and type II cells in FAS-induced apoptosis.[162] In cells requiring mitochondrial involvment, blockade of the mitochondrial pathway may change TRAIL sensitivity. For example, BAX-null HCT-116 are completely resistant to TRAIL-induced apoptosis.

Other cell signaling pathways may also be involved in the regulation of TRAIL sensitivity, including the AKT, Myc, NF-κB or p53 pathways. Constitutive activation of AKT in several cancer cells has been shown to confer resistance to TRAIL.[163,164] This Inhibition of TRAIL-induced apoptosis by activated AKT may occur at the level of BID cleavage. Suppression of AKT activity in these cells sensitizes them to TRAIL-induced apoptosis. Activation of AKT also rescues human gastric cancer cells from TRAIL-induced apoptosis via upregulation of FLIPs.[165] Myc is more likely a positive regulator of TRAIL sensitivity. An RNAi library screen for genes regulating TRAIL sensitivity identified Myc and its transcriptional activator TCF4 as required for TRAIL sensitivity.[166] Several cancer cell lines showed a correlation between TRAIL sensitivity and expression level of Myc. c-Myc-mediated a transcriptional repression of FLIP which sensitized cells to death receptor stimuli.[151] Myc also has been shown to upregulate expression of TRAIL receptors in some tumor cells.[167] c-Myc enhances the apoptotic activity of death receptor signaling proteins by engaging the mitochondrial apoptotic pathway.[168] Thus, myc may serve as a indicator of potential TRAIL sensitive tumor cells in clinical trials. p53 is another important regulator of TRAIL sensitivity. KILLER/DR5 was originally discovered as DNA damage inducible, p53 regulated gene.[127] p53 also may regulate the expression of DR4 in a limited number of tumors.[169] Considering p53 is a central player of the mitochondrial apoptotic pathway, it is not so surprising that p53 can broadly sensitize the tumor cells to TRAIL through a mitochondrial amplification loop. TRAIL can activate NF-κB weakly both in vivo and in vitro. Although most studies have shown that NF-κB acts as an inhibitor of TRAIL-induced apoptosis,[170,171] its role as an activator has also been reported.[172] It is likely that various subunits of NF-κB may have different effects on the regulation of the cellular response to TRAIL. It has been shown that the RelA/p65 subunit of NF-κB transduces a survival signal by inhibiting expression of DR4/5 and caspase-8 as well as upregulating cIAPs, whereas c-Rel acts as a proapoptotic factor by enhancing DR4/5 and Bcl-Xs, and inhibiting cIAPs after TRAIL treatment.

Selective cytotoxicity to tumor cells by TRAIL makes it a promising death ligand for clinical application. However, many cancer cells are resistant to TRAIL-induced apoptosis. Application of TRAIL alone may not be effective in these cancers. Intensive effort has been made to sensitize TRAIL-resistant tumor cells. Various means have been found to act synergistically with TRAIL. These include (1) conventional chemotherapeutic drugs, (2) irradiation, (3) histone deacetylase inhibitors, (4) stress inducers, (5) proteasome inhibitors, (6) interferon, (7) small molecules like peptide mimicking Smac or Bax, (8) others like retinoid or cyclooxygenase-2 inhibitors.[163] The mechanisms underlying these synergies vary between therapies but all appear to be related to the TRAIL signaling pathway or regulatory mechanisms introduced above. Many of these treatments have been applied in clinical cancer therapy, but toxicity is still a problem. Sub-toxic doses of some agents listed above often arrest tumor cells in aspecific cell cycle phase instead of inducing apoptosis. Combined application of low doses of these agents with TRAIL may provide a way to efficiently kill tumor cells with less toxicity to normal cells. Such strategies suggest the importance of understanding the relationship between cell cycle position and TRAIL sensitivity.

Although extensive studies have focused on the molecular mechanisms of TRAIL resistance, few of them explored the acquired resistance after TRAIL treatment. Acquired resistance could be different from the intrinsic resistance. Demonstration of the mechanisms of acquired TRAIL resistance may benefit the successful application of TRAIL and prevention of cancer relapse after TRAIL treatment in patients.

INTRINSIC APOPTOTIC PATHWAYS

Mitochondria as a central regulator of intrinsic apoptotic pathways

Intrinsic apoptotic pathways are initiated inside cells. The most important turning point in the course of the intrinsic apoptotic process occurs in the mitochondria. Although it is arguable whether the mitochondrial event triggers the apoptosis, it appears to be the key event in apoptosis. A pivotal event in the mitochondrial pathway is mitochondrial outer membrane permeabilization (MOMP). MOMP is mainly mediated and controlled by Bcl-2 family members. Once MOMP occurs, it precipitates cell death through either the release of molecules involved in apoptosis, or the loss of mitochondrial functions essential for cell survival.[173]

About 20 Bcl-2 family members in mammals can be divided into at least three groups. They all contain at least one of four relatively conserved Bcl-2 Homology (BH) domains. Anti-apoptotic members, including Bcl-2 and four relatives (Bcl-Xl, Bcl-w, A1, and Mcl-1) promote cell survival. Another two groups of pro-apoptotic members instead elicit cell death. The "multi-BH domain" Bax, Bak, and Bok share three domains (BH1, BH2, and BH3) with Bcl-2 and also have a similar 3D structure featuring a globular bundle of -helices with a hydrophobic surface groove.[174,175] In contrast, the eight or more diverse BH3-only proteins, such as Bid, Bad, and Bim, posses only

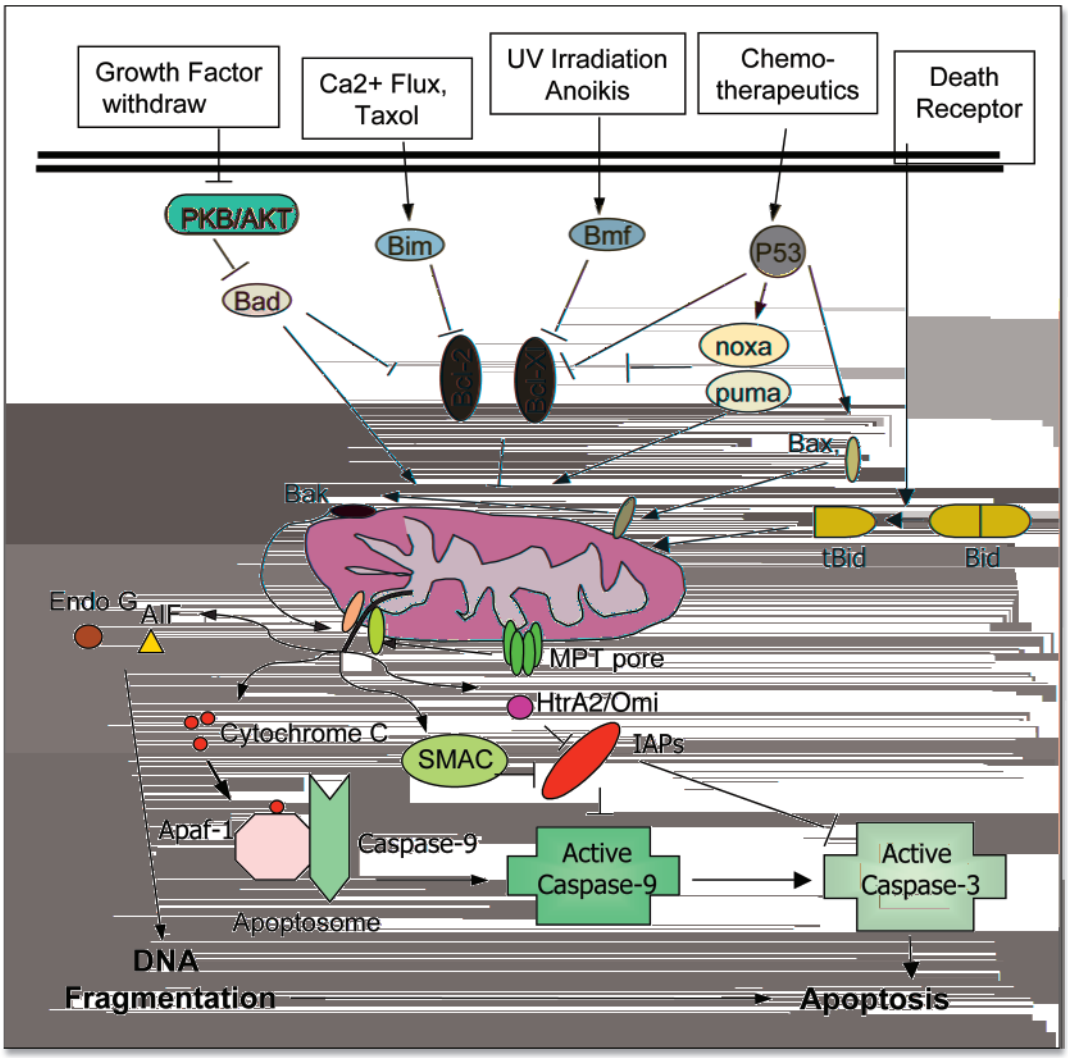


Figure 5. Intrinsic pathway mediated by mitochondria. See text for details.

the short BH3 interaction domain, which is necessary and probably sufficient for the induction of apoptosis. Both pro-apoptotic groups seem to be required for launching apoptosis. The damage-sensing BH3-only proteins clearly lie upstream of Bax/Bak, because they cannot kill cells lacking both Bax and Bak.[176,177]

The pro-survival Bcl-2 is a 26-kDa protein that is localized in the mitochondria, endoplasmic reticulum and perinuclear membranes.[178] Bcl-2 belongs to the family of proto-oncogenes, but the ability of bcl-2 to promote tumor growth is different from other oncogenes; it can rescue cells that are destined to die without affecting the proliferation rate of the cell.[179] It is possible that the location of Bcl-2 might be a key feature of its function. Bcl-2 is involved in maintaining homeostatsis, including the mitochondrial membrane status and balance of the interactions between the members of the Bcl-2 protein family (e.g., Bax). In addition, bcl-2 has a role in the regulation of calcium homeostasis in the cell.[180] The Bcl-2 family members can inhibit cell death by sequestering or neutralizing the BH123 molecules. The BH1, BH2 and BH3 domains in BCL-Xl are in close proximity and create hydrophobic pocket that can accommodate a BH3 domain of the pro-apoptotic members. The ratio of anti- to pro-apoptotic molecules constitutes a rheostat that sets the threshold of susceptibility to apoptosis for the intrinsic pathway.[180] Evidence for a critical role of Bcl-2 in maintaining normal cellular homeostasis comes from the analysis of Bcl-2 knockout mice. Bcl-2 deficient mice display apoptosis of lymphocytes, developmental renal cell death and loss of melanocytes.[181] Bcl-2 transgenic mice show enhanced haematopoietic cell survival. [182,183]

The BH3-only proteins trigger apoptosis in response to developmental cues, insufficient trophic support, and intracellular damage. They play distinct roles in these different death stimuli-induced apoptotic pathways. The loss of Bim renders cells refractory to cytokine deprivation, calcium flux, Taxol, and the T-cell receptor signals that kill self-reactive lymphocytes, but not to γ-irradiation.[184] Conversely, the p53-induced Noxa and Puma may mediate the response to genotoxic damage, as demonstrated for Puma in a cancer cell line. BH3-only proteins can serve as sentinels for damage to particular intracellular structures, but they remain inactive without stimulation by diverse mechanisms. Noxa and Puma are controlled primarily at the transcriptional level and can be transactivated by p53 in response to DNA damage. p53 also mediates apoptosis by transcriptionally regulating many other genes including Bax, Apaf-1, Fas, killer/DR5, or pidd.[185] Additionally recent studies support a transcriptionally independent pathway involving localization of p53 to the mitochondria, followed by binding and inhibition of Bcl-xl and/or bcl-2 or activating Bax directly.[186] Bad is regulated by its phosphorylation and sequestration by 14-3-3 proteins. Deprivation from growth factors or cytokines results in the inactivation of AKT,[187] which induces the de-phosphorylation and activation of Bad. Bid remains inactive until cleaved to expose its BH3 domain. Cleavage of Bid by caspase-8 in death receptor-mediated apoptosis results in the subsequent myristoylation favoring its translocation to mitochondrial membranes.[29,30] Bim and Bmf are normally sequestered to the microtubules and actin cytoskeleton, respectively. Bmf may have role in the suspension-induced apoptosis (anoikis).[188]

Death signals activate some BH3-only members of the Bcl-2 protein family and other proteins, which in turn induce oligomerization of the pro-apoptotic, BH123 proteins like Bax and Bak to insert into the outer mitochondrial membrane (OMM). Other BH3-only proteins can act indirectly by releasing the BH123 proteins from the anti-apoptotic Bcl-2 family proteins that sequester them.

Bax and Bak appear to permeabilize the outer mitochondrial membrane, allowing efflux of apoptogenic proteins.[189] Whereas Bax is predominantly a cytosolic monomer in healthy cells, during apoptosis it undergoes conformational changes at both termini, translocates to the outer mitochondrial membrane, and oligomerizes. Bak is present as monomer in mitochondrial membrane even in healthy cells, but also changes conformation and forms larger aggregates during apoptosis. A small proportion of Bak molecules are bound to VDAC2 in healthy cells. In the absence of inhibition by VDAC2, BAK demonstrates an enhanced allosteric conformational activation resulting in the release of cytochrome c, caspase activation, and mitochondrial dysfunction responsible for an increased susceptibility to apoptotic death.[180]

The BH123 proteins engage either PT (permeability transition)-dependent or PT-independent mechanisms for MOMP. In PT-dependent MOMP, apoptotic signals open the putative PT pore composed of ANT in the inner mitochondrial membrane (IMM) and VDAC in the OMM. This so-called permeability transition pore complexes (PTPC) may also contain cyclophilin D and peripheral benzodiazepine receptor (PBR). Opening the pore cause water to enter the matrix and ions to equilibrate, dissipating $\Delta\Psi m$ at least transiently. The matrix swells, rupturing the OMM to release proteins of the mitochondrial intermembrane space (IMS). In PT-independent MOMP, BH123 proteins, perhaps with other proteins like VDAC, cause the formation of pores in the OMM through which IMS proteins are released.[173]

MOMP results in the cell death through two mechanisms including release of soluble mitochondrial intramembrane proteins (SIMPs), such as cytochrome c (cyt c) and disruption of essential mitochondrial functions (Fig. 5).[173]

Among the released molecules, most are involved in the induction of caspase-dependent apoptosis, but some can prompt cell death in a caspase-independent way. The first identified protein released from the mitochondrion is cytochrome C,[190] which was previously known to function within the electron transport chain. Subsequent binding of cytochrome c to Apaf-1 results in the formation of a complex known as the apoptosome in the presence of ATP/dATP. The apoptosome can recruit procaspase-9 and thereby activate it through oligomerization which then leads to the activation of executioner caspase-3, -6 and -7.[191,192] In vivo studies showed that the Apaf-1, caspase-9 or caspase-3 knock-out MEFs are resistant to various apoptotic stimuli.[21,25,193,194]

The second important group of proteins found to release from the mitochondria upon MOMP are the IAP antagonists including Smac/DIABLO, HtrA2/Omi and GSPT1/eRF3. IAPs act as a brake for apoptosis by inhibiting activated caspases. Smac/DIABLO, HtrA2/Omi and GSPT1/eRF3 bind IAPs in a manner similar to caspases, therefore functioning as competitive inhibitors.[65,195,196] The caspase coactivator Smac/Diablo is released along with cytochrome c during apoptosis to neutralize the inhibitory activity of IAPs and promote cytochrome *c*-dependent caspase activation. Smac/DIABLO seems also to play an important protective role in death receptor-mediated apoptosis.[132] The first four amino acids of mature SMAC/DIABLO, AVPI interact tightly with the BIR3 domain of XIAP. Peptides mimicking this sequence have proven to be strong inhibitors of IAPs and greatly sensitize to TNF and TRAIL-induced apoptosis.[159] Omi/HtrA2 can also proteolytically cleave and inactivate IAP proteins and therefore it is presumably a more efficient IAP suppressor.[197] But recent data showed that cells from Omi/HtrA2 transgenic mice (*mnd2* mice) containing the mutation in a noncatalytic serine residue were more sensitive to apoptosis induced by tunicamycin, etoposide, and hydrogen peroxide.[198] These seemingly conflicting results suggest that Omi/HtrA2 is a dual agent, which promotes DNA fragmentation under apoptotic conditions and maintains normal mitochondrial functions in living cells in a fashion similar to that of cytochrome c.

Processed caspase-9, -3, and -2 are also released together with cytochrome c from mitochondria in vitro by disruption of $\Delta\psi$ with an uncoupler or in cell death caused by staurosporine. These findings imply that the mitochondrial procaspase molecules participate in apoptosis induction.[199]

In addition to proteins that can trigger or enhance caspase activation, mitochondria also release pro-apoptotic proteins unrelated to caspase activation. AIF, once released to the cytosol, translocates to the nucleus in response to death stimuli including pneumococcus, p53, UVB and *N*-methyl-*N*-nitro-*N*-nitrosoguanidine, H_2O_2, and *N*-methyl-aspartate. In the nucleus, AIF induces peripheral chromatin condensation and large-scale DNA fragmentation. AIF in association with a heat-labile cytosolic factor may also permeabilize mitochondrial membranes. None of these AIF effects can be prevented by addition of zVAD.fmk indicating that they are caspase-independent, but the cell death is characterized by various apoptotic features including cell shrinkage, cell surface exposure of phosphatidylserine, loss of mitochondrial membrane potential and large-scale DNA fragmentation.[200] Definitive genetic evidence establishes an essential role for AIF during early mammalian development.[201] AIF was found to be essential for the first wave of programmed cell death required for embryonic morphogenesis and cavitation.

In response to apoptotic stimuli, endoG is also released from the mitochondria into the cytosol where it translocates to the nucleus and generates oligonucleosomal DNA fragmentation. It is essential for DNA fragmentation, particularly during caspase-independent apoptosis.[202] Unlike caspase inhibitors, Bcl-2 effectively protects cells from both caspase-dependent and -independent apoptosis. This is more likely through its ability to prevent mitochondrial membrane permeabilization and the resultant release of potent pro-apoptotic molecules like endo G (Fig. 5).[203]

The ER stress apoptotic pathway

Besides the mitochondria, the endoplasmic reticulum (ER) is a second compartment participating in intrinsic apoptosis. In the ER, quality control mechanisms ensure that only properly folded proteins are passed along the secretory pathway. Stress to the ER including oxidative stress, chemical toxicity, treatment with Ca^{2+} ionophores or exposure to inhibitors of glycosylation can result in misfolded proteins and perturbed calcium homeostasis, which provokes the unfolded protein response (UPR). UPR involves (1) reduced protein synthesis to prevent further aggregation and accumulation of unfolded proteins; (2) induction of ER-resident chaperones and folding catalysts; and (3) activation of ER-associated protein degradation (ERAD) to eliminate aggregates. However, if this response persists and stress can not be resolved, induction of necrosis or apoptosis becomes inevitable.[204]

Release of calcium from the ER into the cytosol is required for stress-induced apoptosis in many cases. The ER is the major intracellular store of Ca^{2+} ions. Efflux of Ca^{2+} ions from the ER is often associated with uptake into the mitochondria. In staurosporine and ceramide-induced apoptosis, calcium acts as a messenger that coordinates the amplification loop between the mitochondria and the

ER. A small amount of cytochrome *c* released from the mitochondria diffuses to the adjacent endoplasmic reticulum (ER) and binds to $InsP_3$ receptors, thereby enhancing calcium release from the endoplasmic reticulum. The released calcium causes a mass exodus of cytochrome *c* from all mitochondria. The positive feedback finally results in the dramatic activation of caspases. This process may be an important event in disorders such as myocardial infarction, Alzheimer's disease and stroke.[205]

Increasing evidence suggests that Bcl-2 family members also function on the ER. Bcl-2 targeted specifically to the ER interrupted the cross-talk from the ER to mitochondria produced by ER stress and certain other stimuli. Either Bax or Bak is required for ER stress-induced apoptosis. Recent findings suggest that both of them localize and function at the ER. Bax and Bak have been implicated in maintaining homeostatic concentrations of Ca^{2+} in the ER. Release of Ca^{2+} from ER stores and its uptake into mitochondria is impaired in Bax or Bak double knockout mice, which results in reduced apoptosis in response to certain death stimuli.[206] Bax or Bak apparently can function directly on the ER to promote caspase-12 activation. Indeed, a full apoptotic response to certain cytotoxic stimuli (e.g., staurosporine and etoposide) may require that Bax/Bak act both in the ER to discharge Ca^{2+} ions and in the mitochondria to permeabilize its outer membrane.[207,208]

Other molecules, such as GADD153/CHOP, ALG-2, VCP and BAP31, may also be involved in ER stress-induced apoptosis. However, the mechanism underlying their function is not very clear.[205]

So far, only caspase-12 activation during ER-mediated apoptosis has been reported. Upon activation, caspase-12 translocates from the ER to the cytosol, where it directly cleaves pro-caspase-9 to activate caspase-3. Caspase-$12^{-/-}$ mice were also found to be resistant to Aβ peptide-induced apoptosis in an Alzheimer's disease model. However, functional caspase-12 has only been cloned from the mouse and rat, and the existence of a human isoform of caspase-12 remains controversial.[209]

Neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease, ALS (amyotrophic lateral sclerosis, or Lou Gehrig's disease) and prion protein diseases all feature misfolded proteins and the aggregation of misfolded proteins. ER stress plays an important pathogenetic role in these diseases.[210]

CROSSTALK BETWEEN EXTRINSIC AND INTRINSIC PATHWAYS

Two major apoptotic pathways exist, namely the extrinsic pathway (death receptor pathway) and the intrinsic pathway (the mitochondrial pathway) within a cell. Despite the difference in the initiation manner, they converge on the activation of effector caspases.[180] Crosstalk between the two pathways at both the execution and initiation levels has been reported.

Based on the requirement of the mitochondrial pathway for apoptosis induced by death receptors, cells can be divided into type I and type II cells. In type I cells, activation of caspase-8 in the DISC is sufficient to induce apoptosis. However, in type II cells, only a small amount of FADD and caspase-8 are recruited to DISC, so insufficient activation of caspase-8 require involvement of mitochondria to finally induce apoptosis.[211] Death receptor-mediated apoptotic signaling can activate the mitochondrial pathway through the BH-3 only protein, Bid. Caspase-8 activated in the DISC is sufficient to cleave cytosolic Bid. Cleaved Bid (tBid) then translocates to the mitochondria, which leads to mitochondrial dysfunction and apoptosome formation (Fig. 4).[29,30] Although the apoptotic mitochondrial events occurs in both types of cells, only the apoptosis in type II cells is abrogated if the mitochondrial pathway is blocked by overexpression of Bcl-2.[180] Genetic evidence further proved the existence of these two cell types. Hepatocytes from Bid$^{-/-}$ mice behave like type II cells after treatment with Fas, but thymocytes from Bid$^{-/-}$ mice respond to Fas in a type I manner.[212] Tumor cells and hepatocytes also showed different requirements for the mitochondrial pathway (type I vs. type II) in TRAIL-induced apoptosis.[162]

It was originally believed that the activation of capspase-9 and -3 in the apoptosome would function as the amplification loop which can compensate the weak DISC formation and caspase-8 activation.[14] However, the evidence that cells like hepatocytes derived from the Apaf-$1^{-/-}$ and caspase-$9^{-/-}$ mice exhibit the similar sensitivity to Fas-mediated apoptosis suggests that other factors required for the amplification.[194,213]

This controversy is reconciled by the finding that the second group of molecules including Smac/Diablo are released from the mitochondria during death receptor-mediated apoptosis. This protein interacts with and sequesters XIAP to remove its inhibition from caspase-3 and caspase-9.[132] Caspase-3 then can undergo a second proteolytic step to be fully activated. This portion of fully activated caspase-3 subsequently cleaves many proteins, such as XIAP, to facilitate the apoptotic feedback functioning as amplification loop finally results in irreversible apoptosis.[214]

Despite the major role of caspase-8 in extrinsic apoptosis, it's activation in response to some intrinsic cell death stimuli also facilitates the further caspase activation and accelerates death kinetics.[215,216] Caspase-9 and -3 is required for the activation of four other caspases (-2, -6, -8, and -10) in cell-free extracts in response to cytochrome c. Inhibition of caspase-6 activity in cells significantly inhibited caspase-8 cleavage and apoptosis, therefore establishing caspase-6 as a major activator of caspase-8 in vivo and confirming that this pathway can have a critical role in promotion of apoptosis.[217]

Not only does mitochondrial dysfunction influence caspase activation in death receptor-mediated apoptosis, but crosstalk exists between the upstream component of the extrinsic and the intrinsic pathways. The typical example is p53-mediated apoptosis. In response to DNA damage, p53 not only targets the intrinsic apoptotic molecules like the Bax and Puma to activate the mitochondrial apoptotic pathway, but also upregulates the extrinsic pathway genes like FasL and killer/DR5.[127,218] It was shown that DNA damage-induced apoptosis can proceed through death receptor signaling.[219] These reports would argue for a contribution of death receptor signaling to the intrinsic apoptotic pathway.

The connections between the extrinsic and the intrinsic pathway suggests that many chemotherapeutic agents or stress-inducers can sensitize cells to death ligand-induced apoptosis. Sensitivity to TRAIL could be achieved or enhanced by many intrinsic death stimuli including (1) the transcription inhibitor actinomycin D or the protein synthesis inhibitor cycloheximide; (2) DNA damaging chemotherapeutic agents such as doxorubicin, cis-diammine-dichloroplatinum or etoposide; (3) ER stress inducers such as tunicamycin or animomycin; and (4) ionizing radiation.[163]

REGULATORY MECHANISMS IN APOPTOTIC SIGNALING

While the initiation and execution of apoptosis depend on activation of the receptor- and/or mitochondrial-dependent death pathways, the apoptotic process is affected by many other signaling

pathways including the Myc, MAPK/ JNK, PI-3K/AKT and NF-κB pathway.

Myc pathway

Mammals have three Myc proteins: c-Myc, N-Myc and L-Myc. These proteins are basic helix-loop-helix leucine-zipper (bHLH-Zip) transcription factors that bind to the bHLH-Zip protein Max. Myc–Max heterodimers regulate the transcription of many target genes. Myc family members have important roles in the control of both cell proliferation and cell death in animals. The Myc proteins seem to regulate the rates of cell growth and G_1-S progression through coordinated effects on many genes.[220]

Activation of Myc can lead to a net loss of cells through apoptosis.[221] Similarly, overexpression of Myc in flies induces cell proliferation and compensatory apoptosis.[222] It seems that c-Myc sensitizes cells to the pro-apoptotic effects of diverse cellular stresses by several means. These include: 1. activation of the intrinsic pathway and the release of cytochrome *c* from mitochondria (possibly by suppression of levels of the pro-survival proteins Bcl-2 and Bcl-X_L and/or by induction of the pro-apoptotic protein Bax).[223,224] Myc-induced apoptosis is dependent on p53 function. c-Myc-induced apoptosis is suppressed in mice harboring p53 mutation, which results in enhanced tumor formation. However, increased p53 signaling inhibits tumor formation. Proliferative signals and p53 activation by Myc in mammalian cells are linked by ARF. The *ARF* promoter is responsive to Myc.[225] Activation of c-Myc upregulates ARF transcription and protein levels. In turn, ARF promotes the p53 response by inhibiting MDM2, which blocks p53-induced transcription and targets p53 for degradation.[226,227] ARF induction therefore sets a threshold for cellular proliferative signaling. Cells that receive excessive proliferative signals are prone to apoptosis due to the induction of p53-dependent apoptotic signaling by ARF. ARF also binds with c-Myc and dramatically blocks c-Myc's ability to activate transcription and induce hyperproliferation and transformation. In contrast, c-Myc's ability to repress transcription is unaffected by ARF and c-Myc-mediated apoptosis is enhanced.[228] These differential effects of ARF on c-Myc function suggest that separate molecular mechanisms mediate c-Myc-induced hyperproliferation and apoptosis. This direct feedback mechanism represents a p53-independent checkpoint to prevent c-Myc-mediated tumorigenesis. (2) Cooperation with extrinsic death-receptor signaling; c-Myc-induced apoptosis has been shown to correlate with Fas ligand and Fas receptor expression.[168,229] c-myc down-regulation of FLIP expression provides a universal mechanism to explain the ability of c-myc to sensitize cells to death receptor stimuli.[151] 3. The generation of reactive oxygen species and damage to DNA. One target for transcriptional stimulation by c-Myc is ornithine decarboxylase (*ODC*) which can cause apoptosis when overexpressed.[230,231]

NF-κB

NF-κB was discovered as a factor in the nucleus of B cells that binds to the enhancer of the kappa light chain of immunoglobulin.[232] NF-κB is known to consist of a family of Rel-domain-containing proteins; e.g., Rel A (also called p65), Rel B, c-Rel, p50 (also called NF-κB1), and p52 (also called NF-κB2). It is activated when it is released from IκB and translocates to the nucleus, where it regulates the expression of many immune, growth, inflammation and anti-apoptosis genes.[233]

The effects of NF-κB on apoptosis has far-reaching consequences for normal development and/or tissue homeostasis. NF-κB's implication in apoptosis is also vital to the outcome of viral infections, the onset and progression of many cancers and their response to radiation and chemotherapy. While NF-κB is generally believed to mediate survival signals, there are a number of instances where it is proapoptotic depending on the inducing stimulus and the cell context.[234] A large body of evidence supports an anti-apoptotic effect of Rel/NF-κB factors. Overexpression of constitutive Rel/NF-κB was shown to protect transformed cells against apoptosis induced by TNF , FasL or TRAIL.[235,236] Conversely, inhibiting the Rel/NF-κB activity by overexpressing IκB sensitizes transformed cells to apoptosis induced by death ligands, ionizing radiations or chemotherapeutic drugs.[81,237] In vivo, the inactivation of the genes encoding RelA, IKK2 or NEMO leads to embryonic lethality by massive liver apoptosis, due to enhanced TNF sensitivity.[82,238,239] Nuclear factor kappa B (NF-κB) prevents cells from undergoing apoptosis via activation of anti-apoptotic genes including XIAP, FLIP, Bcl-Xl, et al.[240]

However, besides these anti-apoptotic effects, numerous reports have in contrast revealed an ability of Rel/NF-κB factors to induce apoptosis. For example, $CD4^+/CD8^+$ thymocytes from transgenic mice overexpressing IκB become resistant to CD3-mediated apoptosis.[241] Activation of Rel/NF-κB factors by cytokine withdrawal, paclitaxel, focal ischemia, p53 or the viral protein LMP1 were shown to promote apoptosis.[240,242]

It was recently discovered that NF-κB also became a repressor of transcription when induced by commonly employed DNA damaging anticancer drugs, doxorubicin (adriamycin) and daunorubicin (daunomycin).[243] These findings are striking in light of the past literature that described these genotoxins as inducers of NF-κB-dependent transcription.[244] This new finding underscores the need to specifically examine the action of NF-κB in an individual cancer cell context.

JNK

c-Jun N-terminal protein kinase JNK/SAPK is a member of the mitogen-activated protein kinase (MAPK) superfamily. JNK is activated by sequential protein phosphorylation through a MAP kinase module, i.e., MAP3K-MAP2K-MAPK. Two MAP2Ks (JNKK1/MKK4/SEK1 and JNKK2/MKK7) for JNK have been identified to phosphorylate JNK on Thr 183 and Tyr185, which result in the activation of JNK. JNK also can be negatively regulated by NF-κB-mediated inhibition. JNK is a key regulator of c-jun activity. JNK specifically phosphorylates the transcription factor c-Jun on its N-terminal transactivation domain at Ser63 and Ser73 resulting in the augmentation of its transcriptional activity. This phosphorylation can be induced by a variety of extracellular stimuli such as TNF exposure.[75,245]

The JNK signaling pathway is involved in regulation of many cellular events, including growth control, transformation and apoptosis. The role of JNK activation in apoptosis is highly controversial. JNK has been implicated in a pro-apoptotic effect, anti-apoptotic effect or as having no role in cell death. The role of the JNK pathway in apoptosis may depend on cell type, the nature of the death stimulus, the duration of its activation and the activity of other signaling pathways, especially NF-κB and p53.[246]

The JNK pathway may play a pro-apoptotic role in neurons. Neurons from mice in which endogenous c-jun alleles were replaced with a nonphosphorylatable c-junAA63/73 mutant are resistant to apoptosis induced by UV and the excitotoxic glutamate-receptor agonist kainite.[247] JNK also has been shown to initiate apoptosis by phosphorylating and thereby inactivating BCL2 and BCL-Xl[248,249] and by phosphorylating and activating the BH3-only protein BIM.[250] However, the role of JNK in the phosphorylation of Bcl-2 and Bcl-Xl in response to cell death stimuli has been challenged by a

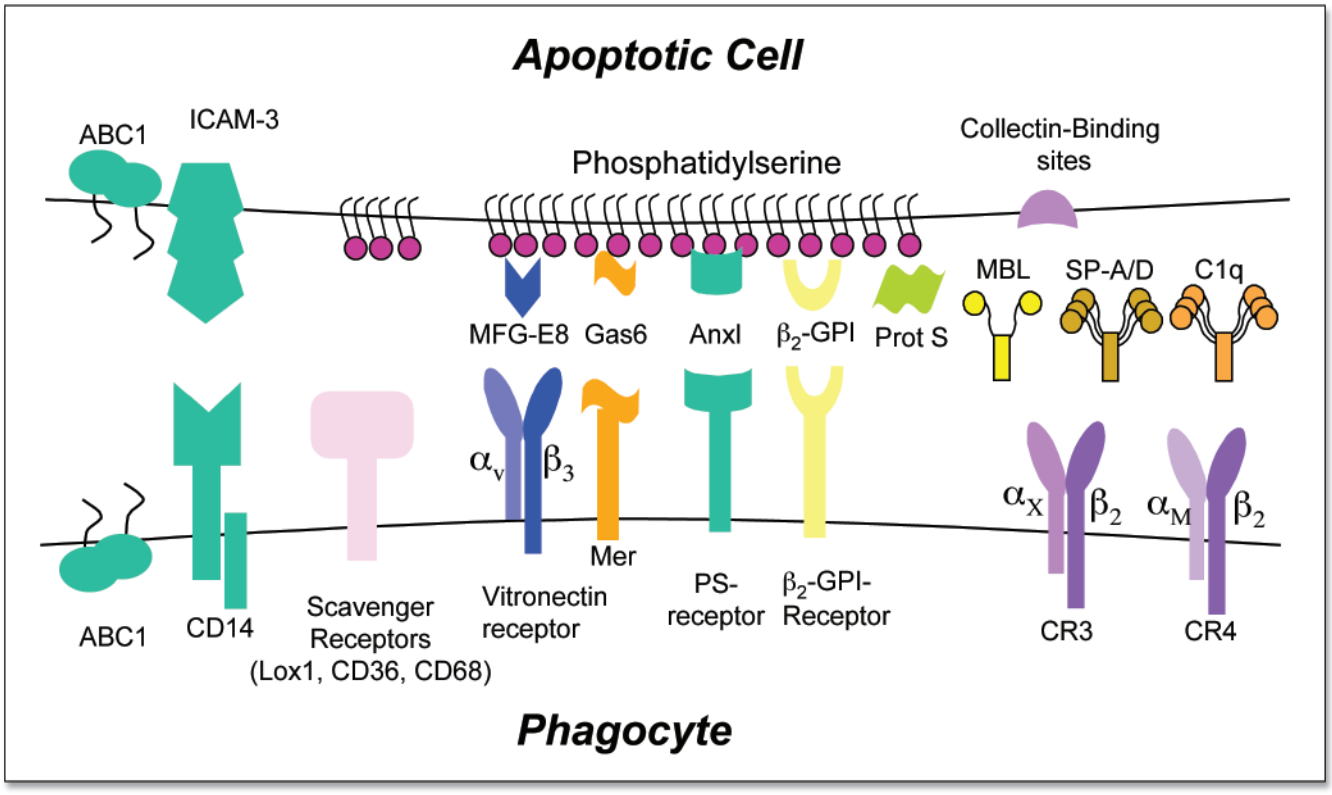


Figure 6. Clearence of apoptotic cells by phagocyte. See text for details.

recent study.[251] JNK also induces increased expression of pro-apoptotic p53 and FAS/FASL molecules under certain situations.[252] C-Jun induction is has also been implicated in the exit from p53-dependent cell cycle arrest followed by apoptosis in response to UV.[253] MEFs isolated from nonphosphorlylatable c-jun AA63/73 mutant transgenic mice displayed resistance to excitotoxic agonists and UV.[254] Recent studies showed that the JNK pathway is required for TNF-induced apoptosis. TNF activates the JNK pathway through Mkk7 to induce the cleavage of Bid to produce j-Bid, and j-Bid then translocates to the mitochondria to induce the release of SMAC from the mitochondria, which finally results in cell death.[92]

Other evidence suggests that JNK activation may be required for survival/anti-apoptosis under certain circumstances. In jnk-$1^{-/-}$jnk-$2^{-/-}$ mice, apoptosis was increased in the hindbrain and forebrain regions.[221] In addition, immature thymocytes and peripheral mature T cells deficient in JNK kinase1 (SEK1/MKK4) were highly susceptible to Fas/CD95- and CD3-mediated apoptosis.[255] This suggests that JNKK1 may play an anti-apoptotic role in T cells. Biochemical analysis also revealed that JNK can phosphorylate the proapoptotic Bcl-2 family protein BAD at threonine 201, thereby inhibiting BAD association with the anti-apoptotic molecule BCL-X_L. This inactivation of BAD is required for IL-3-mediated cell survival.[256] It has been reported that activation of the JNK pathway can inhibit p53-induced cell cycle arrest and therefore promote p53-induced apoptosis. This might explain why JNK only exerts its anti-apoptotic function in p53-deficient tumor cells.[253]

AKT pathway

The AKT pathway is a survival signal in many different cell death paradigms, including withdrawal of extracellular signaling factors, oxidative and osmotic stress, irradiation and treatment of cells with chemotherapeutic drugs and ischemic shock.[257] Moreover, AKT activation has been related, in tumor cells, with increased resistance to apoptosis induced by TRAIL/APO-2L.[258,259]

Receptor protein tyrosine kinase (RPTK) activation results in PI(3,4,5)P_3 and PI(3,4)P_2 production by PI3K at the inner side of the plasma membrane. AKT interacts with these phospholipids, causing its translocation to the inner membrane, where it is phosphorylated and activated by PDK1 and PDK2. AKT activation induces different cell survival mechanisms.[260]

Stimulation of AKT acts principally to attenuate the activation of the intrinsic apoptotic pathway. AKT phosphorylates pro-apoptotic BH3 only protein BAD on serine 136.[187,261] As a result of phosphorylation, BAD interacts with 14-3-3 proteins and is prevented from blocking Bcl-2 death-protective function. Human caspase-9 has been reported to be phosphorylated by AKT, resulting in attenuation of its activity.[55] AKT inactivates by phosphorylation the Forkhead family of transcription factors that induce the expression of proapoptotic factors such as Fas ligand and Bim.[262] In addition, AKT activates by phosphorylation the transcription factor cyclic AMP response element-binding protein (CREB), and the IκB kinase (IKK), a positive regulator of NF-κB, both of them regulating the expression of genes with anti-apoptotic activity (Downward, 2004).

THE FATE OF APOPTOTIC CELLS

Efficient elimination of cells undergoing apoptosis is crucial for normal tissue homeostasis and for the regulation of immune responses. Apoptotic cells must be properly recognized and rapidly cleared before loss of membrane integrity to prevent leakage of potentially cytotoxic or antigenic contents.[263] Indeed, defects in

apoptotic corpse clearance have been closely associated with autoimmune and inflammatory diseases.[264] The engulfment of apoptotic cells also has important consequences for immune responses. For example, the uptake of apoptotic cells can induce the release of anti-inflammatory mediators, such as transforming growth factorβ (TGFβ), interleukin-10 (IL-10) and prostaglandin E_2 (PGE_2), and can inhibit the secretion of pro-inflammatory mediators, such as tumor necrosis factor α (TNF-α) from phagocytes.[265] This clearance of dying cells is thought to prevent immune responses to self-antigens derived from dying cells. By contrast, the uptake of necrotic cells is pro-inflammatory.[266]

Among the surface changes on apoptotic cells, the most common and best characterized is the loss of phospholipid asymmetry in the plasma membrane and the translocation of PtdSer from the inner to the outer leaflet of the lipid bilayer. The exposure of PtdSer occurs very early during the apoptotic process and is almost universally required for engulfment.[267] PtdSer and annexin I (AnxI) translocate and colocalize in discrete patches on the surface of the outer membrane where they function as 'eat-me' signals to trigger phagocyte recognition and uptake.[268] Extracellular bridging molecules in serum, such as milk-fat-globule-EGF-factor 8 (MFG-E8), β2 glycoprotein I (β2GPI), serum protein S and growth arrest-specific 6 (GAS6), bind to phospholipids like PtdSer on the apoptotic cell surface and act as extracellular bridging molecules to link them to phagocytes.[269-271] This makes apoptotic cells more susceptible to phagocytosis by providing recognition sites for a greater number of phagocyte receptors. Other extracellular bridging molecules known to facilitate engulfment have also been linked to the recognition of altered sugars on the surface of apoptotic cells. These include certain members of the collectin family, such as mannose-binding lectin (MBL), as well as the collectin-related first component of the classical complement cascade, C1q. Humans with C1q deficiency and C1q-knockout mice both have defects in clearance of apoptotic cells in vivo and display an increased risk of developing autoimmune diseases such as systemic lupus erythrematosus (SLE) and glomerulonephritis (Fig. 6).[272,273]

MFG-E8 is a bridging protein secreted by macrophages. Binding of MFG-E8 to apoptotic cells stimulates their removal by phagocytic cells. Engagement of the MFG-E8 receptor (possibly integrin $\alpha_v\beta_3$ or $\alpha_2\beta_5$) expressed by macrophages not only induces internalization of MFG-E8, but also may inhibit macrophage activation, thereby preventing the development of an autoimmune response against apoptotic cell epitopes. In the absence of MFG-E8, apoptotic cells still bind to macrophages via other signals or receptors, but they cannot be engulfed. MFG-E8 is specifically required to initiate the subsequent cytoskeletal rearrangements needed for phagocytosis.[266] In addition, the mice lacking MFG-E8 suffer from late-onset autoimmune disease, a defect often associated with impaired clearance of apoptotic cells.[274]

Many different cell-surface receptors in phagocytes can recognize and respond to eat-me signals. These receptors include: certain scavenger receptors, such as CD36, CD68 and lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), all of which can recognize PtdSer in vitro;[275-277] certain integrins, such as the $\alpha_v\beta_3$ integrin receptor, which can bind to MFG-E8.[275,276,278] The receptor tyrosine kinase MER, which binds to the GAS6 bridging molecule;[279,280] LRP (also called CD91), which interacts with CRT bound to MBL and C1q as described above;[272] the LPS receptor CD14, which binds to the Ig-superfamily member adhesion molecule ICAM-3; and the PtdSer receptor (PSR) (Fig. 6).[281] In addition, evidence has been found for a "don't eat me" signal involving CD31, which repulses the binding to phagocytes, exists on the surface of healthy cells. Loss of the "don't eat me" signal is sometimes required for the clearance of apoptotic cells by phagocytes.[281,282]

The "eat-me" and "don't-eat-me" signals are crucial for the ability of phagocytes to recognize apoptotic cells. However, these signals can only apply when the phagocyte and apoptotic cell are in close proximity. It is therefore reasonable to predict that attraction signals must exist to induce the migration of professional phagocytes to sites of apoptosis to facilitate the rapid removal of dying cells. It has been shown that the supernatants of several types of apoptotic cells contain a chemo-attractant activity for monocytes and primary human macrophages. Apoptotic cells, but not nonapoptotic cells, secrete a chemotactic factor into the supernatant. Interestingly, caspase-3-mediated activation of Ca^{2+}-independent phospholipase A2 (iPLA2) in apoptotic cells has been linked to the release of this chemo-attractant lysophosphatidylcholine (LPC).[283]

NONTYPICAL APOPTOSIS AND NECROSIS

Necrosis

Necrosis has traditionally been considered an unregulated, energy-independent form of cell death, and has been well-characterized in a wide range of pathologic states. Extensive failure of normal physiological pathways that are essential for maintaining cellular homeostasis, such as regulation of ion transport, energy production and pH balance can lead to necrosis. Necrosis is characterized morphologically by vacuolation of the cytoplasm, breakdown of the plasma membrane and induction of inflammation around the dying cell due to the release of cellular contents and pro-inflammatory molecules.[284]

A classic example of necrotic conditions is ischemia that leads to a drastic depletion of oxygen, glucose, and other trophic factors and evokes massive necrotic death of endothelial cells and nonproliferating cells of surrounding tissues. Necrosis can be induced by microbial infections, neuronal excitotoxins, or reactive oxygen species (ROS).[285] The expression of Bax or treatment with tumor necrosis factor (TNF) or Fas ligand can elicit nonapoptotic cell death in the presence of nonspecific pan-caspase inhibitors such as zVAD-fmk or anti-apoptotic molecules like Bcl-XL that prevent caspase activation.[286,287] Under these conditions, cells that would normally die by apoptosis exhibit the hallmarks of necrosis. Studies on necrosis are generally performed under condition of inhibition of apoptosis either by pharmacological or genetic methods. A recent observation showed a biologically relevant role for necrosis.[288] In these studies, cells infected with vaccinia virus encoding an anti-apoptotic protein were used to illustrate a natural condition in which necrosis takes over when apoptosis is inhibited. This result also suggests an important function for necrosis in fighting microbial infection.

Recent cytological data indicate that necrotic death occurs not only during pathological events, but is also a component of some physiological processes. For example, during renewal of the small intestine, both apoptosis and necrosis of enterocytes contribute to cell loss.[288] Genetic evidence also indicates that necrotic cell death can potentially substitute for apoptosis during normal development. For instance, genetic deletion of caspase-3 and caspase-9 does not affect normal loss of spinal cord and brain stem neurons during development, although the morphology of the forebrain suffers a marked perturbation.[289]

Until recently, necrosis has often been viewed as an accidental and uncontrolled cell death process. Nevertheless, growing evidence supports the idea that necrotic cell death may also be programmed.[290] Cellular signaling events have been identified to initiate necrotic destruction that could be blocked by inhibiting discrete cellular processes. Integration of the BH3 protein BNIP3 tomitochondria triggers a necrotic-like form of PCD.[291] The necrosis-like cell death triggered by engagement of the Fas/FADD signal transduction system in mammalian cells was reported to depend on the kinase RIP as an effector molecule.[292] Ca^{2+}, ceramide, and the JNK/p38 pathway have also been shown to be mediators of necrosis.[284] Excessive activation of PARP resulting from profound induction of DNA breaks is believed to be a cause of necrotic cell death due to ATP depletion.[293] Surprisingly, programmed necrosis in response to DNA alkylating agents initiated by PARP only occurrs in actively proliferating cells. This selectivity may be due to the differential energy source utilized by vegetative or proliferating cells. The cell fate in response to a DNA-alkylating agent is determined by cellular metabolic status.[47] During apoptosis, PARP is inactivated by caspase-mediated cleavage to shut down the necrosis pathway.

Many human tumors carry mutations that inactivate apoptotic pathways. Inactivation of apoptosis allows tumor cells to proliferate beyond normal homeostatic control. Necrosis represents an alternative pathway for tumor cells to be eliminated. The inflammatory component of necrotic death has the potential advantage of stimulating an immune response that could increase the efficiency of tumor cell death.[290] The balance between apoptotic and necrotic cell death may be modulated to potentiate a patient's immune response to a tumor. However, inflammatory responses induced by necrosis may also be associated with systemic toxicity.

Autophagy

Autophagy is an evolutionarily conserved lysosomal pathway involved in the turnover of long-lived proteins and organelles. Functions of autophagy include: (1) remodeling during development and differentiation; (2) the production of amino acids when nutrients are limiting; (3) elimination of unwanted or damaged organelles and molecules. These functions are important for maintenance of cytoplasmic homeostasis. The conservation of autophagy genes throughout phylogeny suggests that this form of death might have a role in many eukaryotes.[284] Autophagy can be stimulated in response to different situations of stress, such as starvation, changes in cell volume, oxidative stress, accumulation of misfolded proteins, hormonal signaling, irradiation, xenobiotic or TRAIL treatment.[294] TRAIL has been shown to positively regulate lumen formation in acini formed in vitro by the breast cancer cell line MCF-10 via induction of autophagy.[295] The activity of the p70S6-kinase is regulated by the mTor kinase, and inhibition of S6 phosphorylation caused by inactivation of mTor with rapamycin induces autophagy even under nutrient-rich conditions. Autophagy can a also be induced when apoptosis is inhibited, such as treatment with z-VAD, or etoposide in Bax/Bak double knockout MEFs.[290] Three groups of diseases, i.e., myopathies, neurodegenerative disorders and cancer, may be related to alterations in the autophagic pathway and/or deficiency in autophagy genes.[296]

Two major systems are responsible for the degradation of proteins in cells: the ubiquitin-proteasome pathway and the vacuolar system. One is the familiar ubiquitin-mediated proteolysis that takes place in proteasomes. Autophagy is an intracellular lysosome-mediated catabolic mechanism that is responsible for the bulk degradation and recycling of damaged or dysfunctional cytoplasmic components and intracellular organelles.[297] Autophagy may also switch cells to a catabolic program in which cellular constituents are degraded for energy production as a survival mechanism during periods of nutrient stress. Following the induction of autophagy, autophagic vesicles or autophagosomes are formed through the assembly and expansion of double-layered, membrane-bound structures of endoplasmic reticulum around whole organelles and isolated proteins. The autophagosome encapsulates the cytosolic materials, then docks and fuses with lysosomes or other vacuoles, causing degradation of the autophagosomal contents. At the molecular level, the signaling pathway that leads to autophagy involves at least the activities of phosphatidylinositol 3-kinase (PI3K) and the kinase target of rapamycin (TOR). Class-III PI3K activity is particularly important for the early stages of autophagic vesicle formation. By contrast, TOR negatively regulates autophagosome formation and expansion.[298]

The discovery of the *APG* genes and the *AUT* genes that regulate autophagy in the yeast *Saccharomyces cerevisiae* has contributed to our understanding of the molecular control of autophagy.[299,300] Two key autophagy genes, *ATG7* and *beclin 1*, are necessary for this nonapoptotic death pathway in mammalian cells. Beclin 1 is the mammalian homolog of the yeast Atg6 protein that is involved in the early steps of autophagic vesicle formation. Beclin 1 knockouts cause an unexplained increase in spontaneous tumors.[301] It is possible that beclin 1 may act as a tumor suppressor by causing autophagic cell death. Activation of RIP and MKK7 has been shown to be involved in autophagy induced in the presence of z-vad.[302] An interesting unresolved question is the molecular connection between RIP and the JNK signaling pathway. Inhibition of caspase-8 seems to facilitate autophagic death. The suppression of autophagic death by caspase-8 in mammalian cells indicates that caspases can regulate both apoptotic and nonapoptotic cell death. Recent data also demonstrated that Bcl-Xl also modulates autophagic death induced by etoposide in Bax/Bak double-null MEFs, possibly through regulation of Beclin1 to induce APG5-APG12.[303]

Mitotic catastrophe

Mitotic catastrophe has taken on a broader definition and has been used to explain a type of mammalian cell death that is caused by aberrant mitosis. Mitotic catastrophe is associated with the formation of multinucleate, giant cells that contain uncondensed chromosomes, and is morphologically distinct from apoptosis, necrosis or autophagy.[304]

The G_2 checkpoint of the cell cycle is responsible for blocking mitosis when DNA of cells is exposed to sustained insults. DNA damage activates a number of molecules that promote cellular activities such as cell-cycle arrest, DNA repair or apoptosis, if the damage is too harsh to be repaired. However, if the G_2 checkpoint is defective, a cell can enter mitosis prematurely, before DNA replication is complete or DNA damage is repaired. This aberrant mitosis causes the cell to undergo death by mitotic catastrophe.

The inhibition or inactivation of any of these G_2-checkpoint genes including those that encode ATR, CHK1, p53, WAF1 and 14-3-3 may lead to mitotic catastrophe in cells with unrepaired DNA damage.[305-309]

Mitotic catastrophe is a highly conserved stress-response mechanism. Agents that damage microtubules and disrupt the mitotic spindle also cause mitotic catastrophe. For example, the drug paclitaxel induces an abnormal metaphase in which the sister chromatids fail to segregate properly. CDK1 activation is prolonged abnormally in paclitaxel-treated cells, which undergo death by mitotic catastrophe.[310] Defects in genes that are required for inducing mitotic catastrophe can contribute to tumorigenesis.

DISEASE AS A CONSEQUENCE OF DYSREGULATED APOPTOSIS

Many diseases may involve too much apoptosis (e.g., neurodegenerative diseases, or AIDS) or too little apoptosis (e.g., cancer due to mutations of p53 or overexprssion of many anti-apoptotic proteins).

Cancer

Tumorigenesis is a multistep process involving genetic alterations that confer a growth advantage to transformed cells. These changes drive the progressive transformation of normal human cells into highly malignant derivatives. Six essential alterations have been identified to collectively lead to malignant transformation: (1) self-sufficient growth signals, (2) insensitivity to growth-inhibitory signals, (3) evasion of programmed cell death, (4) indefinite replication, (5) sustained angiogenesis, and (6) tissue invasion and metastasis.[311]

Acquired resistance toward apoptosis is a hallmark of perhaps all types of cancer. Overexpression of many oncogenes induces massive apoptosis. These oncogenes include ras, E1A, myc, e2f1, possibly through the ARF-mdm2-p53 pathway. Transgenic mice in which the pRb tumor suppressor has been functionally inactivated exhibit high apoptotic rates.[220] Thus elimination of cells bearing activated oncogenes or inactivated tumor suppressor genes by apoptosis may provide a selection pressure that requires cells to evade apoptosis.

Tumor cells can acquire resistance to apoptosis by the upregulation or overactivation of anti-apoptotic proteins or by the downregulation or mutation of pro-apoptotic proteins.

Dysregulation of tumor resistance-related anti-apoptotic signaling includes anti-apoptotic Bcl-2 family members, IAPs, the PI-3K-AKT pathway, or NF-κB pathways. A common feature of follicular B-cell lymphoma is the chromosomal translocation t(14;18), which couples the *BCL2* gene to the immunoglobulin heavy chain locus, leading to enhanced *BCL2* expression.[312] The expression level of the BCL-2 protein in some tumor cells correlates with malignancy of tumors and response to chemotherapy.[313] The PI-3K-AKT pathway is a strong pro-survival pathway. In many tumors this pathway is overactivated by loss-of-function mutation of the PTEN tumor suppressor gene [314] or gain-of-function mutation of the oncogenes such as Ras, BCR-ABL[315] or growth factor receptors such as EGFR.[257] Recently evidence was showen that activation of the AKT pathway by upregulation of tyrosine kinase receptor factors like TrkB or insulin receptor substrate 2 facilitate mammary tumor metastasis.[316,317] This effect is associated with the ability of AKT to suppress anoikis. Overactivation of the AKT pathway renders tumor cells resistant to apoptosis by several different mechanisms (see "Regulatory Mechanisms in Apoptotic Signaling"). Another important factor influencing apoptosis of tumor cells is the transcription factor nuclear factor κB (NF-κB) (see "Regulatory Mechanisms in Apoptotic Signaling"). NF-κB and proteins regulated by it could promote tumorigenesis through suppression of apoptosis. Genes encoding NF-κB or IκB proteins are amplified or translocated in human cancer.[318] Constitutively active NF-κB has been identified not only in human cell lines but also in tumor tissues derived from patients with multiple myeloma, acute myelogenous leukemia, acute lymphocyte leukemia, chronic myelogenous leukemia, prostate or breast cancers. Furthermore, genetic evidence that NF-κB mediates tumorigenesis has also been provided.[319] Karin and coworkers recently showed that activation of NF-κB by endotoxin in cancer cells produces inflammation-induced tumor growth through expression of TNF, whereas inhibition of NF-κB mediates tumor regression through TRAIL.[171] Upregulation of other anti-apoptotic proteins such as FLIP or survivin have also been reported in different human tumors.[110,320]

Inactivation of the pro-apoptotic signaling pathways provides another efficient way for tumor cells to acquire resistance to apoptosis. Both intrinsic and extrinsic apoptotic pathways can be shut down in tumors through different mechanisms. p53, a major mediator of the intrinsic pathway, is very frequently mutated gene in cancer. Moreover, dysregulation of many other genes facilitates tumorgenesis by interfering with the downstream function of p53. These include mutation in the effector Bax, DR5 or alterations in the upstream regulators ATM, mdm2 or ARF. P53 knockout mice exhibit both reduced sensitivity to -irradiation or chemotherapy and accelerated tumor development.[321] Other key mediators of the intrinsic apoptotic pathway including Bax, Bak or Apaf-1 also do not function normally in certain cancer types. For Bax, frameshift mutations that lead to loss-of-expression, and mutations in the BH domains that result in loss-of-function have been observed in tumors.[322,323] Reduced Bax expression has been associated with a poor response rate to chemotherapy and shorter survival in some situations.[324] Loss of expression of Apaf-1 has been found in metastatic melanomas and proposed as a way to escape mitochondria-dependent apoptosis.[325] Moreover, death receptor pathways are downregulated or inactivated in many tumors. The expression of the death receptor CD95 is reduced in some tumor cells—for example, in hepatocellular carcinomas, neoplastic colon epithelium and melanomas.[326] Deletions and mutations of the death receptors TRAIL-R1 and TRAIL-R2 have also been observed in tumors.[327] The frequent deletion of the chromosomal region 8p21-22 in head and neck cancer and in non-small-cell lung cancers affects the *DR5* gene. Mutations have been found in the ectodomain or the death domain of DR4 or DR5. Loss of CD95 or TRAIL might contribute to immune escape, which facilitates the metastasis of tumors.[122] Resistance to treatment downstream of apoptosis induction might be linked to absence of initiator or executioner caspases. For example, loss of pro-caspase-3 expression in breast cancer cells and a deficiency of apical caspase-8 has recently been described in small-cell lung cancer (SCLC) and in neuroblastomas resulting in resistance to some, but not all apoptosis-inducing treatments.

The resistance to apoptosis in tumor cells hinders the success of cancer therapy. Overcoming therapeutic resistance represents a big challenge. Fortunately most regulatory and mediatory components of apoptosis are present in redundant forms. This redundancy holds important implications for the development of novel types of anti-tumor therapy, since tumor cells that have lost pro-apoptotic components are likely to retain other similar ones. We anticipate that new technologies will be able to display the apoptotic pathways still operative in specific types of cancer cells. Apoptosis research has provided several new cytotoxic agents that might circumvent the pathways that are blocked in tumors. The death ligand TRAIL has attracted a great deal of attention in this regard because most cancer cell lines are sensitive to TRAIL, whereas normal cells are resistant. In SCID mice, treatment of tumor xenografts with TRAIL suppresses tumor growth, with no adverse effects. Chemotherapy or irradiation could sensitize resistant cells to TRAIL-induced apoptosis in vitro and in vivo. Moreover, TRAIL-induced apoptosis is not dependent on the status of p53, which is mutated in many tumor cells. Another strategy to exploit insights into tumor resistance

mechanisms is to downregulate anti-apoptotic molecules with anti-sense, RNAi, small peptide or chemical inhibitors.

Neuro-degenerative disease

Under normal conditions, neuron possess strong intrinsic anti-apoptotic factors and survive for the lifetime of the organism. Pathological PCD occurs if metabolic stress, damage or genetic abnormalities overwhelm these survival factors. Premature death of adult neurons leads to irreversible functional deficits ending up with neuro-degeneration, as seen in Alzheimer's and Parkinson's disease. The remaining neurons have no capacity for regeneration to compensate for the loss.[328]

The mode of cell death in neurodegenerative disorders remains a matter of controversy, and it is possible that both apoptotic and nonapoptotic cell death coexist in the brains of affected patients. Like the apoptosis in other cell types, two main pathways lead to apoptosis in mature neurons—the 'extrinsic' or death receptor-initiated pathway, and the 'intrinsic' or mitochondrial pathway. Specific genes are involved in the death of different types of neurons, and a given neuron might activate distinct death pathways in response to different stimuli.[329]

Changes in the expression of death ligands and receptors in specific neuronal subtypes in neurodegenerative diseases indicate putative death pathways. Basal FAS expression is low, but its induction in various neurological disorders causes both direct and bystander damage to neurons and glia. The FAS–FASL pathway has roles both in maintaining the immune-suppressed status of the CNS and in inducing neuronal death in Alzheimer's disease, Huntington's disease and Parkinson's disease, as well as amyotyophic lateral sclerosis (ALS).[330,331] FASL- and FADD-mediated neuronal death was found in models of motor neuron death and Parkinson's disease.[332,333] TNFα-mediated death occurs in the brains of patients with Alzheimer's disease.[334] TNFα and TNFα-receptors (TNFR) are expressed at low levels in the brain under normal conditions, but are a key component of the inflammatory response in Parkinson's disease, Alzheimer's disease, prion disease, HIV-associated dementia and the neuronal injury produced by trauma and stroke. Death occurs by both the FAS- and TNFα-mediated pathways following ischemic brain injury.[335,336]

AIF is released from mitochondria in the intrinsic apoptotic pathway. AIF acts as a downstream mediator of PARP1-induced caspase-independent neuronal cell death in response to NMDA.[337] In sympathetic and cerebellar granule cells, BIM can be phosphorylated by JNK to potentiate a BAX-dependent corelease of cytochrome *c* and SMAC/DIABLO, which finally lead to apoptosis.[338]

Alzheimer's disease, Huntington's disease, Parkinson's disease and prion disease, as well as ALS, all have features of misfolded proteins. Misfolded proteins are highly toxic to neurons and can induce ER stress in these neurons. ER-stress-mediated apoptosis might have an

8. Green DR, Knight RA, Melino G, Finazzi-Agro A, Orrenius S. Ten years of publication in cell death. Cell Death Differ 2004; 11:2-3.
9. Ashkenazi A, Dixit VM. Death receptors: Signaling and modulation. Science 1998; 281:1305-8.
10. Shi Y. Mechanisms of caspase activation and inhibition during apoptosis. Mol Cell 2002; 9:459-70.
11. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. Nat Med 2004; 10:789-99.
12. Yuan J, Shaham S, Ledoux S, Ellis HM, Horvitz HR. The C. elegans cell death gene CED-3 encodes a protein similar to mammalian interleukin-1 β-converting enzyme. Cell 1993; 75:641-52.
13. Miura M, Zhu H, Rotello R, Hartwieg EA, Yuan J. Induction of apoptosis in fibroblasts by il-1 β-converting enzyme, a mammalian homolog of the C. elegans cell death gene CED-3. Cell 1993; 75:653-60.
14. Stennicke HR, Salvesen GS. Caspases - controlling intracellular signals by protease zymogen activation. Biochim Biophys Acta 2000; 1477:299-306.
15. Degterev A, Boyce M, Yuan J. A decade of caspases. Oncogene 2003; 22:8543-67.
16. Boatright KM, Renatus M, Scott FL, Sperandio S, Shin H, Pedersen IM, Ricci JE, Edris WA, Sutherlin DP, Green DR, Salvesen GS. A unified model for apical caspase activation. Mol Cell 2003; 11:529-41.
17. Donepudi M, Sweeney AM, Briand C, Grutter MG. Insights into the regulatory mechanism for caspase-8 activation. Mol Cell 2003; 11:543-9.
18. Thornberry NA, Lazebnik Y. Caspases: Enemies within. Science 1998; 281:1312-6.
19. Varfolomeev EE, Schuchmann M, Luria V, Chiannilkulchai N, Beckmann JS, Mett IL, Rebrikov D, Brodianski VM, Kemper OC, Kollet O, Lapidot T, Soffer D, Sobe T, Avraham KB, Goncharov T, Holtmann H, Lonai P, Wallach D. Targeted disruption of the mouse caspase 8 gene ablates cell death induction by the TNF receptors, FAS/apo1, and dr3 and is lethal prenatally. Immunity 1998; 9:267-76.
20. Kuida K, Haydar TF, Kuan CY, Gu Y, Taya C, Karasuyama H, Su MS, Rakic P, Flavell RA. Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. Cell 1998; 94:325-37.
21. Hakem R, Hakem A, Duncan GS, Henderson JT, Woo M, Soengas MS, Elia A, de la Pompa JL, Kagi D, Khoo W, Potter J, Yoshida R, Kaufman SA, Lowe SW, Penninger JM, Mak TW. Differential requirement for caspase 9 in apoptotic pathways in vivo. Cell 1998; 94:339-52.
22. Thornberry NA, Rano TA, Peterson EP, Rasper DM, Timkey T, Garcia-Calvo M, Houtzager VM, Nordstrom PA, Roy S, Vaillancourt JP, Chapman KT, Nicholson DW. A combinatorial approach defines specificities of members of the caspase family and granzyme B. Functional relationships established for key mediators of apoptosis. J Biol Chem 1997; 272:17907-11.
23. Slee EA, Harte MT, Kluck RM, Wolf BB, Casiano CA, Newmeyer DD, Wang HG, Reed JC, Nicholson DW, Alnemri ES, Green DR, Martin SJ. Ordering the cytochrome C-initiated caspase cascade: Hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner. J Cell Biol 1999; 144:281-92.
24. Woo M, Hakem R, Soengas MS, Duncan GS, Shahinian A, Kagi D, Hakem A, McCurrach M, Khoo W, Kaufman SA, Senaldi G, Howard T, Lowe SW, Mak TW. Essential contribution of caspase 3/cpp32 to apoptosis and its associated nuclear changes. Genes Dev 1998; 12:806-19.
25. Kuida K, Zheng TS, Na S, Kuan C, Yang D, Karasuyama H, Rakic P, Flavell RA. Decreased apoptosis in the brain and premature lethality in cpp32-deficient mice. Nature 1996; 384:368-72.
26. Zheng TS, Hunot S, Kuida K, Momoi T, Srinivasan A, Nicholson DW, Lazebnik Y, Flavell RA. Deficiency in caspase-9 or caspase-3 induces compensatory caspase activation. Nat Med 2000; 6:1241-7.
27. Martinon F, Burns K, Tschopp J. The inflammasome: A molecular platform triggering activation of inflammatory caspases and processing of proil-β. Mol Cell 2002; 10:417-26.
28. Mariathasan S, Newton K, Monack DM, Vucic D, French DM, Lee WP, Roose-Girma M, Erickson S, Dixit VM.. Differential activation of the inflammasome by caspase-1 adaptors asc and ipaf. Nature 2004; 430:213-8.
29. Li H, Zhu H, Xu CJ, Yuan J. Cleavage of bid by caspase 8 mediates the mitochondrial damage in the FAS pathway of apoptosis. Cell 1998; 94:491-501.
30. Luo X, Budihardjo I, Zou H, Slaughter C, Wang X. Bid, a bcl2 interacting protein, mediates cytochrome C release from mitochondria in response to activation of cell surface death receptors. Cell 1998; 94:481-90.
31. Enari M, Sakahira H, Yokoyama H, Okawa K, Iwamatsu A, Nagata S. A caspase-activated dnase that degrades DNA during apoptosis, and its inhibitor icad. Nature 1998; 391:43-50.
32. Sakahira H, Enari M, Nagata S. Cleavage of cad inhibitor in cad activation and DNA degradation during apoptosis. Nature 1998; 391:96-9.
33. Ricci JE, Munoz-Pinedo C, Fitzgerald P, Bailly-Maitre B, Perkins GA, Yadava N, Scheffler IE, Ellisman MH, Green DR.. Disruption of mitochondrial function during apoptosis is mediated by caspase cleavage of the p75 subunit of complex i of the electron transport chain. Cell 2004; 117:773-86.
34. Bachelder RE, Wendt MA, Fujita N, Tsuruo T, Mercurio AM. The cleavage of AKT/protein kinase B by death receptor signaling is an important event in detachment-induced apoptosis. J Biol Chem 2001; 276:34702-7.
35. Lin Y, Devin A, Rodriguez Y, Liu ZG. Cleavage of the death domain kinase RIP by caspase-8 prompts TNF-induced apoptosis. Genes Dev 1999; 13:2514-26.
36. Wen LP, Fahrni JA, Troie S, Guan JL, Orth K, Rosen GD. Cleavage of focal adhesion kinase by caspases during apoptosis. J Biol Chem 1997; 272:26056-61.
37. Levkau B, Scatena M, Giachelli CM, Ross R, Raines EW. Apoptosis overrides survival signals through a caspase-mediated dominant-negative NF-κB loop. Nat Cell Biol 1999; 1:227-33.
38. Tang G, Yang J, Minemoto Y, Lin A. Blocking caspase-3-mediated proteolysis of ikkβ suppresses TNF-α-induced apoptosis. Mol Cell 2001; 8:1005-16.
39. Coleman ML, Sahai EA, Yeo M, Bosch M, Dewar A, Olson MF. Membrane blebbing during apoptosis results from caspase-mediated activation of rock I. Nat Cell Biol 2001; 3:339-45.
40. Rudel T, Bokoch GM. Membrane and morphological changes in apoptotic cells regulated by caspase-mediated activation of Pak2. Science 1997; 276:1571-4.
41. Deak JC, Cross JV, Lewis M, Qian Y, Parrott LA, Distelhorst CW, Templeton DJ. FAS-induced proteolytic activation and intracellular redistribution of the stress-signaling kinase Mekk1. Proc Natl Acad Sci USA 1998; 95:5595-600.
42. Kothakota S, Azuma T, Reinhard C, Klippel A, Tang J, Chu K, McGarry TJ, Kirschner MW, Koths K, Kwiatkowski DJ, Williams LT. Caspase-3-generated fragment of gelsolin: Effector of morphological change in apoptosis. Science 1997; 278:294-8.
43. Rao L, Perez D, White E. Lamin proteolysis facilitates nuclear events during apoptosis. J Cell Biol 1996; 135:1441-55.
44. Ku NO, Liao J, Omary MB. Apoptosis generates stable fragments of human type I keratins. J Biol Chem 1997; 272:33197-203.
45. Schmeiser K, Hammond EM, Roberts S, Grand RJ. Specific cleavage of gamma catenin by caspases during apoptosis. Febs Lett 1998; 433:51-7.
46. Hotti A, Jarvinen K, Siivola P, Holtta E. Caspases and mitochondria in c-Myc-induced apoptosis: Identification of Atm as a new target of caspases. Oncogene 2000; 19:2354-62.
47. Zong WX, Ditsworth D, Bauer DE, Wang ZQ, Thompson CB. Alkylating DNA damage stimulates a regulated form of necrotic cell death. Genes Dev 2004; 18:1272-82.
48. Levkau B, Koyama H, Raines EW, Clurman BE, Herren B, Orth K, Roberts JM, Ross R. Cleavage of P21cip1/Waf1 and P27kip1 mediates apoptosis in endothelial cells through activation of Cdk2: Role of a caspase cascade. Mol Cell 1998; 1:553-63.
49. Zhou BB, Li H, Yuan J, Kirschner MW. Caspase-dependent activation of cyclin-dependent kinases during FAS-induced apoptosis in jurkat cells. Proc Natl Acad Sci USA 1998; 95:6785-90.
50. Janicke RU, Walker PA, Lin XY, Porter AG. Specific cleavage of the retinoblastoma protein by an ice-like protease in apoptosis. Embo J 1996; 15:6969-78.
51. Nahle Z, Polakoff J, Davuluri RV, McCurrach ME, Jacobson MD, Narita M, Zhang MQ, Lazebnik Y, Bar-Sagi D, Lowe SW. Direct coupling of the cell cycle and cell death machinery by E2f. Nat Cell Biol 2002; 4:859-64.
52. Konishi Y, Lehtinen M, Donovan N, Bonni A. Cdc2 phosphorylation of bad links the cell cycle to the cell death machinery. Mol Cell 2002; 9:1005-16.
53. Maclachlan TK, El-Deiry WS. Apoptotic threshold is lowered by p53 transactivation of caspase-6. Proc Natl Acad Sci USA 2002; 99:9492-7.
54. Rikhof B, Corn PG, El-Deiry WS. Caspase 10 levels are increased following DNA damage in a p53-dependent manner. Cancer Biol Ther 2003; 2:707-12.
55. Cardone MH, Roy N, Stennicke HR, Salvesen GS, Franke TF, Stanbridge E, Frisch S, Reed JC. Regulation of cell death protease caspase-9 by phosphorylation. Science 1998; 282:1318-21.
56. Mcdonald IIIrd ER, El-Deiry WS. Suppression of caspase-8- and -10-associated ring proteins results in sensitization to death ligands and inhibition of tumor cell growth. Proc Natl Acad Sci USA 2004; 101:6170-5.
57. Irusta PM, Chen YB, Hardwick JM. Viral modulators of cell death provide new links to old pathways. Curr Opin Cell Biol 2003; 15:700-5.
58. Leblanc AC. Natural cellular inhibitors of caspases. Prog Neuropsychopharmacol Biol Psychiatry 2003; 27:215-29.
59. Deveraux QL, Takahashi R, Salvesen GS, Reed JC. X-linked IAP is a direct inhibitor of cell-death proteases. Nature 1997; 388:300-4.
60. Takahashi R, Deveraux Q, Tamm I, Welsh K, Assa-Munt N, Salvesen GS, Reed JC. A single bir domain of XIAP sufficient for inhibiting caspases. J Biol Chem 1998; 273:7787-90.
61. Chai J, Shiozaki E, Srinivasula SM, Wu Q, Datta P, Alnemri ES, Shi Y, Dataa P. Structural basis of caspase-7 inhibition by XIAP. Cell 2001; 104:769-80.
62. Suzuki Y, Nakabayashi Y, Takahashi R. Ubiquitin-protein ligase activity of x-linked inhibitor of apoptosis protein promotes proteasomal degradation of caspase-3 and enhances its anti-apoptotic effect in FAS-induced cell death. Proc Natl Acad Sci USA 2001; 98:8662-7.
63. Huang H, Joazeiro CA, Bonfoco E, Kamada S, Leverson JD, Hunter T. The inhibitor of apoptosis, Ciap2, functions as a ubiquitin-protein ligase and promotes in vitro monoubiquitination of caspases 3 and 7. J Biol Chem 2000; 275:26661-4.
64. Deveraux QL, Leo E, Stennicke HR, Welsh K, Salvesen GS, Reed JC. Cleavage of human inhibitor of apoptosis protein XIAP results in fragments with distinct specificities for caspases. EMBO J 1999; 18:5242-51.
65. Du C, Fang M, Li Y, Li L, Wang X. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by Eliminating IAP inhibition. Cell 2000; 102:33-42.
66. Creagh EM, Murphy BM, Duriez PJ, Duckett CS, Martin SJ. Smac/Diablo antagonizes ubiquitin ligase activity of inhibitor of apoptosis proteins. J Biol Chem 2004; 279:26906-14.
67. Baud V, Karin M. Signal transduction by tumor necrosis factor and its relatives. Trends Cell Biol 2001; 11:372-7.

68. Chan FK, Chun HJ, Zheng L, Siegel RM, Bui KL, Lenardo MJ. A domain in TNF receptors that mediates ligand-independent receptor assembly and signaling. Science 2000; 288:2351-4.
69. Walczak H, Krammer PH. The CD95 (Apo-1/FAS) and the trail (Apo-2l) apoptosis systems. Exp Cell Res 2000; 256:58-66.
70. Algeciras-Schimnich A, Shen L, Barnhart BC, Murmann AE, Burkhardt JK, Peter ME. Molecular ordering of the initial signaling events of CD95. Mol Cell Biol 2002; 22:207-20.
71. Wajant H, Pfizenmaier K, Scheurich P. Tumor necrosis factor signaling. Cell Death Differ 2003; 10:45-65.
72. Jiang Y, Woronicz JD, Liu W, Goeddel DV. Prevention of constitutive TNF receptor 1 signaling by silencer of death domains. Science 1999; 283:543-6.
73. Hsu H, Xiong J, Goeddel DV. The TNF receptor 1-associated protein tradd signals cell death and NF-κB activation. Cell 1995; 81:495-504.
74. Chen G, Goeddel DV. TNF-R1 signaling: A beautiful pathway. Science 2002; 296:1634-5.
75. Devin A, Lin Y, Liu ZG. The role of the death-domain kinase RIP in tumour-necrosis-factor-induced activation of mitogen-activated protein kinases. Embo Rep 2003; 4:623-7.
76. Aggarwal BB. Signalling pathways of the TNF superfamily: A double-edged sword. Nat Rev Immunol 2003; 3.
77. Lee SY, Reichlin A, Santana A, Sokol KA, Nussenzweig MC, Choi Y. TRAF2 is essential for JNK but not NF-κB activation and regulates lymphocyte proliferation and survival. Immunity 1997; 7:703-13.
78. Tournier C, Dong C, Turner TK, Jones SN, Flavell RA, Davis RJ. MKK7 is an essential component of the JNK signal transduction pathway activated by proinflammatory cytokines. Genes Dev 2001; 15:1419-26.
79. Shaulian E, Karin M. AP-1 as a regulator of cell life and death. Nat Cell Biol 2002; 4:E131-6.
80. Stehlik C, De Martin R, Kumabashiri I, Schmid JA, Binder BR, Lipp J. Nuclear factor (NF)-κB-regulated X-chromosome-linked IAP gene expression protects endothelial cells from tumor necrosis factor α-induced apoptosis. J Exp Med 1998; 188:211-6.
81. Wang CY, Mayo MW, Baldwin Jr AS. TNF- and cancer therapy-induced apoptosis: Potentiation by inhibition of NF-κB. Science 1996; 274:784-7.
82. Beg AA, Sha WC, Bronson RT, Ghosh S, Baltimore D. Embryonic lethality and liver degeneration in mice lacking the rela component of NF-κB. Nature 1995; 376:167-70.
83. Yeh WC, Pompa JL, McCurrach ME, Shu HB, Elia AJ, Shahinian A, Ng M, Wakeham A, Khoo W, Mitchell K, El-Deiry WS, Lowe SW, Goeddel DV, Mak TW. FADD: Essential for embryo development and signaling from some, but not all, inducers of apoptosis. Science 1998; 279:1954-8.
84. Thorburn A. Death receptor-induced cell killing. Cell Signal 2004; 16:139-44.
85. Harper N, Hughes M, Macfarlane M, Cohen GM. FAS-associated death domain protein and caspase-8 are not recruited to the tumor necrosis factor receptor 1 signaling complex during tumor necrosis factor-induced apoptosis. J Biol Chem 2003; 278:25534-41.
86. Micheau O, Tschopp J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. Cell 2003; 114:181-90.
87. Duan H, Dixit VM. RAIDD is a new 'death' adaptor molecule. Nature 1997; 385:86-9.
88. Kim JW, Choi EJ, Joe CO. Activation of death-inducing signaling complex (DISC) by pro-apoptotic C-terminal fragment of RIP. Oncogene 2000; 19:4491-9.
89. Holler N, Zaru R, Micheau O, Thome M, Attinger A, Valitutti S, Bodmer JL, Schneider P, Seed B, Tschopp J. FAS triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. Nat Immunol 2000; 1:489-95.
90. Hochedlinger K, Wagner EF, Sabapathy K. Differential effects of JNK1 and JNK2 on signal specific induction of apoptosis. Oncogene 2002; 21:2441-5.
91. Tang G, Minemoto Y, Dibling B, Purcell NH, Li Z, Karin M, Lin A. Inhibition of JNK activation through NF-κB target genes. Nature 2001; 414:313-7.
92. Deng Y, Ren X, Yang L, Lin Y, Wu X. A JNK-dependent pathway is required for TNFα-induced apoptosis. Cell 2003; 115:61-70.
93. Ventura JJ, Cogswell P, Flavell RA, Baldwin AS Jr, Davis RJ. JNK potentiates TNF-stimulated necrosis by increasing the production of cytotoxic reactive oxygen species. Genes Dev 2004; 18:2905-15.
94. Sakon S, Xue X, Takekawa M, Sasazuki T, Okazaki T, Kojima Y, Piao JH, Yagita H, Okumura K, Doi T, Nakano H. NF-κB inhibits TNF-induced accumulation of ROS that mediate prolonged MAPK activation and necrotic cell death. Embo J 2003; 22:3898-909.
95. Pham CG, Bubici C, Zazzeroni F, Papa S, Jones J, Alvarez K, Jayawardena S, De Smaele E, Cong R, Beaumont C, Torti FM, Torti SV, Franzoso G. Ferritin heavy chain upregulation by NF-κB inhibits TNFα-induced apoptosis by suppressing reactive oxygen species. Cell 2004; 119:529-42.
96. Nagata S. FAS ligand-induced apoptosis. Annu Rev Genet 1999; 33:29-55.
97. Takahashi T, Tanaka M, Brannan CI, Jenkins NA, Copeland NG, Suda T, Nagata S. Generalized lymphoproliferative disease in mice, caused by a point mutation in the FAS ligand. Cell 1994; 76:969-76.
98. Wu J, Wilson J, He J, Xiang L, Schur PH, Mountz JD. FAS ligand mutation in a patient with systemic lupus erythematosus and lymphoproliferative disease. J Clin Invest 1996; 98:1107-13.
99. Nagata S. Apoptosis by death factor. Cell 1997; 88:355-65.
100. Kischkel FC, Hellbardt S, Behrmann I, Germer M, Pawlita M, Krammer PH, Peter ME. Cytotoxicity-dependent Apo-1 (FAS/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. EMBO J 1995; 14:5579-88.
101. Chinnaiyan AM, O'rourke K, Tewari M, Dixit VM. FADD, a novel death domain-containing protein, interacts with the death domain of FAS and initiates apoptosis. Cell 1995; 81:505-12.
102. Alnemri ES, Livingston DJ, Nicholson DW, Salvesen G, Thornberry NA, Wong WW, Yuan J. Human Ice/CED-3 protease nomenclature. Cell 1996; 87:171.
103. Medema JP, Scaffidi C, Kischkel FC, Shevchenko A, Mann M, Krammer PH, Peter ME. Flice is activated by association with the CD95 death-inducing signaling complex (DISC). EMBO J 1997; 16:2794-804.
104. Lavrik I, Krueger A, Schmitz I, Baumann S, Weyd H, Krammer PH, Kirchhoff S. The active caspase-8 heterotetramer is formed at the CD95 DISC. Cell Death Differ 2003; 10:144-5.
105. Zhang J, Cado D, Chen A, Kabra NH, Winoto A. FAS-mediated apoptosis and activation-induced T-cell proliferation are defective in mice lacking FADD/Mort1. Nature 1998; 392:296-300.
106. Scaffidi C, Fulda S, Srinivasan A, Friesen C, Li F, Tomaselli KJ, Debatin KM, Krammer PH, Peter ME. Two CD95 (Apo-1/FAS) signaling pathways. Embo J 1998; 17:1675-87.
107. Wang X. The expanding role of mitochondria in apoptosis. Genes Dev 2001; 15:2922-33.
108. Sprick MR, Rieser E, Stahl H, Grosse-Wilde A, Weigand MA, Walczak H. Caspase-10 is recruited to and activated at the native trail and CD95 death-inducing signalling complexes in a FADD-dependent manner but can not functionally substitute caspase-8. Embo J 2002; 21:4520-30.
109. Wang J, Zheng L, Lobito A, Chan FK, Dale J, Sneller M, Yao X, Puck JM, Straus SE, Lenardo MJ. Inherited human caspase 10 mutations underlie defective lymphocyte and dendritic cell apoptosis in autoimmune lymphoproliferative syndrome type II. Cell 1999; 98:47-58.
110. Irmler M, Thome M, Hahne M, Schneider P, Hofmann K, Steiner V, Bodmer JL, Schroter M, Burns K, Mattmann C, Rimoldi D, French LE, Tschopp J. Inhibition of death receptor signals by cellular flip. Nature 1997; 388:190-5.
111. Inohara N, Koseki T, Hu Y, Chen S, Nunez G. Clarp, a death effector domain-containing protein interacts with caspase-8 and regulates apoptosis. Proc Natl Acad Sci USA 1997; 94:10717-22.
112. Krueger A, Schmitz I, Baumann S, Krammer PH, Kirchhoff S. Cellular flice-inhibitory protein splice variants inhibit different steps of caspase-8 activation at the CD95 death-inducing signaling complex. J Biol Chem 2001; 276:20633-40.
113. Jin Z, Mcdonald IIIrd ER, Dicker DT, El-Deiry WS. Deficient tumor necrosis factor-related apoptosis-inducing ligand (Trail) death receptor transport to the cell surface in human colon cancer cells selected for resistance to trail-induced apoptosis. J Biol Chem 2004; 279:35829-39.
114. Yeh WC, Itie A, Elia AJ, Ng M, Shu HB, Wakeham A, Mirtsos C, Suzuki N, Bonnard M, Goeddel DV, Mak TW. Requirement for casper (C-Flip) in regulation of death receptor-induced apoptosis and embryonic development. Immunity 2000; 12:633-42.
115. Micheau O, Thome M, Schneider P, Holler N, Tschopp J, Nicholson DW, Briand C, Grutter MG. The long form of flip is an activator of caspase-8 at the FAS death-inducing signaling complex. J Biol Chem 2002; 277:45162-71.
116. Chang DW, Xing Z, Pan Y, Algeciras-Schimnich A, Barnhart BC, Yaish-Ohad S, Peter ME, Yang X. C-Flip(L) is a dual function regulator for caspase-8 activation and CD95-mediated apoptosis. Embo J 2002; 21:3704-14.
117. Yang X, Khosravi-Far R, Chang HY, Baltimore D. Daxx, a novel FAS-binding protein that activates JNK and apoptosis. Cell 1997; 89:1067-76.
118. Sato T, Irie S, Kitada S, Reed JC. FAP-1: A protein tyrosine phosphatase that associates with FAS. Science 1995; 268:411-5.
119. Stanger BZ, Leder P, Lee TH, Kim E, Seed B. RIP: A novel protein containing a death domain that interacts with FAS/Apo-1 (CD95) in yeast and causes cell death. Cell 1995; 81:513-23.
120. Thomas WD, Hersey P. TNF-related apoptosis-inducing ligand (Trail) induces apoptosis in FAS ligand-resistant melanoma cells and mediates CD4 T cell killing of target cells. J Immunol 1998; 161:2195-200.
121. Smyth MJ, Cretney E, Takeda K, Wiltrout RH, Sedger LM, Kayagaki N, Yagita H, Okumura K.. Tumor necrosis factor-related apoptosis-inducing ligand (Trail) contributes to interferon gamma-dependent natural killer cell protection from tumor metastasis. J Exp Med 2001; 193:661-70.
122. Takeda K, Hayakawa Y, Smyth MJ, Kayagaki N, Yamaguchi N, Kakuta S, Iwakura Y, Yagita H, Okumura K.. Involvement of tumor necrosis factor-related apoptosis-inducing ligand in surveillance of tumor metastasis by liver natural killer cells. Nat Med 2001; 7:94-100.
123. Cretney E, Takeda K, Yagita H, Glaccum M, Peschon JJ, Smyth MJ. Increased susceptibility to tumor initiation and metastasis in TNF-related apoptosis-inducing ligand-deficient mice. J Immunol 2002; 168:1356-61.
124. Takeda K, Smyth MJ, Cretney E, Hayakawa Y, Kayagaki N, Yagita H, Okumura K.. Critical role for tumor necrosis factor-related apoptosis-inducing ligand in immune surveillance against tumor development. J Exp Med 2002; 195:161-9.
125. Pan G, O'Rourke K, Chinnaiyan AM, Gentz R, Ebner R, Ni J, Dixit VM. The receptor for the cytotoxic ligand trail. Science 1997; 276:111-3.
126. Walczak H, Degli-Esposti MA, Johnson RS, Smolak PJ, Waugh JY, Boiani N, Timour MS, Gerhart MJ, Schooley KA, Smith CA, Goodwin RG, Rauch CT. Trail-R2: A novel apoptosis-mediating receptor for trail. Embo J 1997; 16:5386-97.

127. Wu GS, Burns TF, McDonald ER, 3rd, Jiang W, Meng R, Krantz ID, Kao G, Gan DD, Zhou JY, Muschel R, Hamilton SR, Spinner NB, Markowitz S, Wu G, el-Deiry. Killer/DR5 is a DNA damage-inducible p53-regulated death receptor gene. Nat Genet 1997; 17:141-3.
128. Sheridan JP, Marsters SA, Pitti RM, Gurney A, Skubatch M, Baldwin D, Ramakrishnan L, Gray CL, Baker K, Wood WI, Goddard AD, Godowski P, Ashkenazi A. Control of trail-induced apoptosis by a family of signaling and decoy receptors. Science 1997; 277:818-21.
129. Pan G, Ni J, Wei YF, Yu G, Gentz R, Dixit VM. An antagonist decoy receptor and a death domain-containing receptor for trail. Science 1997; 277:815-8.
130. Emery JG, McDonnell P, Burke MB, Deen KC, Lyn S, Silverman C, Dul E, Appelbaum ER, Eichman C, DiPrinzio R, Dodds RA, James IE, Rosenberg M, Lee JC, Young PR. Osteoprotegerin is a receptor for the cytotoxic ligand trail. J Biol Chem 1998; 273:14363-7.
131. Ashkenazi A. Targeting death and decoy receptors of the tumour-necrosis factor superfamily. Nat Rev Cancer 2002; 2:420-30.
132. Deng Y, Lin Y, Wu X. Trail-induced apoptosis requires bax-dependent mitochondrial release of Smac/Diablo. Genes Dev 2002; 16:33-45.
133. Lin Y, Devin A, Cook A, Keane MM, Kelliher M, Lipkowitz S, Liu ZG. The death domain kinase RIP is essential for trail (Apo2l)-induced activation of Iκb kinase and C-jun N-terminal kinase. Mol Cell Biol 2000; 20:6638-45.
134. Ashkenazi A, Pai RC, Fong S, Leung S, Lawrence DA, Marsters SA, Blackie C, Chang L, McMurtrey AE, Hebert A, DeForge L, Koumenis IL, Lewis D, Harris L, Bussiere J, Koeppen H, Shahrokh Z, Schwall RH. Safety and antitumor activity of recombinant soluble apo2 ligand. J Clin Invest 1999; 104:155-62.
135. Jo M, Kim TH, Seol DW, Esplen JE, Dorko K, Billiar TR, Strom SC. Apoptosis induced in normal human hepatocytes by tumor necrosis factor-related apoptosis-inducing ligand. Nat Med 2000; 6:564-7.
136. Lawrence D, Shahrokh Z, Marsters S, Achilles K, Shih D, Mounho B, Hillan K, Totpal K, DeForge L, Schow P, Hooley J, Sherwood S, Pai R, Leung S, Khan L, Gliniak B, Bussiere J, Smith CA, Strom SS, Kelley S, Fox JA, Thomas D, Ashkenazi A. Differential hepatocyte toxicity of recombinant apo2l/trail versions. Nat Med 2001; 7:383-5.
137. Ozoren N, Fisher MJ, Kim K, Liu CX, Genin A, Shifman Y, Dicker DT, Spinner NB, Lisitsyn NA, El-Deiry WS. Homozygous deletion of the death receptor DR4 gene in a nasopharyngeal cancer cell line is associated with trail resistance. Int J Oncol 2000; 16:917-25.
138. Ichikawa K, Liu W, Zhao L, Wang Z, Liu D, Ohtsuka T, Zhang H, Mountz JD, Koopman WJ, Kimberly RP, Zhou T. Tumoricidal activity of a novel anti-human DR5 monoclonal antibody without hepatocyte cytotoxicity. Nat Med 2001; 7:954-60.
139. Kim SH, Kim K, Kwagh JG, Dicker DT, Herlyn M, Rustgi AK, Chen Y, El-Deiry WS. Death Induction by recombinant native trail and its prevention by a caspase 9 inhibitor in primary human esophageal epithelial cells. J Biol Chem 2004; 279:40044-52.
140. Ozoren N, Kim K, Burns TF, Dicker DT, Moscioni AD, El-Deiry WS. The caspase 9 inhibitor Z-Lehd-Fmk protects human liver cells while permitting death of cancer cells exposed to tumor necrosis factor-related apoptosis-inducing ligand. Cancer Res 2000; 60:6259-65.
141. Jin Z, Mcdonald IIIrd ER, Dicker DT, El-Deiry WS. Deficient tumor necrosis factor-related apoptosis-inducing ligand (Trail) death receptor transport to the cell surface in human colon cancer cells selected for resistance to trail-induced apoptosis. J Biol Chem 2004; 279:35829-39.
142. Ren YG, Wagner KW, Knee DA, Aza-Blanc P, Nasoff M, Deveraux QL. Differential regulation of the trail death receptors DR4 and DR5 by the signal recognition particle. Mol Biol Cell 2004; 15:5064-74.
143. Zhang XD, Franco A, Myers K, Gray C, Nguyen T, Hersey P. Relation of TNF-related apoptosis-inducing ligand (Trail) receptor and flice-inhibitory protein expression to trail-induced apoptosis of melanoma. Cancer Res 1999; 59:2747-53.
144. Mandruzzato S, Brasseur F, Andry G, Boon T, Van Der Bruggen P. A casp-8 mutation recognized by cytolytic t lymphocytes on a human head and neck carcinoma. J Exp Med 1997; 186:785-93.
145. Janicke RU, Ng P, Sprengart ML, Porter AG. Caspase-3 is required for α-fodrin cleavage but dispensable for cleavage of other death substrates in apoptosis. J Biol Chem 1998; 273:15540-5.
146. Yang XH, Sladek TL, Liu X, Butler BR, Froelich CJ, Thor AD. Reconstitution of caspase 3 sensitizes MCF-7 breast cancer cells to doxorubicin- and etoposide-induced apoptosis. Cancer Res 2001; 61:348-54.
147. Srinivasula SM, Ahmad M, Guo Y, Zhan Y, Lazebnik Y, Fernandes-Alnemri T, Alnemri ES. Identification of an endogenous dominant-negative short isoform of caspase-9 that can regulate apoptosis. Cancer Res 1999; 59:999-1002.
148. Baylin S, Bestor TH. Altered methylation patterns in cancer cell genomes: Cause or consequence? Cancer Cell 2002; 1:299-305.
149. Philchenkov A, Zavelevich M, Kroczak TJ, Los M. Caspases and cancer: Mechanisms of inactivation and new treatment modalities. Exp Oncol 2004; 26:82-97.
150. Kim JH, Ajaz M, Lokshin A, Lee YJ. Role of antiapoptotic proteins in tumor necrosis factor-related apoptosis-inducing ligand and cisplatin-augmented apoptosis. Clin Cancer Res 2003; 9:3134-41.
151. Ricci MS, Jin Z, Dews M, Yu D, Thomas-Tikhonenko A, Dicker DT, El-Deiry WS. Direct repression of flip expression by c-Myc is a major determinant of trail sensitivity. Mol Cell Biol 2004; 24:8541-55.
152. Burns TF, El-Deiry WS. Identification of inhibitors of trail-induced death (Itids) in the trail-sensitive colon carcinoma cell line Sw480 using a genetic approach. J Biol Chem 2001; 276:37879-86.
153. Griffith TS, Chin WA, Jackson GC, Lynch DH, Kubin MZ. Intracellular regulation of trail-induced apoptosis in human melanoma cells. J Immunol 1998; 161:2833-40.
154. Kim Y, Suh N, Sporn M, Reed JC. An inducible pathway for degradation of flip protein sensitizes tumor cells to trail-induced apoptosis. J Biol Chem 2002; 277:22320-9.
155. Mitsiades N, Mitsiades CS, Poulaki V, Anderson KC, Treon SP. Intracellular regulation of tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in human multiple myeloma cells. Blood 2002; 99:2162-71.
156. Suliman A, Lam A, Datta R, Srivastava RK. Intracellular mechanisms of trail: Apoptosis through mitochondrial-dependent and -independent pathways. Oncogene 2001; 20:2122-33.
157. Zhang XD, Zhang XY, Gray CP, Nguyen T, Hersey P. Tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis of human melanoma is regulated by Smac/Diablo Release from mitochondria. Cancer Res 2001; 61:7339-48.
158. Fulda S, Wick W, Weller M, Debatin KM. Smac agonists sensitize for Apo2l/Trail- or anticancer drug-induced apoptosis and induce regression of malignant glioma in vivo. Nat Med 2002; 8:808-15.
159. Li L, Thomas RM, Suzuki H, De Brabander JK, Wang X, Harran PG. A small molecule smac mimic potentiates trail- and TNFα-mediated cell death. Science 2004; 305:1471-4.
160. Keogh SA, Walczak H, Bouchier-Hayes L, Martin SJ. failure of Bcl-2 to block cytochrome C redistribution during trail-induced apoptosis. Febs Lett 2000; 471:93-8.
161. Munshi A, Pappas G, Honda T, McDonnell TJ, Younes A, Li Y, Meyn RE. Trail (Apo-2l) induces apoptosis in human prostate cancer cells that is inhibitable by Bcl-2. Oncogene 2001; 20:3757-65.
162. Ozoren N, El-Deiry WS. Defining characteristics of types I and II apoptotic cells in response to trail. Neoplasia 2002; 4:551-7.
163. Shankar S, Srivastava RK. Enhancement of therapeutic potential of trail by cancer chemotherapy and irradiation: Mechanisms and clinical implications. Drug Resist Updat 2004; 7:139-56.
164. Martelli AM, Tazzari PL, Tabellini G, Bortul R, Billi AM, Manzoli L, Ruggeri A, Conte R, Cocco L. A new selective AKT pharmacological inhibitor reduces resistance to chemotherapeutic drugs, trail, all-trans-retinoic acid, and ionizing radiation of human leukemia cells. Leukemia 2003; 17:1794-805.
165. Nam SY, Jung GA, Hur GC, Chung HY, Kim WH, Seol DW, Lee BL. Upregulation of flip(S) by AKT, a possible inhibition mechanism of trail-induced apoptosis in human gastric cancers. Cancer Sci 2003; 94:1066-73.
166. Tewari M, Vidal M. RNAi on the apoptosis trail: The mammalian cell genetic screen comes of age. Dev Cell 2003; 5:534-5.
167. Wang Y, Engels IH, Knee DA, Nasoff M, Deveraux QL, Quon KC. Synthetic lethal targeting of Myc by activation of the DR5 death receptor pathway. Cancer Cell 2004; 5:501-12.
168. Klefstrom J, Verschuren EW, Evan G. c-Myc augments the apoptotic activity of cytosolic death receptor signaling proteins by engaging the mitochondrial apoptotic pathway. J Biol Chem 2002; 277:43224-32.
169. Liu X, Yue P, Khuri FR, Sun SY. p53 upregulates death receptor 4 expression through an intronic p53 binding site. Cancer Res 2004; 64:5078-83.
170. Eid MA, Lewis RW, Abdel-Mageed AB, Kumar MV. Reduced response of prostate cancer cells to trail is modulated by NF κB-mediated inhibition of caspases and bid activation. Int J Oncol 2002; 21:111-7.
171. Luo JL, Maeda S, Hsu LC, Yagita H, Karin M. Inhibition of NF-κB in cancer cells converts inflammation- induced tumor growth mediated by tnfα to trail-mediated tumor regression. Cancer Cell 2004; 6:297-305.
172. Shetty S, Gladden JB, Henson ES, Hu X, Villanueva J, Haney N, Gibson SB. Tumor necrosis factor-related apoptosis inducing ligand (Trail) up-regulates death receptor 5 (DR5) mediated by NF κB activation in epithelial derived cell lines. Apoptosis 2002; 7:413-20.
173. Green DR, Kroemer G. The pathophysiology of mitochondrial cell death. Science 2004; 305:626-9.
174. Fesik SW. Insights into programmed cell death through structural biology. Cell 2000; 103:273-82.
175. Hengartner MO. The biochemistry of apoptosis. Nature 2000; 407:770-6.
176. Zong WX, Lindsten T, Ross AJ, Macgregor GR, Thompson CB. BH3-only proteins that bind pro-survival Bcl-2 family members fail to induce apoptosis in the absence of BAX and BAK. Genes Dev 2001; 15:1481-6.
177. Cheng EH, Wei MC, Weiler S, Flavell RA, Mak TW, Lindsten T, Korsmeyer SJ. Bcl-2, Bcl-X(L) sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis. Mol Cell 2001; 8:705-11.
178. Korsmeyer SJ. Regulators of cell death. Trends Genet 1995; 11:101-5.
179. Kinloch RA, Treherne JM, Furness LM, Hajimohamadreza I. The pharmacology of apoptosis. Trends Pharmacol Sci 1999; 20:35-42.
180. Danial NN, Korsmeyer SJ. Cell death: Critical control points. Cell 2004; 116:205-19.
181. Veis DJ, Sorenson CM, Shutter JR, Korsmeyer SJ. Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. Cell 1993; 75:229-40.
182. Sentman CL, Shutter JR, Hockenbery D, Kanagawa O, Korsmeyer SJ. Bcl-2 inhibits multiple forms of apoptosis but not negative selection in thymocytes. Cell 1991; 67:879-88.
183. Strasser A, Harris AW, Cory S. Bcl-2 transgene inhibits t cell death and perturbs thymic self-censorship. Cell 1991; 67:889-99.

184. Harada H, Grant S. Apoptosis regulators. Rev Clin Exp Hematol 2003; 7:117-38.
185. Schuler M, Green DR. Mechanisms of p53-dependent apoptosis. Biochem Soc Trans 2001; 7:684-8.
186. Chipuk JE, Green DR. p53's believe it or not: Lessons on transcription-independent death. J Clin Immunol 2003; 23:355-61.
187. Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME. AKT phosphorylation of bad couples survival signals to the cell-intrinsic death machinery. Cell 1997; 91:231-41.
188. Bouillet P, Metcalf D, Huang DC, Tarlinton DM, Kay TW, Kontgen F, Adams JM, Strasser A. Proapoptotic Bcl-2 relative bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. Science 1999; 286:1735-8.
189. Newmeyer DD, Ferguson-Miller S. Mitochondria: Releasing power for life and unleashing the machineries of death. Cell 2003; 112:481-90.
190. Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in cell-free extracts: Requirement for dATP and Cytochrome C. Cell 1996; 86:147-57.
191. Zou H, Henzel WJ, Liu X, Lutschg A, Wang X. Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome C-dependent activation of caspase-3. Cell 1997; 90:405-13.
192. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X. Cytochrome C And dATP-dependent formation of Apaf-1/Caspase-9 complex initiates an apoptotic protease cascade. Cell 1997; 91:479-89.
193. Cecconi F, Alvarez-Bolado G, Meyer BI, Roth KA, Gruss P. Apaf1 (CED-4 Homolog) regulates programmed cell death in mammalian development. Cell 1998; 94:727-37.
194. Yoshida H, Kong YY, Yoshida R, Elia AJ, Hakem A, Hakem R, Penninger JM, Mak TW. Apaf1 is required for mitochondrial pathways of apoptosis and brain development. Cell 1998; 94:739-50.
195. Suzuki Y, Imai Y, Nakayama H, Takahashi K, Takio K, Takahashi R. A serine protease, Htra2, is released from the mitochondria and interacts with XIAP, inducing cell death. Mol Cell 2001; 8:613-21.
196. Hegde R, Srinivasula SM, Datta P, Madesh M, Wassell R, Zhang Z, Cheong N, Nejmeh J, Fernandes-Alnemri T, Hoshino S, Alnemri ES. The polypeptide chain-releasing factor GSPT1/eRF3 is proteolytically processed into an iap-binding protein. J Biol Chem 2003; 278:38699-706.
197. Yang QH, Church-Hajduk R, Ren J, Newton ML, Du C. Omi/Htra2 catalytic cleavage of inhibitor of apoptosis (IAP) irreversibly inactivates IAPs and facilitates caspase activity in apoptosis. Genes Dev 2003; 17:1487-96.
198. Jones JM, Datta P, Srinivasula SM, Ji W, Gupta S, Zhang Z, Davies E, Hajnoczky G, Saunders TL, Van Keuren ML, Fernandes-Alnemri T, Meisler MH, Alnemri ES. Loss of Omi mitochondrial protease activity causes the neuromuscular disorder of Mnd2 mutant mice. Nature 2003; 425:721-7.
199. Katoh I, Tomimori Y, Ikawa Y, Kurata S. Dimerization and processing of procaspase-9 by redox stress in mitochondria. J Biol Chem 2004; 279:15515-23.
200. Susin SA, Daugas E, Ravagnan L, Samejima K, Zamzami N, Loeffler M, Costantini P, Ferri KF, Irinopoulou T, Prevost MC, Brothers G, Mak TW, Penninger J, Earnshaw WC, Kroemer G. Two distinct pathways leading to nuclear apoptosis. J Exp Med 2000; 192:571-80.
201. Joza N, Susin SA, Daugas E, Stanford WL, Cho SK, Li CY, Sasaki T, Elia AJ, Cheng HY, Ravagnan L, Ferri KF, Zamzami N, Wakeham A, Hakem R, Yoshida H, Kong YY, Mak TW, Zuniga-Pflucker JC, Kroemer G, Penninger JM. Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death. Nature 2001; 410:549-54.
202. Li LY, Luo X, Wang X. Endonuclease G is an apoptotic dnase when released from mitochondria. Nature 2001; 412:95-9.
203. Donovan M, Cotter TG. Control of mitochondrial integrity by Bcl-2 family members and caspase-independent cell death. Biochim Biophys Acta 2004; 1644:133-47.
204. Breckenridge DG, Germain M, Mathai JP, Nguyen M, Shore GC. Regulation of apoptosis by endoplasmic reticulum pathways. Oncogene 2003; 22:8608-18.
205. Rao RV, Ellerby HM, Bredesen DE. Coupling endoplasmic reticulum stress to the cell death program. Cell Death Differ 2004; 11:372-80.
206. Nutt LK, Pataer A, Pahler J, Fang B, Roth J, McConkey DJ, Swisher SG. BAX and BAK promote apoptosis by modulating endoplasmic reticular and mitochondrial Ca2+ stores. J Biol Chem 2002; 277:9219-25.
207. Scorrano L, Oakes SA, Opferman JT, Cheng EH, Sorcinelli MD, Pozzan T, Korsmeyer SJ. BAX and BAK regulation of endoplasmic reticulum Ca2+: A control point for apoptosis. Science 2003; 300:135-9.
208. Zong WX, Li C, Hatzivassiliou G, Lindsten T, Yu QC, Yuan J, Thompson CB. BAX and BAK can localize to the endoplasmic reticulum to initiate apoptosis. J Cell Biol 2003; 162:59-69.
209. Szegezdi E, Fitzgerald U, Samali A. Caspase-12 and Er-Stress-Mediated apoptosis: The story so far. Ann NY Acad Sci 2003; 1010:186-94.
210. Verkhratsky A, Toescu EC. Endoplasmic reticulum Ca^{2+} homeostasis and neuronal death. J Cell Mol Med 2003; 7:351-61.
211. Yin XM, Wang K, Gross A, Zhao Y, Zinkel S, Klocke B, Roth KA, Korsmeyer SJ. Two CD95 (Apo-1/FAS) signaling pathways. Embo J 1998; 17:1675-87.
212. Zheng TS, Flavell RA. Divinations and surprises: genetic analysis of caspase function in mice. Exp Cell Res 2000; 256:67-73.
213. Zou H, Yang R, Hao J, Wang J, Sun C, Fesik SW, Wu JC, Tomaselli KJ, Armstrong RC. Regulation of the Apaf-1/Caspase-9 Apoptosome by Caspase-3 and XIAP. J Biol Chem 2003; 278:8091-8.
214. Engels IH, Stepczynska A, Stroh C, Lauber K, Berg C, Schwenzer R, Wajant H, Janicke RU, Porter AG, Belka C, Gregor M, Schulze-Osthoff K, Wesselborg S. Caspase-8/FLICE functions as an executioner caspase in anticancer drug-induced apoptosis. Oncogene 2000; 19:4563-73.
215. Tang D, Lahti JM, Kidd VJ. Caspase-8 activation and bid cleavage contribute to MCF7 cellular execution in a caspase-3-dependent manner during staurosporine-mediated apoptosis. J Biol Chem 2000; 275:9303-7.
216. Cowling V, Downward J. Caspase-6 is the direct activator of caspase-8 in the cytochrome c-induced apoptosis pathway: absolute requirement for removal of caspase-6 prodomain. Cell Death Differ 2002; 9:1046-56.
217. Bennett M, Macdonald K, Chan SW, Luzio JP, Simari R, Weissberg P. Cell surface trafficking of Fas: a rapid mechanism of p53-mediated apoptosis. Science 1998; 282:290-3.
218. Kasibhatla S, Brunner T, Genestier L, Echeverri F, Mahboubi A, Green DR. DNA damaging agents induce expression of Fas ligand and subsequent apoptosis in T lymphocytes via the activation of NF-kappa B and AP-1. Mol Cell 1998; 1:543-51.
219. Hipfner DR, Cohen SM. Connecting proliferation and apoptosis in development and disease. Nat Rev Mol Cell Biol 2004; 5:805-15.
220. Pelengaris S, Khan M, Evan GI. Suppression of Myc-induced apoptosis in beta cells exposes multiple oncogenic properties of Myc and triggers carcinogenic progression. Cell 2002; 109:321-34.
221. de la Cova C, Abril M, Bellosta P, Gallant P, Johnston LA. Drosophila myc regulates organ size by inducing cell competition. Cell 2004; 117:107-16.
222. Juin P, Hueber AO, Littlewood T, Evan G. c-Myc-induced sensitization to apoptosis is mediated through cytochrome c release. Genes Dev 1999; 13:1367-81.
223. Juin P, Hunt A, Littlewood T, Griffiths B, Swigart LB, Korsmeyer S, Evan G. c-Myc functionally cooperates with Bax to induce apoptosis. Mol Cell Biol 2002; 22:6158-69.
224. Zindy F, Eischen CM, Randle DH, Kamijo T, Cleveland JL, Sherr CJ, Roussel MF. Myc signaling via the ARF tumor suppressor regulates p53-dependent apoptosis and immortalization. Genes Dev 1998; 12:2424-33.
225. Eischen CM, Weber JD, Roussel MF, Sherr CJ, Cleveland JL. Disruption of the ARF-Mdm2-p53 tumor suppressor pathway in Myc-induced lymphomagenesis. Genes Dev 1999; 13:2658-69.
226. Alt JR, Greiner TC, Cleveland JL, Eischen CM. Mdm2 haplo-insufficiency profoundly inhibits Myc-induced lymphomagenesis. Embo J 2003; 22:1442-50.
227. Qi Y, Gregory MA, Li Z, Brousal JP, West K, Hann SR. p19ARF directly and differentially controls the functions of c-Myc independently of p53. Nature 2004; 431:712-7.
228. Hueber AO, Zornig M, Lyon D, Suda T, Nagata S, Evan GI. Requirement for the CD95 receptor-ligand pathway in c-Myc-induced apoptosis. Science 1997; 278:1305-9.
229. Vafa O, Wade M, Kern S, Beeche M, Pandita TK, Hampton GM, Wahl GM. c-Myc can induce DNA damage, increase reactive oxygen species, and mitigate p53 function: a mechanism for oncogene-induced genetic instability. Mol Cell 2002; 9:1031-44.
230. Tanaka H, Matsumura I, Ezoe S, Satoh Y, Sakamaki T, Albanese C, Machii T, Pestell RG, Kanakura Y. E2F1 and c-Myc potentiate apoptosis through inhibition of NF-kappaB activity that facilitates MnSOD-mediated ROS elimination. Mol Cell 2002; 9:1017-29.
231. Sen R, Baltimore D. Multiple nuclear factors interact with the immunoglobulin enhancer sequences. Cell 1986; 46:705-16.
232. Stancovski I, Baltimore D. NF-kappaB activation: the I kappaB kinase revealed? Cell 1997; 91:299-302.
233. Kucharczak J, Simmons MJ, Fan Y, Gelinas C. To be, or not to be: NF-kappaB is the answer--role of Rel/NF-kappaB in the regulation of apoptosis. Oncogene 2003; 22:8961-82.
234. Liu ZG, Hsu H, Goeddel DV, Karin M. Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-kappaB activation prevents cell death. Cell 1996; 87:565-76.
235. Zong WX, Bash J, Gelinas C. Rel blocks both anti-Fas- and TNF alpha-induced apoptosis and an intact Rel transactivation domain is essential for this effect. Cell Death Differ 1998; 5:963-72.
236. Wang CY, Cusack JC, Jr., Liu R, Baldwin AS, Jr. Control of inducible chemoresistance: enhanced anti-tumor therapy through increased apoptosis by inhibition of NF-kappaB. Nat Med 1999; 5:412-7.
237. Li Q, Van Antwerp D, Mercurio F, Lee KF, Verma IM. Severe liver degeneration in mice lacking the IkappaB kinase 2 gene. Science 1999; 284:321-5.
238. Rudolph D, Yeh WC, Wakeham A, Rudolph B, Nallainathan D, Potter J, Elia AJ, Mak TW. Severe liver degeneration and lack of NF-kappaB activation in NEMO/IKKgamma-deficient mice. Genes Dev 2000; 14:854-62.
239. Gosselin K, Abbadie C. Involvement of Rel/NF-kappa B transcription factors in senescence. Exp Gerontol 2003; 38:1271-83.
240. Hettmann T, DiDonato J, Karin M, Leiden JM. An essential role for nuclear factor kappaB in promoting double positive thymocyte apoptosis. J Exp Med 1999; 189:145-58.
241. Ryan KM, Ernst MK, Rice NR, Vousden KH. Role of NF-kappaB in p53-mediated programmed cell death. Nature 2000; 404:892-7.
242. Perkins ND. NF-kappaB: tumor promoter or suppressor? Trends Cell Biol 2004; 14:64-9.
243. Kato T, Jr., Delhase M, Hoffmann A, Karin M. CK2 Is a C-Terminal IkappaB Kinase Responsible for NF-kappaB Activation during the UV Response. Mol Cell 2003; 12:829-39.
244. Varfolomeev EE, Ashkenazi A. Tumor necrosis factor: an apoptosis JuNKie? Cell 2004; 116:491-7.

245. Lin A. Activation of the JNK signaling pathway: breaking the brake on apoptosis. Bioessays 2003; 25:17-24.
246. Kuan CY, Yang DD, Samanta Roy DR, Davis RJ, Rakic P, Flavell RA. The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development. Neuron 1999; 22:667-76.
247. Yamamoto K, Ichijo H, Korsmeyer SJ. BCL-2 is phosphorylated and inactivated by an ASK1/Jun N-terminal protein kinase pathway normally activated at G(2)/M. Mol Cell Biol 1999; 19:8469-78.
248. Basu A, Haldar S. Identification of a novel Bcl-xL phosphorylation site regulating the sensitivity of taxol- or 2-methoxyestradiol-induced apoptosis. FEBS Lett 2003; 538:41-7.
249. Becker EB, Howell J, Kodama Y, Barker PA, Bonni A. Characterization of the c-Jun N-terminal kinase-BimEL signaling pathway in neuronal apoptosis. J Neurosci 2004; 24:8762-70.
250. Du L, Lyle CS, Chambers TC. Characterization of vinblastine-induced Bcl-xL and Bcl-2 phosphorylation: evidence for a novel protein kinase and a coordinated phosphorylation/dephosphorylation cycle associated with apoptosis induction. Oncogene 2004.
250. Becker EB, Howell J, Kodama Y, Barker PA, Bonni A. Characterization of the C-Jun N-terminal kinase-bimel signaling pathway in neuronal apoptosis. J Neurosci 2004; 24:8762-70.
251. Du L, Lyle CS, Chambers TC. Characterization of vinblastine-induced Bcl-xL and Bcl-2 phosphorylation: evidence for a novel protein kinase and a coordinated phosphorylation/dephosphorylation cycle associated with apoptosis induction. Oncogene 2005; 24:107-17.
252. Shimada K, Nakamura M, Ishida E, Kishi M, Yonehara S, Konishi N. C-Jun Nh2-terminal kinase-dependent FAS activation contributes to etoposide-induced apoptosis in p53-mutated prostate cancer cells. Prostate 2003; 55:265-80.
253. Shaulian E, Schreiber M, Piu F, Beeche M, Wagner EF, Karin M. The mammalian UV response: C-Jun induction is required for exit from p53-imposed growth arrest. Cell 2000; 103:897-907.
254. Behrens A, Sibilia M, Wagner EF. Amino-terminal phosphorylation of C-Jun regulates stress-induced apoptosis and cellular proliferation. Nat Genet 1999; 21:326-9.
255. 255. Yu C, Minemoto Y, Zhang J, Liu J, Tang F, Bui TN, Xiang J, Lin A. JNK suppresses apoptosis via phosphorylation of the proapoptotic Bcl-2 family protein BAD. Mol Cell 2004; 13:329-40.
256. Downward J. Mechanisms and consequences of activation of protein kinase B/Akt. Curr Opin Cell Biol 1998; 10:262-7.
257. Chen X, Thakkar H, Tyan F, Gim S, Robinson H, Lee C, Pandey SK, Nwokorie C, Onwudiwe N, Srivastava RK. Constitutively active Akt is an important regulator of TRAIL sensitivity in prostate cancer. Oncogene 2001; 20:6073-83.
258. Wang Q, Wang X, Hernandez A, Hellmich MR, Gatalica Z, Evers BM. Regulation of TRAIL expression by the phosphatidylinositol 3-kinase/Akt/GSK-3 pathway in human colon cancer cells. J Biol Chem 2002; 277:36602-10.
259. Downward J. PI 3-kinase, Akt and cell survival. Semin Cell Dev Biol 2004; 15:177-82.
260. del Peso L, Gonzalez-Garcia M, Page C, Herrera R, Nunez G. Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. Science 1997; 278:687-9.
261. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 1999; 96:857-68.
262. Lauber K, Blumenthal SG, Waibel M, Wesselborg S. Clearance of apoptotic cells: getting rid of the corpses. Mol Cell 2004; 14:277-87.
263. Voll RE, Herrmann M, Roth EA, Stach C, Kalden JR, Girkontaite I. Immunosuppressive effects of apoptotic cells. Nature 1997; 390:350-1.
264. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. J Clin Invest 1998; 101:890-8.
265. Grimsley C, Ravichandran KS. Cues for apoptotic cell engulfment: eat-me, don't eat-me and come-get-me signals. Trends Cell Biol 2003; 13:648-56.
266. Fadok VA, Voelker DR, Campbell PA, Cohen JJ, Bratton DL, Henson PM. Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. J Immunol 1992; 148:2207-16.
267. Schlegel RA, Williamson P. Phosphatidylserine, a death knell. Cell Death Differ 2001; 8:551-63.
268. Ishimoto Y, Ohashi K, Mizuno K, Nakano T. Promotion of the uptake of PS liposomes and apoptotic cells by a product of growth arrest-specific gene, gas6. J Biochem (Tokyo) 2000; 127:411-7.
269. Balasubramanian K, Chandra J, Schroit AJ. Immune clearance of phosphatidylserine-expressing cells by phagocytes. The role of beta2-glycoprotein I in macrophage recognition. J Biol Chem 1997; 272:31113-7.
270. Hanayama R, Tanaka M, Miwa K, Shinohara A, Iwamatsu A, Nagata S. Identification of a factor that links apoptotic cells to phagocytes. Nature 2002; 417:182-7.
271. Ogden CA, deCathelineau A, Hoffmann PR, Bratton D, Ghebrehiwet B, Fadok VA, Henson PM. C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. J Exp Med 2001; 194:781-95.
272. Vandivier RW, Ogden CA, Fadok VA, Hoffmann PR, Brown KK, Botto M, Walport MJ, Fisher JH, Henson PM, Greene KE. Role of surfactant proteins A, D, and C1q in the clearance of apoptotic cells in vivo and in vitro: calreticulin and CD91 as a common collectin receptor complex. J Immunol 2002; 169:3978-86.
273. Hanayama R, Tanaka M, Miyasaka K, Aozasa K, Koike M, Uchiyama Y, Nagata S. Autoimmune disease and impaired uptake of apoptotic cells in MFG-E8-deficient mice. Science 2004; 304:1147-50.
274. Sambrano GR, Steinberg D. Recognition of oxidatively damaged and apoptotic cells by an oxidized low density lipoprotein receptor on mouse peritoneal macrophages: role of membrane phosphatidylserine. Proc Natl Acad Sci U S A 1995; 92:1396-400.
275. Oka K, Sawamura T, Kikuta K, Itokawa S, Kume N, Kita T, Masaki T. Lectin-like oxidized low-density lipoprotein receptor 1 mediates phagocytosis of aged/apoptotic cells in endothelial cells. Proc Natl Acad Sci U S A 1998; 95:9535-40.
276. Fadok VA, Warner ML, Bratton DL, Henson PM. CD36 is required for phagocytosis of apoptotic cells by human macrophages that use either a phosphatidylserine receptor or the vitronectin receptor (alpha v beta 3). J Immunol 1998; 161:6250-7.
277. Fadok VA, Bratton DL, Frasch SC, Warner ML, Henson PM. The role of phosphatidylserine in recognition of apoptotic cells by phagocytes. Cell Death Differ 1998; 5:551-62.
278. Cohen PL, Caricchio R, Abraham V, Camenisch TD, Jennette JC, Roubey RA, Earp HS, Matsushima G, Reap EA. Delayed apoptotic cell clearance and lupus-like autoimmunity in mice lacking the c-mer membrane tyrosine kinase. J Exp Med 2002; 196:135-40.
279. Scott RS, McMahon EJ, Pop SM, Reap EA, Caricchio R, Cohen PL, Earp HS, Matsushima GK. Phagocytosis and clearance of apoptotic cells is mediated by MER. Nature 2001; 411:207-11.
280. Devitt A, Moffatt OD, Raykundalia C, Capra JD, Simmons DL, Gregory CD. Human CD14 mediates recognition and phagocytosis of apoptotic cells. Nature 1998; 392:505-9.
281. Brown S, Heinisch I, Ross E, Shaw K, Buckley CD, Savill J. Apoptosis disables CD31-mediated cell detachment from phagocytes promoting binding and engulfment. Nature 2002; 418:200-3.
282. Lauber K, Bohn E, Krober SM, Xiao YJ, Blumenthal SG, Lindemann RK, Marini P, Wiedig C, Zobywalski A, Baksh S, Xu Y, Autenrieth IB, Schulze-Osthoff K, Belka C, Stuhler G, Wesselborg S. Apoptotic cells induce migration of phagocytes via caspase-3-mediated release of a lipid attraction signal. Cell 2003; 113:717-30.
283. Assuncao Guimaraes C, Linden R. Programmed cell deaths. Apoptosis and alternative deathstyles. Eur J Biochem 2004; 271:1638-50.
284. Proskuryakov SY, Gabai VL, Konoplyannikov AG. Necrosis is an active and controlled form of programmed cell death. Biochemistry (Mosc) 2002; 67:387-408.
285. Lockshin RA, Zakeri Z. Caspase-independent cell death? Oncogene 2004; 23:2766-73.
286. Jaattela M, Tschopp J. Caspase-independent cell death in T lymphocytes. Nat Immunol 2003; 4:416-23.
287. Lenardo MJ. Molecular regulation of T lymphocyte homeostasis in the healthy and diseased immune system. Immunol Res 2003; 27:387-98.
288. Oppenheim RW, Flavell RA, Vinsant S, Prevette D, Kuan CY, Rakic P. Programmed cell death of developing mammalian neurons after genetic deletion of caspases. J Neurosci 2001; 21:4752-60.
289. Edinger AL, Thompson CB. Death by design: apoptosis, necrosis and autophagy. Curr Opin Cell Biol 2004; 16:663-9.
290. Vande Velde C, Cizeau J, Dubik D, Alimonti J, Brown T, Israels S, Hakem R, Greenberg AH. BNIP3 and genetic control of necrosis-like cell death through the mitochondrial permeability transition pore. Mol Cell Biol 2000; 20:5454-68.
291. Eliasson M, Brock S, Bengtsson Ahlberg M. Evidence for mitochondrial metabolism of 7,12-dimethylbenz(a)anthracene in porcine ovaries: comparison with microsomal metabolism. Toxicology 1997; 122:11-21.
292. Okada H, Mak TW. Pathways of apoptotic and non-apoptotic death in tumour cells. Nat Rev Cancer 2004; 4:592-603.
293. Mills KR, Reginato M, Debnath J, Queenan B, Brugge JS. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is required for induction of autophagy during lumen formation in vitro. Proc Natl Acad Sci U S A 2004; 101:3438-43.
294. Cuervo AM. Autophagy: in sickness and in health. Trends Cell Biol 2004; 14:70-7.
295. Yoshimori T. Autophagy: a regulated bulk degradation process inside cells. Biochem Biophys Res Commun 2004; 313:453-8.
296. Wang CW, Klionsky DJ. The molecular mechanism of autophagy. Mol Med 2003; 9:65-76.
297. Tsukada M, Ohsumi Y. Isolation and characterization of autophagy-defective mutants of Saccharomyces cerevisiae. FEBS Lett 1993; 333:169-74.
298. Thumm M, Egner R, Koch B, Schlumpberger M, Straub M, Veenhuis M, Wolf DH. Isolation of autophagocytosis mutants of Saccharomyces cerevisiae. FEBS Lett 1994; 349:275-80.
299. Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, Levine B. Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature 1999; 402:672-6.
300. Yu L, Alva A, Su H, Dutt P, Freundt E, Welsh S, Baehrecke EH, Lenardo MJ. Regulation of an ATG7-beclin 1 program of autophagic cell death by caspase-8. Science 2004; 304:1500-2.
300. Yu L, Alva A, Su H, Dutt P, Freundt E, Welsh S, Baehrecke EH, Lenardo MJ. Regulation of an ATG7-beclin 1 program of autophagic cell death by caspase-8. Science 2004; 304:1500-2.
301. Shimizu S, Kanaseki T, Mizushima N, Mizuta T, Arakawa-Kobayashi S, Thompson CB, Tsujimoto Y. Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. Nat Cell Biol 2004; 6:1221-1228.
302. Garrett S, Kapoor TM. Microtubule assembly: catastrophe factors to the rescue. Curr Biol 2003; 13:R810-2.

303. Brown EJ, Baltimore D. ATR disruption leads to chromosomal fragmentation and early embryonic lethality. Genes Dev 2000; 14:397-402.
304. Takai H, Tominaga K, Motoyama N, Minamishima YA, Nagahama H, Tsukiyama T, Ikeda K, Nakayama K, Nakanishi M. Aberrant cell cycle checkpoint function and early embryonic death in Chk1(-/-) mice. Genes Dev 2000; 14:1439-47.
305. Takada S, Kelkar A, Theurkauf WE. Drosophila checkpoint kinase 2 couples centrosome function and spindle assembly to genomic integrity. Cell 2003; 113:87-99.
306. Bunz F, Dutriaux A, Lengauer C, Waldman T, Zhou S, Brown JP, Sedivy JM, Kinzler KW, Vogelstein B. Requirement for p53 and p21 to sustain G2 arrest after DNA damage. Science 1998; 282:1497-501.
307. Chan TA, Hermeking H, Lengauer C, Kinzler KW, Vogelstein B. 14-3-3Sigma is required to prevent mitotic catastrophe after DNA damage. Nature 1999; 401:616-20.
308. Jordan MA, Wendell K, Gardiner S, Derry WB, Copp H, Wilson L. Mitotic block induced in HeLa cells by low concentrations of paclitaxel (Taxol) results in abnormal mitotic exit and apoptotic cell death. Cancer Res 1996; 56:816-25.
309. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100:57-70.
310. McDonnell TJ, Deane N, Platt FM, Nunez G, Jaeger U, McKearn JP, Korsmeyer SJ. bcl-2-immunoglobulin transgenic mice demonstrate extended B cell survival and follicular lymphoproliferation. Cell 1989; 57:79-88.
311. Schmitt CA, Rosenthal CT, Lowe SW. Genetic analysis of chemoresistance in primary murine lymphomas. Nat Med 2000; 6:1029-35.
312. Cantley LC, Neel BG. New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. Proc Natl Acad Sci U S A 1999; 96:4240-5.
313. Kauffmann-Zeh A, Rodriguez-Viciana P, Ulrich E, Gilbert C, Coffer P, Downward J, Evan G. Suppression of c-Myc-induced apoptosis by Ras signalling through PI(3)K and PKB. Nature 1997; 385:544-8.
314. Douma S, Van Laar T, Zevenhoven J, Meuwissen R, Van Garderen E, Peeper DS. Suppression of anoikis and induction of metastasis by the neurotrophic receptor TrkB. Nature 2004; 430:1034-9.
315. Nagle JA, Ma Z, Byrne MA, White MF, Shaw LM. Involvement of insulin receptor substrate 2 in mammary tumor metastasis. Mol Cell Biol 2004; 24:9726-35.
316. Rayet B, Gelinas C. Aberrant rel/nfkb genes and activity in human cancer. Oncogene 1999; 18:6938-47.
317. Aggarwal BB. Nuclear factor-kappaB: the enemy within. Cancer Cell 2004; 6:203-8.
318. Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 1997; 3:917-21.
319. Ryan KM, Phillips AC, Vousden KH. Regulation and function of the p53 tumor suppressor protein. Curr Opin Cell Biol 2001; 13:332-7.
320. Rampino N, Yamamoto H, Ionov Y, Li Y, Sawai H, Reed JC, Perucho M. Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. Science 1997; 275:967-9.
321. Meijerink JP, Mensink EJ, Wang K, Sedlak TW, Sloetjes AW, de Witte T, Waksman G, Korsmeyer SJ. Hematopoietic malignancies demonstrate loss-of-function mutations of BAX. Blood 1998; 91:2991-7.
322. Krajewski S, Blomqvist C, Franssila K, Krajewska M, Wasenius VM, Niskanen E, Nordling S, Reed JC. Reduced expression of proapoptotic gene BAX is associated with poor response rates to combination chemotherapy and shorter survival in women with metastatic breast adenocarcinoma. Cancer Res 1995; 55:4471-8.
323. Soengas MS, Capodieci P, Polsky D, Mora J, Esteller M, Opitz-Araya X, McCombie R, Herman JG, Gerald WL, Lazebnik YA, Cordon-Cardo C, Lowe SW. Inactivation of the apoptosis effector Apaf-1 in malignant melanoma. Nature 2001; 409:207-11.
324. Igney FH, Krammer PH. Death and anti-death: tumour resistance to apoptosis. Nat Rev Cancer 2002; 2:277-88.
325. Shin MS, Kim HS, Lee SH, Park WS, Kim SY, Park JY, Lee JH, Lee SK, Lee SN, Jung SS, Han JY, Kim H, Lee JY, Yoo NJ. Mutations of tumor necrosis factor-related apoptosis-inducing ligand receptor 1 (TRAIL-R1) and receptor 2 (TRAIL-R2) genes in metastatic breast cancers. Cancer Res 2001; 61:4942-6.
326. Benn SC, Woolf CJ. Adult neuron survival strategies--slamming on the brakes. Nat Rev Neurosci 2004; 5:686-700.
327. Pettmann B, Henderson CE. Neuronal cell death. Neuron 1998; 20:633-47.
328. Raoul C, Estevez AG, Nishimune H, Cleveland DW, deLapeyriere O, Henderson CE, Haase G, Pettmann B. Motoneuron death triggered by a specific pathway downstream of Fas. potentiation by ALS-linked SOD1 mutations. Neuron 2002; 35:1067-83.
329. Morishima Y, Gotoh Y, Zieg J, Barrett T, Takano H, Flavell R, Davis RJ, Shirasaki Y, Greenberg ME. Beta-amyloid induces neuronal apoptosis via a mechanism that involves the c-Jun N-terminal kinase pathway and the induction of Fas ligand. J Neurosci 2001; 21:7551-60.
330. Hartmann A, Hirsch EC. Parkinson's disease. The apoptosis hypothesis revisited. Adv Neurol 2001; 86:143-53.
331. Ugolini G, Raoul C, Ferri A, Haenggeli C, Yamamoto Y, Salaun D, Henderson CE, Kato AC, Pettmann B, Hueber AO. Fas/tumor necrosis factor receptor death signaling is required for axotomy-induced death of motoneurons in vivo. J Neurosci 2003; 23:8526-31.
332. Tan J, Town T, Paris D, Mori T, Suo Z, Crawford F, Mattson MP, Flavell RA, Mullan M. Microglial activation resulting from CD40-CD40L interaction after beta-amyloid stimulation. Science 1999; 286:2352-5.
333. Barone FC, Parsons AA. Therapeutic potential of anti-inflammatory drugs in focal stroke. Expert Opin Investig Drugs 2000; 9:2281-306.
334. Perry SW, Dewhurst S, Bellizzi MJ, Gelbard HA. Tumor necrosis factor-alpha in normal and diseased brain: Conflicting effects via intraneuronal receptor crosstalk? J Neurovirol 2002; 8:611-24.
335. Yu SW, Wang H, Poitras MF, Coombs C, Bowers WJ, Federoff HJ, Poirier GG, Dawson TM, Dawson VL. Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. Science 2002; 297:259-63.
336. Putcha GV, Le S, Frank S, Besirli CG, Clark K, Chu B, Alix S, Youle RJ, LaMarche A, Maroney AC, Johnson EM, Jr. JNK-mediated BIM phosphorylation potentiates BAX-dependent apoptosis. Neuron 2003; 38:899-914.
337. Tajiri S, Oyadomari S, Yano S, Morioka M, Gotoh T, Hamada JI, Ushio Y, Mori M. Ischemia-induced neuronal cell death is mediated by the endoplasmic reticulum stress pathway involving CHOP. Cell Death Differ 2004; 11:403-15.
338. Yamashima T. Implication of cysteine proteases calpain, cathepsin and caspase in ischemic neuronal death of primates. Prog Neurobiol 2000; 62:273-95.
339. Todaro M, Zeuner A, Stassi G. Role of apoptosis in autoimmunity. J Clin Immunol 2004; 24:1-11.
340. Lenardo M, Chan KM, Hornung F, McFarland H, Siegel R, Wang J, Zheng L. Mature T lymphocyte apoptosis--immune regulation in a dynamic and unpredictable antigenic environment. Annu Rev Immunol 1999; 17:221-53.
341. Fisher GH, Rosenberg FJ, Straus SE, Dale JK, Middleton LA, Lin AY, Strober W, Lenardo MJ, Puck JM. Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. Cell 1995; 81:935-46.
342. Siegel RM, Chan FK, Chun HJ, Lenardo MJ. The multifaceted role of Fas signaling in immune cell homeostasis and autoimmunity. Nat Immunol 2000; 1:469-74.
343. Song K, Chen Y, Goke R, Wilmen A, Seidel C, Goke A, Hilliard B. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is an inhibitor of autoimmune inflammation and cell cycle progression. J Exp Med 2000; 191:1095-104.
344. Hilliard B, Wilmen A, Seidel C, Liu TS, Goke R, Chen Y. Roles of TNF-related apoptosis-inducing ligand in experimental autoimmune encephalomyelitis. J Immunol 2001; 166:1314-9.
345. Lamhamedi-Cherradi SE, Zheng SJ, Maguschak KA, Peschon J, Chen YH. Defective thymocyte apoptosis and accelerated autoimmune diseases in TRAIL-/- mice. Nat Immunol 2003; 4:255-60.