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SnapShot: Extrinsic Apoptosis Pathways

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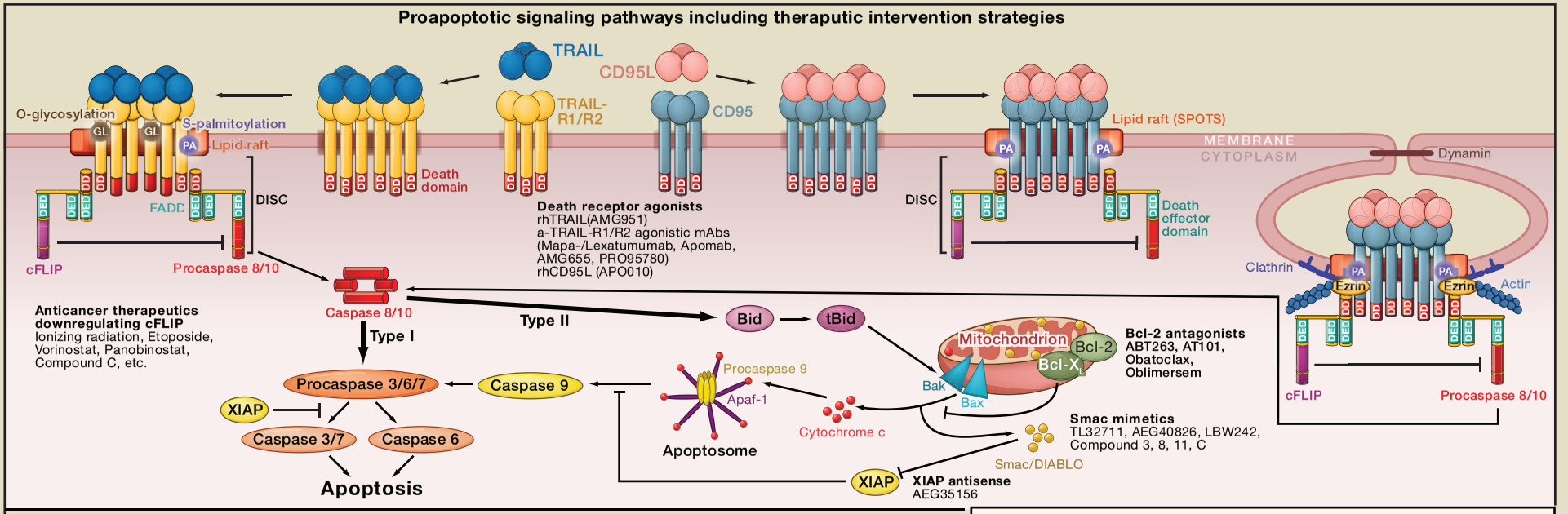
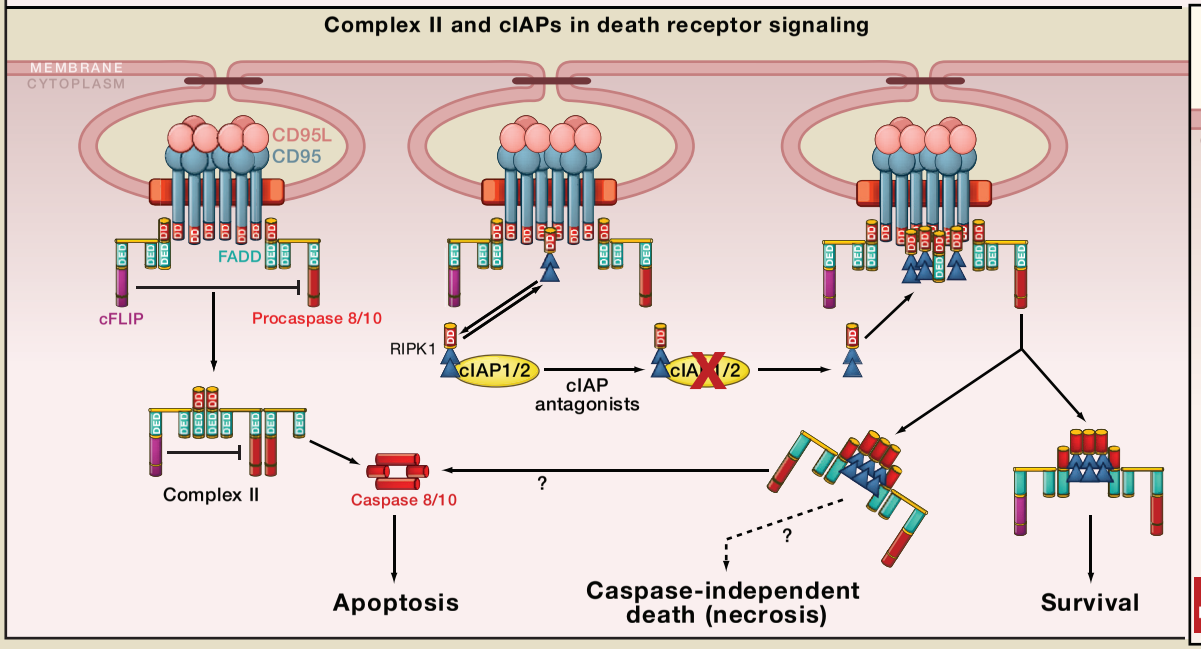
Cell

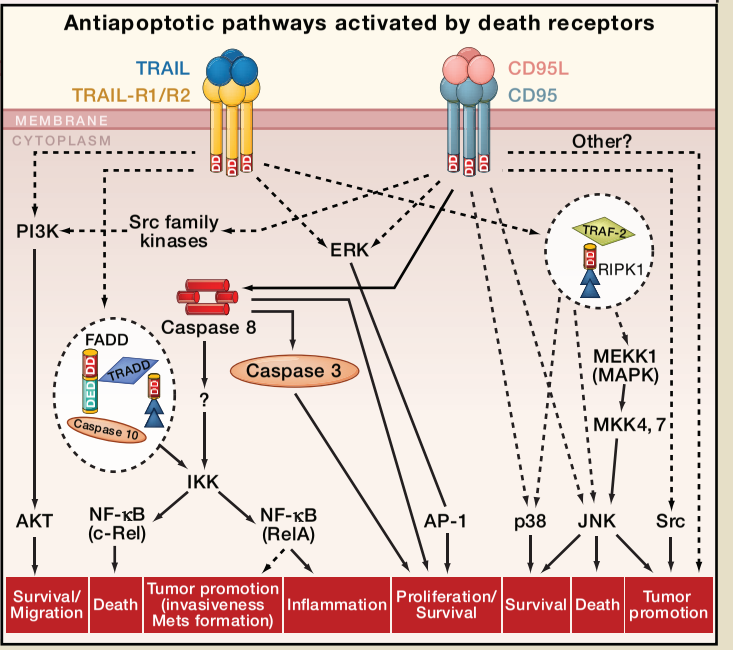
Proapoptotic signaling pathways including theraputic intervention strategies



Complex II and cIAPs in death receptor signaling



Antiapoptotic pathways activated by death receptors



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Apoptosis plays an important role in cell and tissue homeostasis and in growth control. Two separate yet interlinked signaling pathways lead to apoptotic execution: (1) The intrinsic pathway that is activated by diverse stress signals (e.g., DNA-damaging chemotherapeutics, UV radiation, and growth factor withdrawal), and (2) the extrinsic pathway that is activated by proapoptotic receptor signals at the cell surface and is important for biological processes such as lymphocyte homeostasis. The two pathways converge at the level of effector Caspases (e.g., Caspases 3, 6, and 7), which are cysteine proteases that execute apoptosis by cleaving essential cellular proteins.

The intrinsic apoptosis pathway is regulated by proapoptotic (e.g., Bid, Bak, and Bax) and antiapoptotic (e.g., Bcl-2 and Bcl-X_L) members of the Bcl-2 family, which are characterized by at least one conserved Bcl-2 homology (BH) domain. Activation of the intrinsic pathway ultimately leads to mitochondrial permeabilization and subsequent effector Caspase activation. The extrinsic apoptosis pathway is triggered through activation of death receptors of the tumor necrosis factor (TNF) family, including the TNF receptor 1 (TNF-R1) itself, CD95 (APO-1, Fas), TNF-related apoptosis-inducing ligand (TRAIL) receptors (TRAIL-R1 and TRAIL-R2, also known as DR4 and DR5), DR3 and DR6. The extrinsic pathway can link to the intrinsic mitochondrial pathway via cleavage of Bid. In this SnapShot, we describe the apoptotic pathways activated by the CD95 and TRAIL death receptors, as they are considered bona fide death receptors and induce apoptosis using similar pathways.

Cell Death Pathways Induced by Death Receptors

Signaling through both CD95 and TRAIL-R1/-R2 leads to formation of a death-inducing signaling complex (DISC), which activates inducer Caspases 8 and 10. CD95 and TRAIL-R1/-R2 exist as preformed homotrimers on the cell membrane and contain an intracellular death domain (DD). Binding of ligand or agonistic antibodies results in death receptor oligomerization followed by recruitment Fas-associated protein with death domain (FADD) through homotypic interactions between the death domains of the receptors and those of FADD. Subsequently, FADD recruits Procaspases 8 and 10 and cellular FADD-like interleukin-1 β converting enzyme (FLICE)-like inhibitory protein (cFLIP) through homotypic interactions between their death effector domains (DEDs). Together with the death receptors, these proteins form the DISC.

Formation of the DISC and further clustering of death receptors promotes the homodimerization and activation of inducer Caspases 8 and 10, which are then processed into their mature forms. cFLIP is an important regulator of inducer Caspase activation. It closely resembles Procaspases 8 and 10 and competes for FADD binding, but cFLIP lacks the Procaspase 8/10 catalytic site, thereby preventing Procaspase 8/10 homodimerization and activation.

In cells that process only small amounts of Caspase 8 at the DISC, an amplification of the apoptotic signal through the mitochondria is often required. This is known as the "Type II" pathway and is engaged by cells such as hepatocytes. In contrast, cells that process large amounts of Caspase 8 at the DISC can activate effector Caspases directly and do not require mitochondrial amplification for death to occur. This is known as the "Type I" pathway and is utilized by cells such as lymphocytes.

In the Type II pathway, Caspase 8 cleaves the BH3-only protein Bid. Truncated Bid (tBid) activates Bak and Bax at the mitochondria, resulting in permeabilization of the mitochondrial outer membrane and release of proteins such as cytochrome c, Smac (second mitochondria-derived activator of Caspases)/DIABLO (direct inhibitor of apoptosis protein [IAP]-binding protein with low pI), and HtrA2 (high-temperature requirement protein A2)/Omi. Cytochrome c binds to the adaptor molecule APAF-1 (apoptotic protease-activating factor 1), which interacts with Procaspase 9 to form the apoptosome. At the apoptosome, Caspase 9 is activated, processed, and released, leading to the activation of effector Caspases 3, 6, and 7, and eventually disintegration of the cell.

X-linked inhibitor of apoptosis protein (XIAP), a ubiquitously expressed cytoplasmic protein, inhibits apoptosis by binding directly to Caspase 3, 7, and 9, thereby masking their active sites. Smac/DIABLO and HtrA2/Omi are released following mitochondrial permeabilization and antagonize the activity of XIAP by competing for XIAP binding with Caspases, thus promoting Caspase activation and cell death.

In the case of the CD95 pathway, a cytosolic "complex II" consisting of FADD, Procaspase 8, and cFLIP molecules conveys the apoptotic signal. In an alternative pathway, the receptor-interacting serine/threonine kinase 1 (RIPK1, also known as RIP-1) associates with the DISC through its death domain and activates a cell death pathway that is independent of Caspases. This alternative pathway is inhibited by cellular inhibitor of apoptosis proteins (cIAPs), which prevent RIPK1 accumulation in the DISC. In addition, $cFLIP_L$ in the cytosolic RIPK1 complex inhibits RIPK1-mediated death. cIAPs may also inhibit TRAIL-induced death.

The Importance of Receptor and Ligand Modification and Organization

Receptor oligomerization is critical for apoptosis mediated by death receptors, which can depend on the formulation (native or crosslinked ligands or antibodies) and presentation of death receptor agonists. In mice, membrane-bound CD95 ligand (CD95L) induces only apoptosis, whereas antiapoptotic CD95 signaling pathways are activated in mice that express only soluble CD95L, promoting autoimmunity and tumorigenesis.

Posttranslational modifications of death receptors are also required for optimal apoptosis signaling. S-palmitoylation of TRAIL-R1 (at cysteines 261–263) and CD95 (at Cys 199) or O-glycosylation of TRAIL receptors by UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 14 (GALNT14) facilitates receptor oligomerization. This leads to the localization of death receptors into specialized membrane microdomains called "lipid rafts." Lipid rafts stabilize receptor oligomers, forming so-called "signaling protein oligomerization transduction structures" (SPOTS), which are required for inducer Caspase activation. The CD95 complex subsequently requires internalization in Type I cells for apoptosis to proceed. The "rafts" that have formed require dynamin and clathrin to be internalized, which then form specialized membrane structures termed "CD95 receptosomes." Formation of the receptosomes also requires ezrin-mediated actin filament association. In contrast, TRAIL-R1/-R2 appears to signal for apoptosis induction from the cell surface. Although TRAIL-R1/-R2 complexes can be internalized, interfering with receptor internalization still allows apoptosis to occur.

Alternative Signaling Pathways Induced by Death Receptors

Under certain circumstances, death receptors may activate prosurvival and proliferation pathways involving nuclear factor κB (NF-κB), mitogen-activated protein kinases (MAPKs), phosphoinositide 3-kinase (PI3K), and Akt. Numerous factors can determine whether death receptors promote apoptosis or survival. These include the strength or duration of receptor stimulation and the presence or activity of proteins that regulate intracellular signaling. However, the precise molecular events that mediate activation of survival pathways remain unknown.

CD95 activation has been linked to tumorigenesis and tumor invasiveness through activation of a number of signaling proteins, such as Src kinases and NF-κB and through activation of JNK-signaling pathways. Therefore, CD95 does not always act as a dedicated death receptor but instead mediates diverse functions in different tissues and under different conditions.

Clinical Application of the Extrinsic Apoptosis Pathway in Cancer

As tumors frequently acquire mutations that make them resistant to apoptosis induction via the intrinsic pathway, targeting the extrinsic apoptosis pathways is attractive in cancer therapy. Because TRAIL induces apoptosis in a large proportion of long-term established tumor cell lines, but for as yet unknown reasons is generally not toxic to normal tissue, therapeutic targeting of the extrinsic apoptosis pathways has focused primarily on reagents that stimulate TRAIL-R1 and -R2. TRAIL death receptor agonists in clinical trials include recombinant human (rh) TRAIL AMG951 (phase I), α-TRAIL-R1 monoclonal antibody (mAb) Mapatumamab (phase II), α-TRAIL-R2 mAbs Lexatumamab (phase I), Apomab (phase I), and AMG655 (phase I). A CD95 agonist in a phase I clinical trial is APO010, a dimer of CD95L trimers.

In addition, cells can be greatly sensitized to apoptosis signaling mediated by death receptors via the inhibition of prosurvival Bcl-2 family proteins and downregulation of c-FLIP or IAPs. Many anticancer agents that are in use clinically, including but certainly not limited to ionizing radiation, chemotherapeutic drugs (e.g., etoposide), histone deacetylase (HDAC) inhibitors, and small molecule kinase inhibitors, have been shown to downregulate c-FLIP protein levels.

Finally, numerous agents that affect cell death mediated by death receptors are currently in clinical trials. They include IAP antagonists, such as XIAP antisense AEG35156/GEM640 (phase II) and Smac mimetics that target XIAP, cIAP1, and cIAP2. These mimetics include TL32711 (phase I), LBW242 (phase I), AEG40826/HGS1029 (phase I), compound 3 (phase I), compound 11 (phase I), compound C (phase I), and compound 8 (phase I). Bcl-2 antagonists, such as ABT 263 (structural homolog to ABT737, phase II), AT-101 (phase II), Obatoclax (GX15-070, phase II), and Oblimersem (antisense DNA-targeting Bcl-2, phase III), are also in clinical trials for a wide range of tumor types.

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Abbreviations

APAF-1, apoptotic protease-activating factor 1; BH, Bcl-2 homology; cFLIP, cellular FLICE (FADD-like interleukin 1 β)-like inhibitory protein; (c)IAP, (cellular) inhibitor of apoptosis protein; DD, death domain; DED, death effector domain; DIABLO, direct IAP-binding protein with low pI; DISC, death-inducing signaling complex; DR, death receptor; FADD, Fas-associated protein with death domain; GALNT14, UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 14; HtrA2, high-temperature requirement protein A2; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; PI3K, phosphoinositide 3-kinase; rh, recombinant human; RIPK1, receptor interacting serine/threonine kinase 1; Smac, second mitochondria-derived activator of caspases; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; TRAIL-R, TRAIL receptor; XIAP, X-linked inhibitor of apoptosis protein.

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