Chapter 2
Systems Biology of Death Receptor-Induced Apoptosis

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Abstract Programmed cell death, termed apoptosis, plays a fundamental role in the development and homeostasis of multicellular organisms. Dysregulation of apoptosis can lead to numerous diseases, including autoimmune diseases, neurodegenerative diseases, and cancer. In mammalian cells apoptosis can be induced by intra- or extracellular stimuli. Extracellular stimuli comprise death ligands which lead to death receptor-induced apoptosis, referred to as extrinsic pathway. Intracellular signals, such as DNA damage, trigger the intrinsic pathway which results in the activation of Bcl-2 proteins and release of proapoptotic factors from the mitochondria into the cytosol. Apoptosis is executed by a family of cysteine proteases, the caspases, which eventually lead to the apoptotic phenotype, such as chromatin condensation, nuclear fragmentation, membrane blebbing, cell shrinkage, and formation of apoptotic bodies. The focus of this chapter is on understanding the signaling complexity of the extrinsic apoptotic pathway using systems biology. We summarize the main signaling paradigms and the major models of the extrinsic pathway. The development of these models has elucidated new insights into the regulation of apoptosis.

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2.1 Death Receptor-Mediated Apoptosis

Death receptors (DR) belong to the tumor necrosis factor (TNF) family of proteins and are characterized by extracellular cysteine rich domains (CRD) and an intracellular ~80 amino acid long motif, the death domain (DD) (Ashkenazi 1998; Krammer et al. 2007). The best characterized DRs are CD95 (also named Fas/APO-1), TNFR1, TRAIL receptor 1 (TRAIL-R1), and TRAIL-R2 (Ashkenazi 1998; Krammer 2000; Krammer et al. 2007). Other DRs include DR3 and DR6, EDA-R, and NGF-R (Ashkenazi 1998; Krammer et al. 2007; Lavrik et al. 2005a). Death ligands (DL) are assumed to be homotrimeric (Yan and Shi 2005) and exist in a membrane-bound or a soluble form.

The CD95-induced apoptotic pathway is one of the best-studied signaling pathways. The natural ligand of CD95, CD95L, is expressed on a variety of cells, such as cytotoxic T cells, as a type II membrane protein (Krammer 2000; Rathmell et al. 1995). Cleavage of the membrane-bound to the soluble form reduces its apoptosis-inducing potential by more than 1,000-fold (Schneider 1998). Stimulation of CD95 with its ligand or with agonistic anti-CD95 antibodies, such as anti-APO-1 (Trauth et al. 1989), triggers the oligomerization of CD95 (Fig. 2.1). This leads to the recruitment of Fas-associated death domain (FADD) through DD interactions, as well as procaspase-8, procaspase-10, and cellular FLICE inhibitory proteins (c-FLIPs) via N-terminal death effector domains (DED), and formation of the death-inducing signaling complex (DISC). In the DISC procaspase-8/10 are activated by dimerization and internal cleavage (Lavrik et al. 2005b), which is regulated by c-FLIP proteins. Recently, it has been reported that ubiquitylation plays an important role in caspase-8 activation (Jin et al. 2009). Recent structural analyses challenge the concept of a trimeric ligand binding to a trimeric receptor. Scott et al. (2009) reported a tetrameric conformation of the CD95/FADD complex. Wang et al. (2010) suggest that the minimal signaling unit of CD95L is at least hexameric and found an asymmetric conformation of 5–7 CD95 bound to 5 FADD molecules.

Triggering of CD95 has also been reported to induce nonapoptotic pathways, such as NF-κB, AKT, and ERK (Neumann et al. 2010; Peter et al. 2007). However, the detailed mechanisms of the induction of CD95-mediated nonapoptotic pathways are not elucidated yet.

Activated caspase-8 is released from the DISC and activates effector caspases-3 and -7, which cleave a variety of substrates, such as DFF45/ICAD, thus releasing the DNase DFF40/CAD. DFF40/CAD is responsible for the fragmentation of chromosomes (Strasser et al. 2009; Yan and Shi 2005). Additionally, caspase-8 cleaves the BH3-only Bcl-2 protein Bid. The C-terminal part, tBid, then translocates to the mitochondria resulting in mitochondrial outer membrane permeabilization (MOMP) and the release of proapoptotic factors into the cytosol, such as cytochrome c, Apaf-1, or endonuclease G (Li 1998; Yan and Shi 2005). Recently, it has been reported that caspase-8 cleaves Bid in a specific complex at the mitochondria, which involves cardiolipin (Gonzalvez et al. 2008; Schug et al. 2011). This results in the

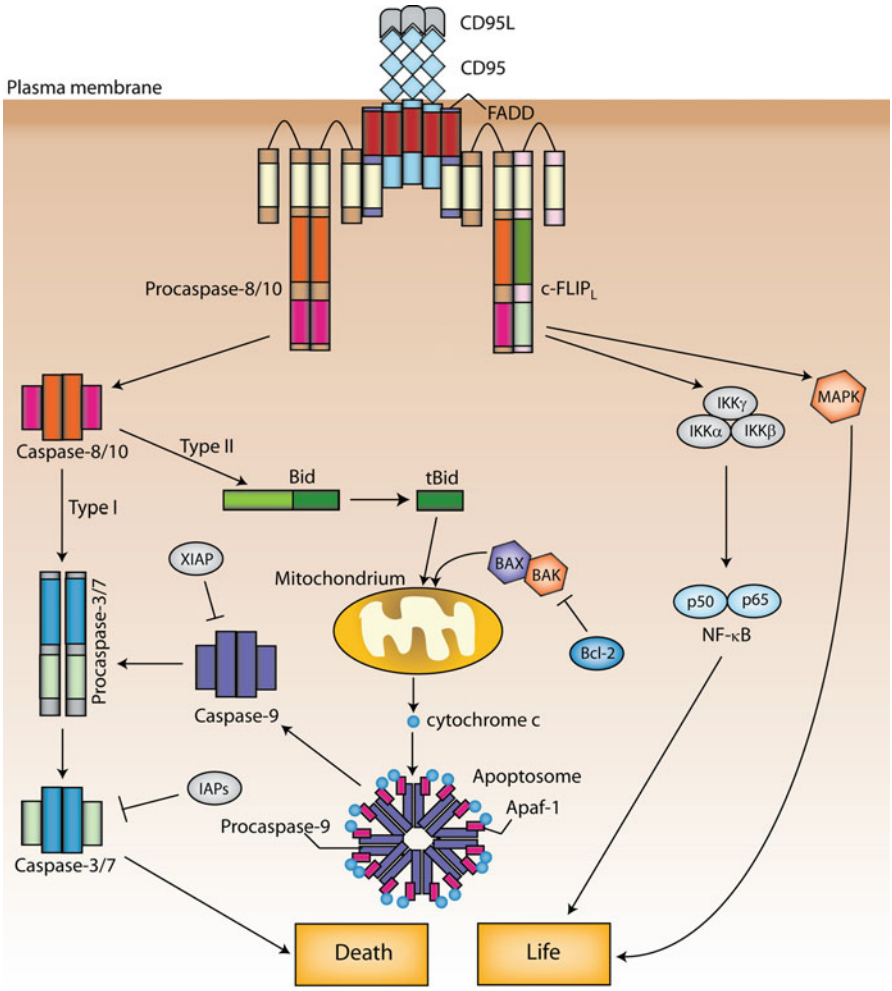

Fig. 2.1 Overview of CD95-induced pathways. Stimulation of CD95 leads to the formation of the death-inducing signaling complex (DISC), which includes at least CD95, FADD, procaspase-8, procaspase-10, and c-FLIP proteins. Caspase-8 is activated at the DISC which is regulated by c-FLIP proteins. In the cytosol caspase-8 cleaves and activates procaspase-3 or the Bcl-2 protein Bid. The truncated form of Bid (tBid) translocates to the mitochondria, which leads to mitochondrial outer membrane permeabilization (MOMP) and the release of cytochrome c, as well as other proapoptotic proteins from the mitochondria into the cytosol. Cytochrome c is involved in apoptosome formation and procaspase-9 activation. Procaspase-9 in turn also activates procaspase-3 resulting in caspase-3 activity and eventually cell death. Additionally CD95 stimulation can lead to the activation of nonapoptotic pathways, such as NF-κB or MAPK and cell survival

formation of another complex, the apoptosome, including cytochrome c, Apaf-1, ATP and procaspase-9, and activation of procaspase-9 in this complex (Boatright et al. 2003; Shi 2002). Caspase-9 also cleaves and activates caspase-3.

In CD95-induced apoptosis two different cell types are distinguished: Type I and Type II (Barnhart et al. 2003; Scaffidi et al. 1998). Type I cells [e.g., thymocytes (Strasser et al. 2009)] are characterized by high amounts of CD95 DISC, which results in very efficient procaspase-8 activation, leading to massive activation of caspase-3 and cell death. Type II cells [e.g., hepatocytes (Strasser et al. 2009)], on the other side, are characterized by lower amounts of CD95 DISC formation, that results in less active procaspase-8 and require signal amplification through tBid-mediated mitochondrial permeabilization.

2.2 Effectors and Regulators of Extrinsic Apoptosis

DR-induced apoptosis is regulated at several levels and involves numerous protein families, from the DISC to effector caspases. The interplay between the different levels of regulation provides a significant complexity, which can be understood better using systems biology. In the following sections, we will give a summary of the different protein families and their roles in extrinsic apoptosis.

2.2.1 Caspases as Major Effector Molecules of Apoptosis Pathway

Caspases are cysteine proteases and are the effector molecules of the apoptotic machinery (Fuentes-Prior and Salvesen 2004; Lavrik et al. 2005b). There are apoptotic, as well as inflammatory caspases. The apoptotic caspases are divided into initiator caspases, including caspase-2, -8, -9, and -10, and effector caspases, such as caspase-7 and -3 (Fuentes-Prior and Salvesen 2004). All caspases are present in the cell as inactive zymogens referred to as procaspases and are activated by internal cleavage (Fuentes-Prior and Salvesen 2004; Yan and Shi 2005). Initiator caspases act upstream of effector caspases and activate them through cleavage. Effector caspases then cleave a variety of cellular substrates, eventually resulting in cell death (Fuentes-Prior and Salvesen 2004). All caspases share a common structure. Caspase monomers consist of a large (~20 kDa) and small (~10 kDa) subunit (Fig. 2.2). Initiator caspases additionally have specific recruitment domains at their N-terminus. Procaspase-8 and -10 have two tandem DED through which they interact with FADD at the DISC, procaspase-9 possesses a caspase-recruitment domain (CARD) which is required for recruitment to the apoptosome via interactions with Apaf-1 (Fuentes-Prior and Salvesen 2004; Krammer et al. 2007; Lavrik et al. 2005b; Yan and Shi 2005).

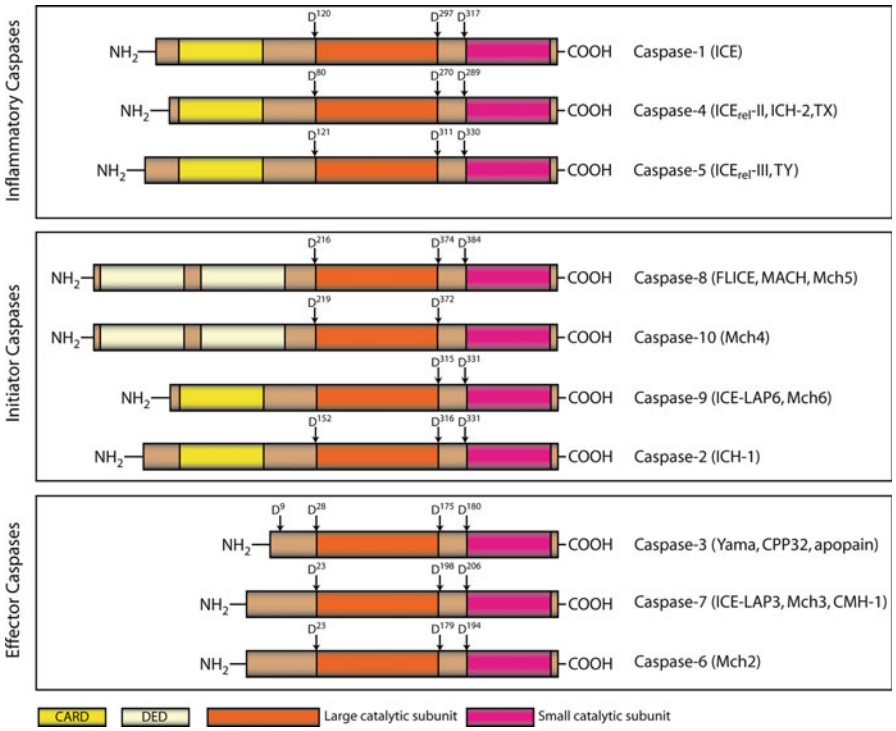

Fig. 2.2 Structural organization of caspases. Caspases are generally divided into inflammatory and apoptotic caspases. Apoptotic caspases are further divided into initiator and effector caspases. Caspases possess a large (p20) and small (p10) subunit. Initiator caspases additionally have DEDs (procaspase-8/10) or CARD domains (procaspase-9) at their N-terminus

Active caspases are heterotetramers consisting of two large and two small subunits (Fig. 2.3). Initiator caspases are present in the cytosol as monomers and are activated by dimerization or oligomerization at caspase-activating platforms and cleavage between the large and small subunits only stabilizes the dimer (Fuentes-Prior and Salvesen 2004; Hughes et al. 2009; Krammer et al. 2007; Lavrik et al. 2005b; Oberst et al. 2010). It was shown that both dimerization and interdomain cleavage are required for full activation of caspase-8 (Hughes et al. 2009; Keller et al. 2009; Oberst et al. 2010). Effector caspases on the contrary are present as inactive dimers and are readily activated by internal cleavage (Fuentes-Prior and Salvesen 2004; Oberst et al. 2010). This cleavage is performed by initiator caspases. The conceptual difference between the two classes is that there is no proteolytic enzyme upstream of initiator caspases. Consequently, initiator caspases exhibit low zymogenicity, which is defined as the ratio of activity between the cleaved and the uncleaved form. While initiator caspases have highest ratios of 10 (caspase-9) or 100 (caspase-8), the ratio for caspase-3 is more than 10,000 (Fuentes-Prior and Salvesen 2004).

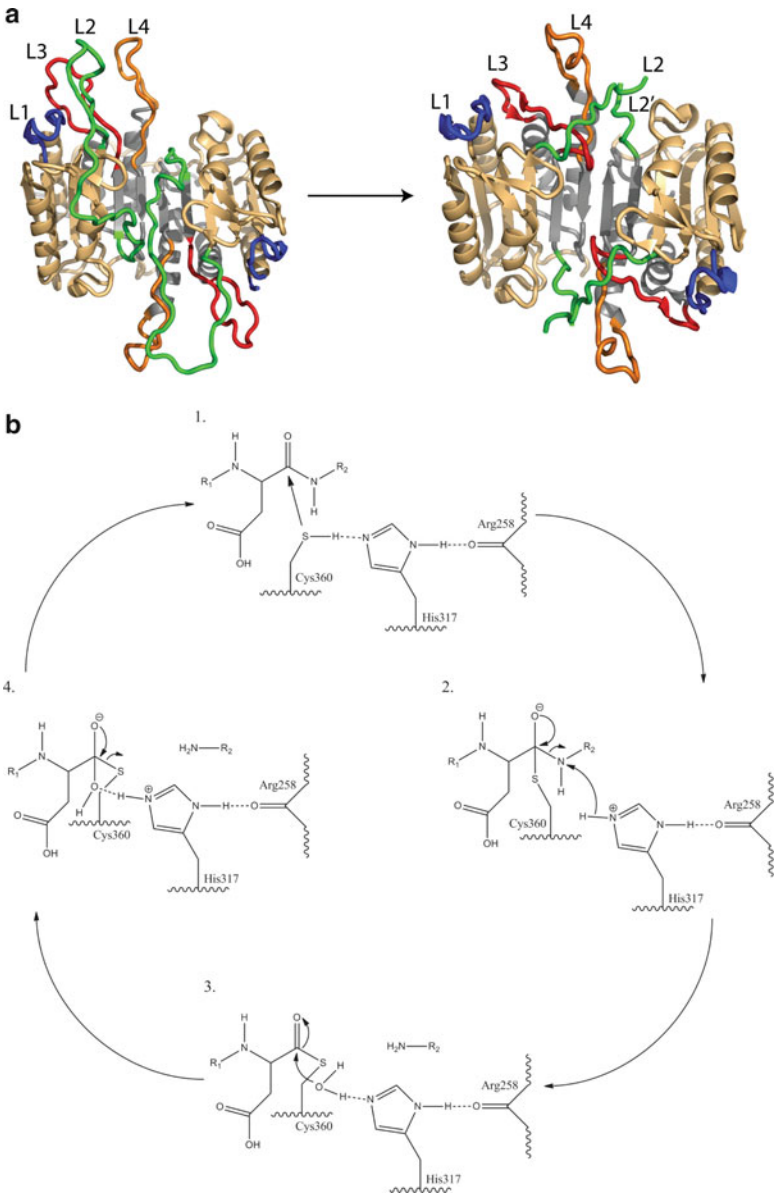

Fig. 2.3 Structure and suggested catalytic mechanism of caspases. (**a**) Crystal structure of procaspase-7 (*left*, PDB 1GQF) and active caspase-7 (*right*, PDB 1RHJ). The large and small subunits are colored *light orange* and *gray*, respectively. Cleavage of the intersubunit linker (L2) results in a conformational change in the active site and activation of caspase-7. The active site is formed by five loops, loops L1–L4 from one monomer and loop L2′ from the adjacent monomer. The structure was generated using PyMOL (Schrodinger, 2010). (**b**) The catalytic mechanism of caspases is suggested to be similar to serine proteases (Fuentes-Prior and Salvesen 2004). The cysteine acts as nucleophile and forms a tetrahedral intermediate with the substrate (step 2). A nearby histidine is critical for the subsequent hydrolysis of the substrate (step 3) [adapted from Fesik (2000)]

The active site of caspases is highly conserved and is formed by five protruding loops: L1, L2, L3, and L4 from one monomer and loop L2′ from the adjacent monomer (Fig. 2.3) (Fuentes-Prior and Salvesen 2004; Yan and Shi 2005). The catalytic cysteine residue is positioned in the loop L2 which determines, together with L1, L3, and L4 substrate specificity, recognizing specific tetrapeptide sequences. The L2′ loop from the adjacent monomer has mainly a stabilizing function of the active site (Fuentes-Prior and Salvesen 2004; Yan and Shi 2005). Initiator and effector caspases have a fundamental difference in active site architecture, which explains their different modes of activation. The intersubunit linker (L2) of initiator caspases is longer than that of effector caspases, which allows the formation of the active site also in its uncleaved form and results in the low zymogenicity (Fuentes-Prior and Salvesen 2004). The catalytic mechanism of caspases is suggested to be similar to serine proteases (Fuentes-Prior and Salvesen 2004). The cysteine acts as nucleophile and forms a tetrahedral intermediate with the substrate (Fig. 2.3b, step 2). A nearby histidine is critical for the subsequent hydrolysis of the substrate (Fig. 2.3b, step 3) (Fesik 2000; Fuentes-Prior and Salvesen 2004).

2.2.1.1 Caspase-8/10 Activation at the DISC

Two isoforms of procaspase-8 procaspase-8a (p55) and -8b (p53) are recruited to the DISC (Scaffidi et al. 1997). After binding to the DISC, procaspase-8a/b (p55/p53) undergoes processing, thus generating active caspase-8 (Lavrik et al. 2005b; Medema et al. 1997; Muzio et al. 1996) (Fig. 2.4). This processing has been suggested to occur via dimerization of two procaspase-8 monomers followed by a conformational change, leading to autoactivation of procaspase-8 homodimers (Chang et al. 2002; Golks et al. 2006b; Hughes et al. 2009; Yu et al. 2009). Procaspase-8a/b (p55/p53) processing at the DISC also results in the generation of the N-terminal cleavage products p43/p41, the prodomains p26/p24, as well as the C-terminal cleavage products p30, p18, and p10 (Golks et al. 2006b; Hoffmann et al. 2009; Hughes et al. 2009; Medema et al. 1997). Active caspase-8 heterotetramers $(p10/p18)_2$ generated at the DISC initiate the execution of apoptotis (Krammer et al. 2007). Recently, it has been reported that the cleavage products p30 and p43/p41 also possess catalytic activity leading to apoptosis initiation (Hoffmann et al. 2009; Hughes et al. 2009). Hence, procaspase-8 processing at the DISC initiates apoptosis through the generation of several catalytically active cleavage products. Recently it has been reported that full activation of caspase-8 at the DISC requires Cullin3-mediated polyubiquitination in the C-terminal part of caspase-8 (Jin et al. 2009).

Three isoforms of caspase-10 namely procaspase-10a, procaspase-10c, and procaspase-10d were reported to be bound to the DISC (Sprick et al. 2002). Procaspase-10 is also activated at the DISC via generation of homodimers, leading to the formation of an active heterotetramer. However, whether caspase-10 can

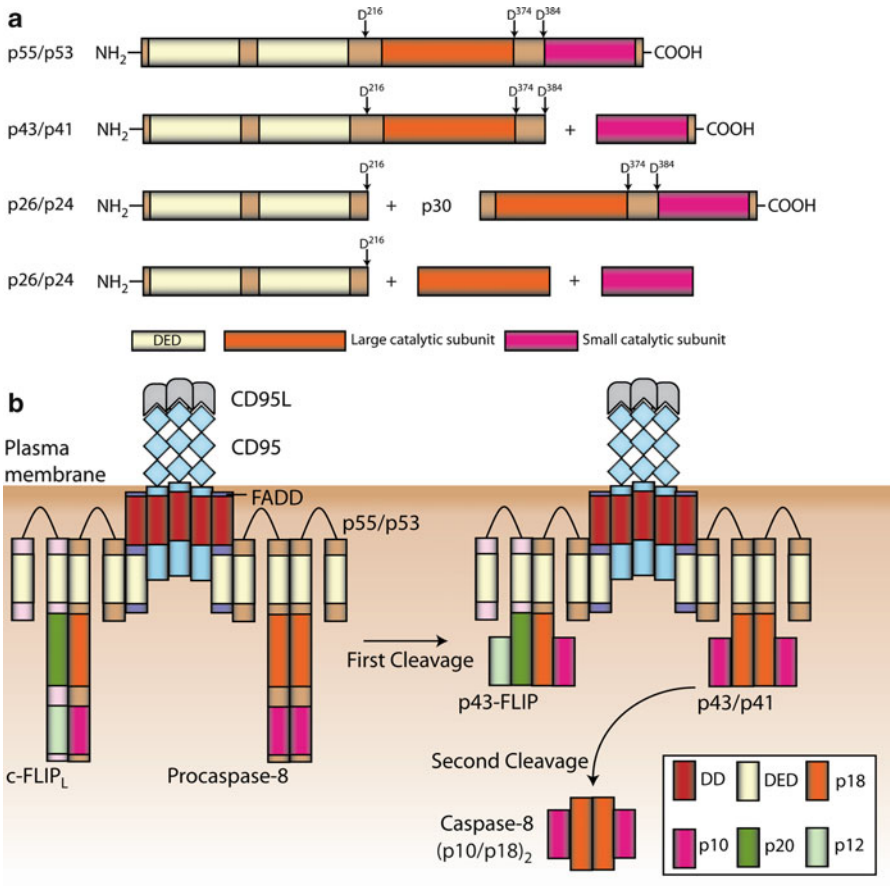

Fig. 2.4 Caspase-8 cleavage products and processing at the DISC. (**a**) Procaspase-8 can be cleaved between the large and small subunit or between the prodomain and the large catalytic subunit, resulting in numerous different cleavage products. (**b**) Procaspase-8 homodimers at the DISC can be processed to the active caspase-8 heterotetramers $(p10/p18)_2$ via the intermediate p43/p41. The procaspase-8/c-$FLIP_L$ heterodimer can only be processed to the intermediate p43-FLIP

trigger cell death in the absence of caspase-8 in response to CD95 stimulation is controversial (Fischer et al. 2006; Mühlethaler-Mottet et al. 2011; Sprick et al. 2002).

2.2.2 c-FLIP Proteins Regulate Caspase Activation at the DISC

Three c-FLIP isoforms and two cleavage products have been characterized so far (Golks et al. 2005, 2006a; Scaffidi et al. 1999; Ueffing et al. 2008) (Fig. 2.5). Three isoforms include one long, c-$FLIP_L$, and two short variants, c-$FLIP_S$ and c-$FLIP_R$. All three c-FLIP isoforms possess two tandem DED domains at their

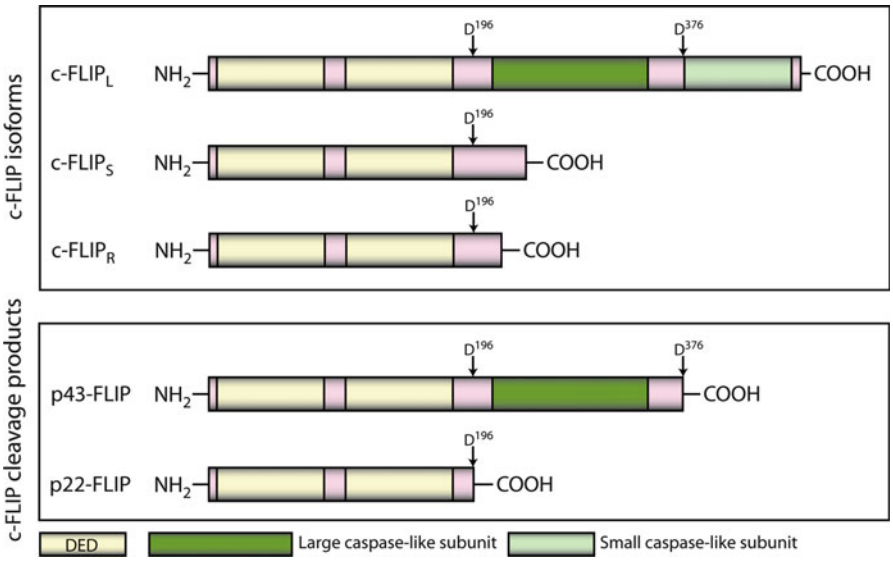

Fig. 2.5 c-FLIP isoforms and cleavage products. Three isoforms of c-FLIP proteins exist, one long (c-FLIP$_L$) and two short variants (c-FLIP$_S$ and c-FLIP$_R$). All isoforms contain two tandem DEDs which are required for DISC recruitment. c-FLIP$_L$ additionally has a large and small caspase-like subunit, which are catalytically inactive. C-FLIP$_L$ can be cleaved by caspase-8 at different positions generating the N-terminal fragment p43-FLIP or N-terminal fragment p22-FLIP

N-terminus. c-FLIP$_L$ additionally contains catalytically inactive caspase-like domains (p20 and p12). The two short isoforms, c-FLIP$_S$ and c-FLIP$_R$, block DR-induced apoptosis by inhibition of procaspase-8 activation at the DISC (Golks et al. 2005; Krueger et al. 2001). This has been suggested to occur through the formation of catalytically inactive procaspase-8/c-FLIP$_{R/S}$ heterodimers. c-FLIP$_L$ can play both a pro- and an antiapoptotic role. It can act as an antiapoptotic molecule, functioning in a way analogous to c-FLIP$_S$ when it is present at high concentrations at the DISC (Chang et al. 2002; Krueger et al. 2001). c-FLIP$_L$ can act proapoptotic when expressed at lower concentrations, in combination with strong receptor stimulation or in the presence of high amounts of either of the short c-FLIP isoforms c-FLIP$_S$ or c-FLIP$_R$. Under these conditions c-FLIP$_L$ facilitates the activation of procaspase-8 at the DISC (Fricker et al. 2010). This occurs via the formation of catalytically active procaspase-8/c-FLIP$_L$ heterodimers in which the procaspase-8 active loop is stabilized by c-FLIP$_L$ (Micheau et al. 2002; Yu et al. 2009), thereby increasing the catalytic activity of procaspase-8.

2.2.3 IAP Family of Proteins

The Inhibitors of Apoptosis (IAP) proteins directly inhibit caspases. They all share a conserved sequence motif of 70–80 amino acids, the baculoviral IAP repeat (BIR)

domain, which is arranged around a coordinated zinc atom (Fuentes-Prior and Salvesen 2004; Shi 2002), of which each family member can possess up to three copies (Deveraux and Reed 1999). There are six human IAPs, which include XIAP, c-IAP1, c-IAP2, NAIP, Bruce, and survivin (Deveraux and Reed 1999). Numerous mammalian IAPs, as well as IAPs in flies and viruses, possess a C-terminal RING domain, however, the requirement of this domain for apoptosis suppression remains unclear (Deveraux and Reed 1999). There are reports that both domains are required for their antiapoptotic function in insects (Clem and Miller 1994; Harvey et al. 1997), however, c-IAP1, c-IAP2, and XIAP in humans could still inhibit apoptosis when lacking the RING domain (Deveraux and Reed 1999; Hay et al. 1995).

XIAP directly inhibits active caspase-3. After MOMP Smac is released from the mitochondria into the cytosol and relieves XIAP-mediated inhibition. This causes a delay between receptor-mediated initiator phase and final commitment to cell death in type II cells (Albeck et al. 2008; Fuentes-Prior and Salvesen 2004). XIAP also contains a RING domain with E3 ubiquitin ligase activity, which promotes caspase-3 degradation by the proteasome (Albeck et al. 2008; Fuentes-Prior and Salvesen 2004).

2.2.4 Bcl-2 Family of Proteins

The Bcl-2 family of proteins play a key role in the regulation of apoptosis at the mitochondrial level and are essential for extrinsic apoptosis in type II cells or intrinsic apoptosis (Adams 1998; Yan and Shi 2005). The Bcl-2 protein family comprises at least 15 members with pro- as well as antiapoptotic functions (Adams 1998). All family members share a conserved structural motif, the Bcl-2 homology domain (BH1-BH4) (Adams 1998; Yan and Shi 2005). Prosurvival family members include Bcl-2 and Bcl-X_L. The proapoptotic Bcl-2 family members can be further subdivided into multidomain proteins, represented by Bax and Bak, and BH3-only proteins, such as Bid (Adams 1998; Yan and Shi 2005). The balance of pro- and antiapoptotic Bcl-2 family members determines the apoptosis induction. Proapoptotic Bcl-2 proteins cause the release of proapoptotic factors from the mitochondria by inducing MOMP. This process is triggered either in the extrinsic pathway through cleavage of Bid by caspase-8 or in the intrinsic pathway which is mainly controlled by the tumor suppressor p53 (Yu and Zhang 2005). The Bcl-2 family is described in detail in the Chaps. 4 and 5.

2.3 CD95-Induced Nonapoptotic Pathways

Accumulating evidence suggests that stimulation of CD95 can also induce nonapoptotic pathways, such as tumor growth and invasion, as well as proliferation and programmed necrosis, termed necroptosis (Chen et al. 2010; Choi et al. 2010; Geserick et al. 2009; Lee et al. 2011; Steller et al. 2011; Strasser et al. 2009; Tang

et al. 2010). CD95-mediated nonapoptotic signaling occurs via induction of NF-κB, Akt and mitogen-activated protein kinases (MAPK) pathways. These, however, are not well understood, but have been reported to require caspase-8 activity (Kober et al. 2011; Lee et al. 2011; Nakajima et al. 2008; Neumann et al. 2010; Shikama et al. 2003; van Raam and Salvesen 2011). C-FLIP proteins play a very important role in the regulation of caspase-8 activation as well as induction of nonapoptotic pathways. It could be shown that the cleavage product of c-$FLIP_L$ p43-FLIP directly interacts with the IKK complex, leading to the activation of NF-κB (Neumann et al. 2010). Other prominent players in nonapoptotic pathways are receptor-interacting protein 1 (RIP1) and RIP3 which are important for CD95-induced necroptosis, as well as activation of NF-κB (Geserick et al. 2009; Kamarajan et al. 2010).

2.3.1 CD95-Mediated NF-κB Activation

The eukaryotic transcription factor NF-κB was originally discovered transcribing the immunoglobulin kappa light chain gene in B cells (Sen and Baltimore 1986). NF-κB can be activated following a variety of stimuli, including bacterial lipopolysaccharide (LPS), T cell receptor (TCR) signaling, different cytokines, such as TNFα, interleukin 1 (IL-1) and IL-2, viral infections, UV and X-ray radiation, nitric oxide, and also CD95 (Ghosh et al. 1998; Hayden and Ghosh 2004; Legembre et al. 2004; Verma et al. 1995). The NF-κB transcription family comprises multiple members, including RelA (p65), NF-κB1 (p50, p105), NF-κB2 (p52, p100), c-Rel, and RelB (Ghosh et al. 1998; Li and Verma 2002; Rasper et al. 1998; Verma et al. 1995). All NF-κB proteins share a conserved N-terminal 300-amino acid motif, the Rel homology domain (RHD), which contains a dimerization, nuclear localization as well as DNA-binding domain (Ghosh and Hayden 2008). NF-κB proteins form homo- or heterodimers with each other, except for RelB. The most prominent dimer which is commonly referred to as NF-κB is the heterodimer of p65 with either p50 or p52 (Li and Verma 2002). Importantly, only c-Rel, RelA, and RelB possess a transactivation domain and thus act as transcriptional activators, while other NF-κB proteins act as transcriptional repressors (Ghosh and Hayden 2008). In the absence of activating stimuli NF-κB dimers are inhibited by IκB (inhibitor of NF-κB) proteins via ankyrin-repeat motifs and masking their nuclear localization signal (Hayden et al. 2006). IκB proteins are phosphorylated by IκB kinases (IKKs) and subsequently degraded by the proteasome (Li and Verma 2002). The IKK complex, consisting of IKKα, IKKβ, and NEMO (IKKγ) regulates NF-κB activation following various stimuli and lack of either IKK complex component blocks NF-κB activation (Ghosh and Hayden 2008).

Besides its well studied proapoptotic function, CD95 also has nonapoptotic functions. It was shown that T cell proliferation under suboptimal CD3 stimulation could be enhanced by CD95 costimulation (Alderson et al. 1993; Paulsen and Janssen 2011; Paulsen et al. 2011). Additionally, CD95-knockout mice had

abnormally low lymphocyte levels (Hao et al. 2004). Further, cancer cells were found to produce CD95L and inhibition of CD95 reduced tumor size in several cancer mouse models (Chen et al. 2010). The mechanism of the nonapoptotic functions of CD95 is largely unknown. It could be shown from different independent groups that CD95 stimulation triggers NF-κB activation, the exact mechanism, however, remains elusive (Kataoka et al. 2000; Kreuz et al. 2004; Neumann et al. 2010; O'Reilly et al. 2004). Numerous groups have reported that CD95-mediated activation of NF-κB, ERK, and MAPK requires caspase-8 activity and is controlled by c-FLIP proteins (Fricker et al. 2010; Golks et al. 2006a; Hughes et al. 2009; Kataoka et al. 2000; Kober et al. 2011; Kreuz et al. 2004; Nakajima et al. 2008; Neumann et al. 2010; Shikama et al. 2003; van Raam and Salvesen 2011). Our group could show in a systems biology study that p43-FLIP mediates NF-κB activation by direct interaction with the IKK complex (Neumann et al. 2010). However, the exact details of molecular mechanism of nonapoptotic signaling of CD95 will be addressed in further studies.

2.3.2 *The Function of RIP in CD95-Mediated Nonapoptotic Pathways*

The receptor-interacting protein (RIP) family plays a major role in CD95-mediated necrosis, as well as in life and death decisions after DR stimulation (Geserick et al. 2009, 2010; Kamarajan et al. 2010; Kreuz et al. 2004; Moquin and Chan 2010; Shikama et al. 2003; Vandenabeele et al. 2010a). The most prominent member is RIP1, a serine/threonine kinase, which also possesses one C-terminal DD, a RIP homotypic interaction motif (RHIM) and a ubiquitylation site (Geserick et al. 2009; Moquin and Chan 2010; Vandenabeele et al. 2010a, b). The DD of RIP1 allows it to interact with DR or FADD. Another important player is RIP3, a homolog of RIP1, lacking the DD. RIP1 and RIP3 can interact via their RHIM domains (Moquin and Chan 2010; Vandenabeele et al. 2010b). RIP1 can form another cytosolic complex, termed ripoptosome, which consists of at least RIP1, Caspase-8/10, and FADD and is regulated by cIAPs and c-FLIP proteins (Feoktistova et al. 2011; Tenev et al. 2011). The essential role of RIP1 for CD95-induced necroptosis, however, is controversial and it may be that both RIP1 and RIP3 are required (Bertrand and Vandenabeele 2010; Wong et al. 2010). While the kinase domain of RIP1 is required for the execution of necroptosis, ubiquitylation by cIAP1/2 has been reported to be needed for its nonapoptotic activities (Moquin and Chan 2010; Vandenabeele et al. 2010a, b). The exact roles of RIP1 and RIP3 in nonapoptotic pathways induced by CD95 are still unresolved and will be addressed in models of extrinsic apoptosis in the chapter by Zhivotovsky (Calzone et al. 2010). Especially the mechanistic interactions leading to nonapoptotic pathways after death receptor stimulation are poorly understood.

2.4 Mathematical Formalisms Used in Modeling of the Extrinsic Pathway

There are numerous different modeling formalisms to describe cellular systems and some of them have been applied for modeling the extrinsic pathway. The simplest model is a graph, which only qualitatively describes a system and cannot be used for simulation. Further, apoptosis signaling has been described by Boolean models (Calzone et al. 2010; Mai and Liu 2009; Saez-Rodriguez et al. 2009; Schlatter et al. 2009). The advantage of Boolean models is that simulation and optimization can be done very fast and no kinetic parameters need to be known, because each variable can be either on or off, represented by 1 or 0, respectively. The disadvantage, however, is that Boolean models are not quantitative and cannot represent reaction kinetics. Other modeling approaches which were also applied to apoptosis modeling include Bayesian models and petri nets (Heiner et al. 2004; Yang 2005). The most common approach used in apoptosis modeling is based on ordinary differential equations (ODEs), which describe cellular reactions based on mass-action kinetics and can describe biochemical networks with adequate accuracy. A first order ODE has the form $\dot{x} = f(t, x(t))$. Coupled differential equations can be used to describe the changes in protein concentrations depending on the system state. ODEs cannot describe diffusion in a cell. Diffusion is mostly neglected when modeling a signaling pathway, based on the general assumption that molecule diffusion is much faster compared to signal transduction. In order to include diffusion, partial differential equations (PDE) can be applied.

Contrary to models based on differential equations, which are deterministic, stochastic models use probabilities that two molecules interact with each other. This can be also used in agent-based models (Bonabeau 2002; Brown et al. 2011; Macal and North 2005, 2009; Roche et al. 2011). Each molecule is represented by an agent with certain properties based on biological knowledge. Agent-based models take into account local heterogeneity of molecules, while other approaches only consider molecule populations.

ODEs are constructed based on mass-action kinetics. The following chemical reaction:

$$A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C$$

transforms into the following system of ODEs:

$$\frac{\mathrm{d}[A]}{\mathrm{d}t} = \frac{\mathrm{d}[B]}{\mathrm{d}t} = -k_1 \cdot A \cdot B + k_2 \cdot C$$
$$\frac{\mathrm{d}[C]}{\mathrm{d}t} = k_1 \cdot A \cdot B - k_2 \cdot C$$

Usually ODEs cannot be solved analytically, but are solved numerically using computer programs, such as Matlab. The larger the model, the more kinetic

parameters the system has. Measuring a large amount of parameters is often not possible resulting in unknown parameters. Either the system needs to be simplified to reduce parameter complexity or parameters need to be estimated. When estimating parameters the system should describe the system as accurately as possible. Parameter estimation can be done by the method of least-squares to fit experimental data to the model. Oftentimes the model includes many kinetic parameters compared to few experimental data, which gives multiple solutions for the parameter estimation. Therefore, sensitivity analysis is applied to test the robustness of the model upon parameter changes.

2.4.1 Modeling Extrinsic Apoptosis

The first model of CD95-mediated apoptosis was published more than a decade ago by Fussenegger et al. (2000), which was not based on experimental data, but only mechanistically described the apoptotic pathway. Albeck et al. (2008) quantitatively described death receptor-induced signaling with a focus on MOMP. Others focused on the intrinsic pathway and mitochondrial permeabilization (Düssmann et al. 2010; Rehm et al. 2009). The following section gives a review of systems biology studies of the CD95 signaling pathway as prototypic extrinsic pathway, all using an ODE-based modeling approach.

2.4.1.1 Revealing a Threshold Mechanism in CD95-Induced Apoptosis

In Bentele et al. (2004) the CD95 pathway topology was reconstructed by literature and database research. Initially, a model was generated consisting of about 70 molecules, 80 reactions, and more than 120 unknown parameters. The relatively high number of parameters did not allow parameter estimation using experimental data. Thus, subunits of varying information qualities were added. Well-known interactions were modeled mechanistically, and two "black boxes" were introduced for mitochondria and the degradation process. Black boxes were defined by their input–output behavior. For simplification the DISC was modeled as one complex, without taking into account its stoichiometry. These steps led to a model of CD95-induced apoptosis with 41 molecules (including complexes, e.g., DISC), 32 reactions, and two black boxes, namely mitochondria and degradation. The mitochondria black box takes the concentration of Bcl-X_L/Bcl-2 and tBid as input and triggers the release of cytochrome c if the concentration of tBid reaches a certain threshold compared to the concentrations of the antiapoptotic proteins. For degradation, a decay function was introduced, depending on the apoptotic activity of the different molecules. This model still had over 50 unknown parameters and was further simplified by applying sensitivity analysis, which tests the system stability upon parameter changes. Due to the large number of unknown parameters, the sensitivities for many randomly chosen points, covering a

range of three orders of magnitude, were calculated. This analysis indicated that most sensitivities of the system were highly robust to changes in parameters. This analysis led to a simplified model of the DISC system in which the different c-FLIP isoforms were not distinguished and it was assumed that c-FLIP proteins block procaspase-8 activation at the DISC. Furthermore, the system dimensionality could be reduced from 58 to 18 unknown parameters. Based on this structured information model of CD95-induced cell death, experiments were designed to measure time-dependent concentrations of 15 different molecules after stimulation of CD95. The experiments were performed in the type I B lymphoblastoid cell line SKW6.4. The cells were stimulated with agonistic anti-APO-1 antibodies and the concentrations of the molecules of interest were measured by Western blot. In the first approach, kinetics of molecule concentrations were measured with an oversaturated ligand concentration of 5 μg/ml anti-APO-1, corresponding to a ligand/receptor ratio of about 5:1. The mathematical model could describe well the experimental data under these conditions. However, the system was still underdetermined, meaning different model parameters could match the same experimental data. Therefore, the same kinetics were measured for lower ligand concentrations and the parameters were estimated based on these different stimulation conditions. The resulting model could fit different stimulation scenarios. Most importantly, the model predicted a critical ligand concentration, below which apoptosis is abrogated, which could be verified experimentally (Bentele et al. 2004; Lavrik et al. 2007). It could be shown experimentally that c-FLIP proteins play a critical role in maintaining the threshold behavior of CD95 signaling. Upon low ligand concentration only few receptors would be activated and c-FLIP proteins could block all binding sites for caspase-8, thus preventing induction of apoptosis. High ligand concentrations, however, could lead to the activation of many receptors and caspase-8 would outnumber c-FLIP proteins at the DISC, allowing its activation and initiation of apoptosis.

2.4.1.2 The Stoichiometry of the DISC Determines Life and Death at CD95

In Neumann et al. (2010) life/death decisions after CD95 stimulation were analyzed. In particular, we focused on the crosstalk between the apoptotic and NF-κB signaling pathways. Initially a model was designed in which a trimerized ligand binds to trimerized CD95 further leading to the recruitment of three FADD molecules and DISC formation. To each of the FADD molecules either procaspase-8, c-FLIP$_L$, or c-FLIP$_S$ could be recruited via DED interactions. The order of binding of the DED proteins to the DISC gave rise to different intermediates. Fully formed DISCs were divided into three groups. The first group contained at least two procaspase-8 molecules leading to activation of procaspase-8 and apoptosis. The second group contained at least one procaspase-8 and one c-FLIP$_L$ molecule and would lead to the activation of NF-κB via generation of the c-FLIP$_L$ cleavage product p43-FLIP. The model assumed a direct interaction between p43-

FLIP and the IKK complex, leading to the phosphorylation and degradation of IκB and translocation of p65 to the nucleus. This prediction could be also verified experimentally. The third group of DISCs comprised all other conformations leading to a blocked state which neither initiates apoptosis nor NF-κB activation. The model was simulated using coupled ODEs and the large number of unknown parameters was reduced by assuming irreversible reactions. The remaining unknown parameters were estimated from experimental Western blot data by the method of least-squares. The model could well describe experimental data and postulated direct interaction between p43-FLIP and the IKK complex. Interestingly effector caspase-activity was not required to reproduce the dynamics of NF-κB activation after CD95 stimulation. In addition, the model postulated that apoptotic and nonapoptotic pathways diverge at the DISC. This hypothesis was tested experimentally by inhibiting the apoptotic branch using the pan-caspase inhibitor zVAD-fmk, which blocks caspase activation downstream of the DISC, but not at the DISC (Golks et al. 2006b; Hughes et al. 2009). We stimulated zVAD-fmk pretreated CD95 overexpressing HeLa cells (HeLa-CD95) and studied NF-κB activation as well as apoptotic signaling. We could see p65 translocation to the nucleus using mCherry-tagged p65 and fluorescence microscopy, but could not detect apoptosis, thus validating the model prediction. In order to study whether the complete model might hide key features due to its complexity we performed a stepwise model reduction. Parameter estimation was done after each reducing step and its performance compared to the original complete model. A simpler model usually gives more reliable parameter estimation, because very complex models are prone to overfitting. We came up with a simplified model, in which CD95 and FADD were assumed to be pretrimerized (RF). Upon ligand (L) binding, the L/RF complex could further recruit procaspase-8, c-FLIP$_L$, or c-FLIP$_S$. Two procaspase-8 molecules in the DISC would lead to the generation of active caspase-8 heterotetramers (p18/p10)$_2$ via p43/p41, while the presence of one molecule of procaspase-8 and c-FLIP$_L$ initiates the NF-κB pathway via the cleavage product p43-FLIP leading to nonlinear dynamics. Recruitment of c-FLIP$_S$ to the DISC leads to inhibition. This model could still well describe experimental data and also fit with the previously found threshold behavior of CD95-signaling (Bentele et al. 2004). Interestingly, the threshold concentration did not depend on the number of CD95 on the cell surface, but determines the rates of apoptotic and nonapoptotic signaling, which was found by comparing HeLa wt and HeLa-CD95 (Neumann et al. 2010). Importantly, we revealed that the stoichiometry of DISC components and especially the ratios of c-FLIP isoforms to procaspase-8 and the concentration of their cleavage products p43/p41 and p43-FLIP play a crucial role in the life/death decisions in CD95 signaling.

2.4.1.3 Dual Function of c-FLIP$_L$ in Procaspase-8 Processing and Cell Death

In Fricker et al. (2010) we also focused on the CD95 DISC and studied the role of c-FLIP proteins in the activation of procaspase-8 and cell death. We studied the

influence of c-FLIP on caspase-8 activation in HeLa-CD95 cells which stably overexpress c-FLIP$_L$ (HeLa-CD95-F$_L$), c-FLIP$_S$ (HeLa-CD95-F$_S$), or c-FLIP$_R$ (HeLa-CD95-F$_R$). Procaspase-8 activation was studied after stimulation of these cell lines for different times with CD95L, followed by CD95 DISC Immunoprecipitation (IP) and Western blot analysis. Overexpression of either of the short isoforms of c-FLIP resulted in inhibition of procaspase-8 processing, even after several hours. Interestingly overexpression of c-FLIP$_L$ strongly accelerated the first cleavage step to p43/p41, which agreed with previous studies (Golks et al. 2005; Krueger et al. 2001; Micheau et al. 2002). This effect was also observed for different concentrations of CD95L. Even though the first cleavage of procaspase-8 was enhanced, c-FLIP$_L$ overexpressing HeLa-CD95 cells were less sensitive to apoptosis induction compared to normal HeLa-CD95. Stimulation with lower concentrations of CD95L resulted in complete inhibition of procaspase-8 processing as well as caspase-3 and PARP cleavage in c-FLIP$_L$ overexpressing HeLa-CD95 cells, but not in normal HeLa-CD95 cells. Stimulation with a high dose of ligand led to accelerated caspase-8 processing and cleavage of caspase-3 and PARP. Essentially, we observed nonlinear effects of c-FLIP$_L$ overexpression on procaspase-8 processing and cell death. To further study these effects, we built a mathematical model of caspase-8 processing at the DISC using coupled ODEs. The model involved CD95L which binds to CD95, further causing the recruitment of FADD to form the DISC. Contrary to the model of Neumann et al. (2010), we considered the complex of CD95L/CD95/FADD as monomer which can further recruit one DED protein. Procaspase-8 can form three different dimers at the DISC: procaspase-8 homodimers, procaspase-8/c-FLIP$_L$ heterodimers, and procaspase-8/c-FLIP$_{S/R}$ heterodimers. Procaspase-8 homodimers could be fully processed to p43/p41 and p18, procaspase-8/c-FLIP$_L$ heterodimers could be only processed to p43/p41, but not p18, and procaspase-8/c-FLIP$_{S/R}$ heterodimers could not be processed at all. This assumption was experimentally validated in HeLa-CD95-c-FLIP$_{L/R}$ cells, overexpressing both the short and long isoforms of c-FLIP. Furthermore, procaspase-8 homodimers and procaspase-8/c-FLIP$_L$ heterodimers were assumed to be catalytically active and cleave other molecules at the DISC. Fully processed caspase-8 heterotetramers would be released into the cytosol and replaced by new DED proteins, causing a turnover of caspase-8 at the DISC. Additionally, to reduce model complexity, procaspase-8a and procaspase-8b were considered as one entity, because they had been reported to have similar catalytic properties (Hughes et al. 2009). In addition, processing of procaspase-8 to p30 was neglected due to much lower amounts compared to p43/p41 (Hoffmann et al. 2009). Essentially, we showed that c-FLIP$_L$ only acts proapoptotic under certain conditions and depends upon the strength of stimulation. At moderate concentrations at the DISC combined with strong stimulation of CD95 or high amounts of one of the short c-FLIP isoforms at the DISC c-FLIP$_L$ plays a proapoptotic role, while high amounts of c-FLIP$_L$ at the DISC results in inhibition of apoptotic signaling. Due to its high affinity to the DISC (Chang et al. 2002), c-FLIP$_L$ could be preferentially recruited to the DISC and compete with procaspase-8 for binding sites. In addition c-FLIP$_L$ could block procaspase-8 turnover at the DISC by blocking the final cleavage step to p18.

Finally, the role of c-FLIP$_L$ in procaspase-8 processing is further modulated by the short c-FLIP isoforms. High levels of c-FLIP$_{R/S}$ in the cell cause a sensitizing effect of c-FLIP$_L$. These findings demonstrate that c-FLIP proteins are critical regulators of life and death decisions in CD95 signaling. Further, they demonstrate the importance of the stoichiometry of the DISC in this decision process. Changes in DISC components could shift the signaling from apoptotic to nonapoptotic or vice versa. In addition the data show the complex interplay of the different components at the DISC, because the action of c-FLIP$_L$ does not only depend on its own concentration but also on the concentration of the short variants at the DISC as well as the strength of stimulation.

2.5 Conclusions

CD95-induced apoptosis is one of the best-studied signaling pathways, making it especially interesting for modeling. Stimulation of CD95 can induce, both, apoptotic and nonapoptotic responses. The apoptotic response upon CD95 stimulation is very well defined. CD95 stimulation leads to the formation of the DISC and activation of the initiator procaspases-8 and -10 that, in turn, triggers the apoptotic cascade.

Apoptosis has been subject of intense modeling, using different modeling formalisms, including Boolean modeling (Calzone et al. 2010; Mai and Liu 2009; Saez-Rodriguez et al. 2009; Schlatter et al. 2009), Bayesian modeling, petri nets (Heiner et al. 2004; Yang 2005), and ODEs (Albeck et al. 2008; Bentele et al. 2004; Cui et al. 2008; Fricker et al. 2010; Fussenegger et al. 2000; Kober et al. 2011; Legewie et al. 2006; Neumann et al. 2010; Rehm et al. 2006; Spencer and Sorger 2011). We could successfully model extrinisic apoptosis using coupled ODEs based on biochemical data and derive reasonable biological conclusions. Using this systems biology approach, we could reveal the threshold mechanism of CD95-induced apoptosis (Bentele et al. 2004; Lavrik et al. 2007). Furthermore, we could show that the stoichiometry of the DISC is central to life and death decisions upon CD95-stimulation (Neumann et al. 2010). In the same study, we showed that p43-FLIP directly interacts with the IKK complex leading to NF-κB activation after CD95 stimulation. In addition, we could gain detailed insights into the role of c-FLIP proteins in the activation of apoptosis or nonapoptotic pathways and showed that c-FLIP$_L$ can exhibit pro- or antiapoptotic functions, depending on the strength of stimulation, as well as its concentration at the DISC (Fricker et al. 2010).

Our results depicted the important function of the DISC in the activation of apoptotic as well as nonapoptotic responses. Especially the DISC stoichiometry and the complex interplay between DISC components decide the signaling outcome. The stoichiometry of the DISC, however, has only been studied for CD95 and FADD (Scott et al. 2009; Wang et al. 2010), but no conclusive data on all major components, including caspase-8/10 as well as c-FLIP proteins are available.

Determining the stoichiometry of the CD95 DISC and comparing apoptotic vs. nonapoptotic cells could provide key insights into this decision process, which is still not understood conclusively. Analysis of the CD95 DISC stoichiometry using mathematical modeling should provide new insights into the regulation of CD95 signaling. Furthermore, it could be possible to alter the DISC stoichiometry pharmacologically, thus switching between the two signaling outcomes in CD95. This would add new therapies for diseases with dysfunctional apoptosis, such as neurodegenerative disorders or cancer.

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References

Adams JM (1998) The Bcl-2 protein family: arbiters of cell survival. Science 281:1322–1326

Albeck JG, Burke JM, Aldridge BB, Zhang M, Lauffenburger DA, Sorger PK (2008) Quantitative analysis of pathways controlling extrinsic apoptosis in single cells. Mol Cell 30:11–25

Alderson MR, Armitage RJ, Maraskovsky E, Tough TW, Roux E, Schooley K, Ramsdell F, Lynch DH (1993) Fas transduces activation signals in normal human T lymphocytes. J Exp Med 178:2231–2235

Ashkenazi A (1998) Death receptors: signaling and modulation. Science 281:1305–1308

Barnhart BC, Alappat EC, Peter ME (2003) The CD95 Type I/Type II model. Semin Immunol 15:185–193

Bentele M, Lavrik I, Ulrich M, Stösser S, Heermann DW, Kalthoff H, Krammer PH, Eils R (2004) Mathematical modeling reveals threshold mechanism in CD95-induced apoptosis. J Cell Biol 166:839–851

Bertrand MJM, Vandenabeele P (2010) RIP1's function in NF-κB activation: from master actor to onlooker. Cell Death Differ 17:379–380

Boatright KM, Renatus M, Scott FL, Sperandio S, Shin H, Pedersen IM, Ricci JE, Edris WA, Sutherlin DP, Green DR, Salvesen GS (2003) A unified model for apical caspase activation. Mol Cell 11:529–541

Bonabeau E (2002) Agent-based modeling: methods and techniques for simulating human systems. Proc Natl Acad Sci USA 99(Suppl 3):7280–7287

Brown BN, Price IM, Toapanta FR, Dealmeida DR, Wiley CA, Ross TM, Oury TD, Vodovotz Y (2011) An agent-based model of inflammation and fibrosis following particulate exposure in the lung. Math Biosci 231(2):186–96

Calzone L, Tournier L, Fourquet S, Thieffry D, Zhivotovsky B, Barillot E, Zinovyev A (2010) Mathematical modeling of cell-fate decision in response to death receptor engagement. PLoS Comput Biol 6:e1000702

Chang DW, Xing Z, Pan Y, Algeciras-Schimnich A, Barnhart BC, Yaish-Ohad S, Peter ME, Yang X (2002) c-FLIP(L) is a dual function regulator for caspase-8 activation and CD95-mediated apoptosis. EMBO J 21:3704–3714

Chen L, Park S-M, Tumanov AV, Hau A, Sawada K, Feig C, Turner JR, Fu Y-X, Romero IL, Lengyel E, Peter ME (2010) CD95 promotes tumour growth. Nature 465:492–496

Choi K, Ni L, Jonakait GM (2010) Fas ligation and tumor necrosis factor alpha activation of murine astrocytes promote heat shock factor-1 activation and heat shock protein expression leading to chemokine induction and cell survival. J Neurochem 116(3):438–448

Clem RJ, Miller LK (1994) Control of programmed cell death by the baculovirus genes p35 and iap. Mol Cell Biol 14:5212–5222

Cui J, Chen C, Lu H, Sun T, Shen P (2008) Two independent positive feedbacks and bistability in the Bcl-2 apoptotic switch. PLoS One 3:e1469

Deveraux QL, Reed JC (1999) IAP family proteins–suppressors of apoptosis. Genes Dev 13:239–252

Düssmann H, Rehm M, Concannon CG, Anguissola S, Würstle M, Kacmar S, Völler P, Huber HJ, Prehn JHM (2010) Single-cell quantification of Bax activation and mathematical modeling suggest pore formation on minimal mitochondrial Bax accumulation. Cell Death Differ 17:278–290

Feoktistova M, Geserick P, Kellert B, Dimitrova DP, Langlais C, Hupe M, Cain K, Macfarlane M, Häcker G, Leverkus M (2011) cIAPs block ripoptosome formation, a RIP1/Caspase-8-containing intracellular cell death complex differentially regulated by cFLIP isoforms. Mol Cell 43(3–6):449–463

Fesik SW (2000) Insights into programmed cell death through structural biology. Cell 103:273–282

Fischer U, Stroh C, Schulze-Osthoff K (2006) Unique and overlapping substrate specificities of caspase-8 and caspase-10. Oncogene 25:152–159

Fricker N, Beaudouin J, Richter P, Eils R, Krammer PH, Lavrik IN (2010) Model-based dissection of CD95 signaling dynamics reveals both a pro- and antiapoptotic role of c-FLIPL. J Cell Biol 190:377–389

Fuentes-Prior P, Salvesen GS (2004) The protein structures that shape caspase activity, specificity, activation and inhibition. Biochem J 384:201–232

Fussenegger M, Bailey JE, Varner J (2000) A mathematical model of caspase function in apoptosis. Nat Biotechnol 18:768–774

Geserick P, Hupe M, Moulin M, Wong WW-L, Feoktistova M, Kellert B, Gollnick H, Silke J, Leverkus M (2009) Cellular IAPs inhibit a cryptic CD95-induced cell death by limiting RIP1 kinase recruitment. J Cell Biol 187:1037–1054

Geserick P, Hupe M, Moulin M, Leverkus M (2010) RIP-in CD95-induced cell death: the control of alternative death receptors pathways by cIAPs. Cell Cycle 9:2689–2691

Ghosh S, Hayden MS (2008) New regulators of NF-κB in inflammation. Nat Rev Immunol 8:837–848

Ghosh S, May MJ, Kopp EB (1998) NF-κ B and Rel proteins: evolutionarily conserved mediators of immune responses. Annu Rev Immunol 16:225–260

Golks A, Brenner D, Fritsch C, Krammer PH, Lavrik IN (2005) c-FLIPR, a new regulator of death receptor-induced apoptosis. J Biol Chem 280:14507–14513

Golks A, Brenner D, Krammer PH, Lavrik IN (2006a) The c-FLIP-NH2 terminus (p22-FLIP) induces NF-κB activation. J Exp Med 203:1295–1305

Golks A, Brenner D, Schmitz I, Watzl C, Krueger A, Krammer PH, Lavrik IN (2006b) The role of CAP3 in CD95 signaling: new insights into the mechanism of procaspase-8 activation. Cell Death Differ 13:489–498

Gonzalvez F, Schug ZT, Houtkooper RH, MacKenzie ED, Brooks DG, Wanders RJA, Petit PX, Vaz FM, Gottlieb E (2008) Cardiolipin provides an essential activating platform for caspase-8 on mitochondria. J Cell Biol 183:681–696

Hao Z, Hampel B, Yagita H, Rajewsky K (2004) T cell-specific ablation of Fas leads to Fas ligand-mediated lymphocyte depletion and inflammatory pulmonary fibrosis. J Exp Med 199:1355–1365

Harvey AJ, Soliman H, Kaiser WJ, Miller LK (1997) Anti- and pro-apoptotic activities of baculovirus and Drosophila IAPs in an insect cell line. Cell Death Differ 4:733–744

Hay BA, Wassarman DA, Rubin GM (1995) Drosophila homologs of baculovirus inhibitor of apoptosis proteins function to block cell death. Cell 83:1253–1262

Hayden MS, Ghosh S (2004) Signaling to NF-κB. Genes Dev 18:2195–2224

Hayden MS, West AP, Ghosh S (2006) NF-κB and the immune response. Oncogene 25:6758–6780

Heiner M, Koch I, Will J (2004) Model validation of biological pathways using Petri nets–demonstrated for apoptosis. Biosystems 75:15–28

Hoffmann JC, Pappa A, Krammer PH, Lavrik IN (2009) A new C-terminal cleavage product of procaspase-8, p30, defines an alternative pathway of procaspase-8 activation. Mol Cell Biol 29:4431–4440

Hughes MA, Harper N, Butterworth M, Cain K, Cohen GM, MacFarlane M (2009) Reconstitution of the death-inducing signaling complex reveals a substrate switch that determines CD95-mediated death or survival. Mol Cell 35:265–279

Jin Z, Li Y, Pitti R, Lawrence D, Pham VC, Lill JR, Ashkenazi A (2009) Cullin3-based polyubiquitination and p62-dependent aggregation of caspase-8 mediate extrinsic apoptosis signaling. Cell 137:721–735

Kamarajan P, Bunek J, Lin Y, Nunez G, Kapila YL (2010) Receptor-interacting protein shuttles between cell death and survival signaling pathways. Mol Biol Cell 21:481–488

Kataoka T, Budd RC, Holler N, Thome M, Martinon F, Irmler M, Burns K, Hahne M, Kennedy N, Kovacsovics M, Tschopp J (2000) The caspase-8 inhibitor FLIP promotes activation of NF-κB and Erk signaling pathways. Curr Biol 10:640–648

Keller N, Mares J, Zerbe O, Grütter MG (2009) Structural and biochemical studies on procaspase-8: new insights on initiator caspase activation. Structure 17:438–448

Kober AMM, Legewie S, Pforr C, Fricker N, Eils R, Krammer PH, Lavrik IN (2011) Caspase-8 activity has an essential role in CD95/Fas-mediated MAPK activation. Cell Death Dis 2:e212

Krammer PH (2000) CD95's deadly mission in the immune system. Nature 407:789–795

Krammer PH, Arnold R, Lavrik IN (2007) Life and death in peripheral T cells. Nat Rev Immunol 7:532–542

Kreuz S, Siegmund D, Rumpf J-J, Samel D, Leverkus M, Janssen O, Häcker G, Dittrich-Breiholz O, Kracht M, Scheurich P, Wajant H (2004) NFkappaB activation by Fas is mediated through FADD, caspase-8, and RIP and is inhibited by FLIP. J Cell Biol 166:369–380

Krueger A, Schmitz I, Baumann S, Krammer PH, Kirchhoff S (2001) Cellular FLICE-inhibitory protein splice variants inhibit different steps of caspase-8 activation at the CD95 death-inducing signaling complex. J Biol Chem 276:20633–20640

Lavrik I, Golks A, Krammer PH (2005a) Death receptor signaling. J Cell Sci 118:265–267

Lavrik IN, Golks A, Krammer PH (2005b) Caspases: pharmacological manipulation of cell death. J Clin Invest 115:2665–2672

Lavrik IN, Golks A, Riess D, Bentele M, Eils R, Krammer PH (2007) Analysis of CD95 threshold signaling: triggering of CD95 (FAS/APO-1) at low concentrations primarily results in survival signaling. J Biol Chem 282:13664–13671

Lee S-M, Kim E-J, Suk K, Lee W-H (2011) Stimulation of Fas (CD95) induces production of pro-inflammatory mediators through ERK/JNK-dependent activation of NF-κB in THP-1 cells. Cell Immunol 271(1):157–62

Legembre P, Barnhart BC, Peter ME (2004) The relevance of NF-κB for CD95 signaling in tumor cells. Cell Cycle 3:1235–1239

Legewie S, Blüthgen N, Herzel H (2006) Mathematical modeling identifies inhibitors of apoptosis as mediators of positive feedback and bistability. PLoS Comput Biol 2:e120

Li H (1998) Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell 94:491–501

Li Q, Verma IM (2002) NF-κB regulation in the immune system. Nat Rev Immunol 2:725–734

Macal CM, North MJ (2005) Tutorial on agent-based modeling and simulation. J Simul 4:151–162

Macal, C.M.; North, M.J.; , "Agent-based modeling and simulation", Proceedings of the 2009 Winter Simulation Conference (WSC), pp.86–98, 13–16Dec. 2009 doi:http://ieeexplore.ieee.org/stamp/stamp.jsp?tp=&arnumber=5429318&isnumber=5429163

Mai Z, Liu H (2009) Boolean network-based analysis of the apoptosis network: irreversible apoptosis and stable surviving. J Theor Biol 259:760–769

Medema JP, Toes RE, Scaffidi C, Zheng TS, Flavell RA, Melief CJ, Peter ME, Offringa R, Krammer PH (1997) Cleavage of FLICE (caspase-8) by granzyme B during cytotoxic T lymphocyte-induced apoptosis. Eur J Immunol 27:3492–3498

Micheau O, Thome M, Schneider P, Holler N, Tschopp J, Nicholson DW, Briand C, Grütter MG (2002) The long form of FLIP is an activator of caspase-8 at the Fas death-inducing signaling complex. J Biol Chem 277:45162–45171

Moquin D, Chan FK-M (2010) The molecular regulation of programmed necrotic cell injury. Trends Biochem Sci 35:434–441

Mühlethaler-Mottet A, Flahaut M, Bourloud KB, Nardou K, Coulon A, Liberman J, Thome M, Gross N (2011) Individual caspase-10 isoforms play distinct and opposing roles in the initiation of death receptor-mediated tumour cell apoptosis. Cell Death Dis 2:e125

Muzio M, Chinnaiyan AM, Kischkel FC, O'Rourke K, Shevchenko A, Ni J, Scaffidi C, Bretz JD, Zhang M, Gentz R et al (1996) FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death–inducing signaling complex. Cell 85:817–827

Nakajima A, Kojima Y, Nakayama M, Yagita H, Okumura K, Nakano H (2008) Downregulation of c-FLIP promotes caspase-dependent JNK activation and reactive oxygen species accumulation in tumor cells. Oncogene 27:76–84

Neumann L, Pforr C, Beaudouin J, Pappa A, Fricker N, Krammer PH, Lavrik IN, Eils R (2010) Dynamics within the CD95 death-inducing signaling complex decide life and death of cells. Mol Syst Biol 6:352

Oberst A, Pop C, Tremblay AG, Blais V, Denault J-B, Salvesen GS, Green DR (2010) Inducible dimerization and inducible cleavage reveal a requirement for both processes in caspase-8 activation. J Biol Chem 285:16632–16642

O'Reilly LA, Divisekera U, Newton K, Scalzo K, Kataoka T, Puthalakath H, Ito M, Huang DCS, Strasser A (2004) Modifications and intracellular trafficking of FADD/MORT1 and caspase-8 after stimulation of T lymphocytes. Cell Death Differ 11:724–736

Paulsen M, Janssen O (2011) Pro- and anti-apoptotic CD95 signaling in T cells. Cell Commun Signal 9:7

Paulsen M, Valentin S, Mathew B, Adam-Klages S, Bertsch U, Lavrik I, Krammer PH, Kabelitz D, Janssen O (2011) Modulation of CD4+ T-cell activation by CD95 co-stimulation. Cell Death Differ 18:619–631

Peter ME, Budd RC, Desbarats J, Hedrick SM, Hueber A-O, Newell MK, Owen LB, Pope RM, Tschopp J, Wajant H et al (2007) The CD95 receptor: apoptosis revisited. Cell 129:447–450

Rasper DM, Vaillancourt JP, Hadano S, Houtzager VM, Seiden I, Keen SL, Tawa P, Xanthoudakis S, Nasir J, Martindale D et al (1998) Cell death attenuation by 'Usurpin', a mammalian DED-caspase homologue that precludes caspase-8 recruitment and activation by the CD-95 (Fas, APO-1) receptor complex. Cell Death Differ 5:271–288

Rathmell JC, Cooke MP, Ho WY, Grein J, Townsend SE, Davis MM, Goodnow CC (1995) CD95 (Fas)-dependent elimination of self-reactive B cells upon interaction with CD4+ T cells. Nature 376:181–184

Rehm M, Huber HJ, Dussmann H, Prehn JHM (2006) Systems analysis of effector caspase activation and its control by X-linked inhibitor of apoptosis protein. EMBO J 25:4338–4349

Rehm M, Huber HJ, Hellwig CT, Anguissola S, Dussmann H, Prehn JHM (2009) Dynamics of outer mitochondrial membrane permeabilization during apoptosis. Cell Death Differ 16:613–623

Roche B, Drake JM, Rohani P (2011) An agent-based model to study the epidemiological and evolutionary dynamics of Influenza viruses. BMC Bioinformatics 12:87

Saez-Rodriguez J, Alexopoulos LG, Epperlein J, Samaga R, Lauffenburger DA, Klamt S, Sorger PK (2009) Discrete logic modeling as a means to link protein signaling networks with functional analysis of mammalian signal transduction. Mol Syst Biol 5:331

Scaffidi C, Medema JP, Krammer PH, Peter ME (1997) FLICE is predominantly expressed as two functionally active isoforms, caspase-8/a and caspase-8/b. J Biol Chem 272:26953–26958

Scaffidi C, Fulda S, Srinivasan A, Friesen C, Li F, Tomaselli KJ, Debatin KM, Krammer PH, Peter ME (1998) Two CD95 (APO-1/Fas) signaling pathways. EMBO J 17:1675–1687

Scaffidi C, Schmitz I, Krammer PH, Peter ME (1999) The role of c-FLIP in modulation of CD95-induced apoptosis. J Biol Chem 274:1541–1548

Schlatter R, Schmich K, Avalos Vizcarra I, Scheurich P, Sauter T, Borner C, Ederer M, Merfort I, Sawodny O (2009) ON/OFF and beyond–a boolean model of apoptosis. PLoS Comput Biol 5: e1000595

Schneider P (1998) Conversion of membrane-bound Fas(CD95) ligand to its soluble form is associated with downregulation of its proapoptotic activity and loss of liver toxicity. J Exp Med 187:1205–1213

Schug ZT, Gonzalvez F, Houtkooper RH, Vaz FM, Gottlieb E (2011) BID is cleaved by caspase-8 within a native complex on the mitochondrial membrane. Cell Death Differ 18:538–548

Schrodinger, LLC (2010). The PyMOL Molecular Graphics System, Version 1.3r1

Scott FL, Stec B, Pop C, Dobaczewska MK, Lee JJ, Monosov E, Robinson H, Salvesen GS, Schwarzenbacher R, Riedl SJ (2009) The Fas-FADD death domain complex structure unravels signaling by receptor clustering. Nature 457:1019–1022

Sen R, Baltimore D (1986) Inducibility of κ immunoglobulin enhancer-binding protein NF-κB by a posttranslational mechanism. Cell 47:921–928

Shi Y (2002) Mechanisms of caspase activation and inhibition during apoptosis. Mol Cell 9:459–470

Shikama Y, Yamada M, Miyashita T (2003) Caspase-8 and caspase-10 activate NF-κB through RIP, NIK and IKKalpha kinases. Eur J Immunol 33:1998–2006

Spencer SL, Sorger PK (2011) Measuring and modeling apoptosis in single cells. Cell 144:926–939

Sprick MR, Rieser E, Stahl H, Grosse-Wilde A, Weigand MA, Walczak H (2002) Caspase-10 is recruited to and activated at the native TRAIL and CD95 death-inducing signaling complexes in a FADD-dependent manner but cannot functionally substitute caspase-8. EMBO J 21:4520–4530

Steller EJ, Ritsma L, Raats DA, Hoogwater FJ, Emmink BL, Govaert KM, Laoukili J, Rinkes IH, van Rheenen J, Kranenburg O (2011) The death receptor CD95 activates the cofilin pathway to stimulate tumour cell invasion. EMBO Rep 12(9):931–937

Strasser A, Jost PJ, Nagata S (2009) The many roles of FAS receptor signaling in the immune system. Immunity 30:180–192

Tang D, Lotze MT, Kang R, Zeh HJ (2010) Apoptosis promotes early tumorigenesis. Oncogene 30(16):1851–4

Tenev T, Bianchi K, Darding M, Broemer M, Langlais C, Wallberg F, Zachariou A, Lopez J, Macfarlane M, Cain K, Meier P (2011) The ripoptosome, a signaling platform that assembles in response to genotoxic stress and loss of IAPs. Mol cell 43(3):432–48

Trauth BC, Klas C, Peters AM, Matzku S, Möller P, Falk W, Debatin KM, Krammer PH (1989) Monoclonal antibody-mediated tumor regression by induction of apoptosis. Science 245:301–305

Ueffing N, Schuster M, Keil E, Schulze-Osthoff K, Schmitz I (2008) Up-regulation of c-FLIP short by NFAT contributes to apoptosis resistance of short-term activated T cells. Blood 112:690–698

van Raam BJ, Salvesen GS (2011) Proliferative versus apoptotic functions of caspase-8 hetero or homo: the caspase-8 dimer controls cell fate. Biochim Biophys Acta 1824(1):113–22

Vandenabeele P, Declercq W, Van Herreweghe F, Vanden Berghe T (2010a) The role of the kinases RIP1 and RIP3 in TNF-induced necrosis. Sci Signal 3(115):re4

Vandenabeele P, Galluzzi L, Vanden Berghe T, Kroemer G (2010b) Molecular mechanisms of necroptosis: an ordered cellular explosion. Nat Rev Mol Cell Biol 11:700–715

Verma IM, Stevenson JK, Schwarz EM, Van Antwerp D, Miyamoto S (1995) Rel/NF-κ B/I kappa B family: intimate tales of association and dissociation. Genes Dev 9:2723–2735

Wang L, Yang JK, Kabaleeswaran V, Rice AJ, Cruz AC, Park AY, Yin Q, Damko E, Jang SB, Raunser S et al (2010) The Fas-FADD death domain complex structure reveals the basis of DISC assembly and disease mutations. Nat Struct Mol Biol 17:1324–1329

Wong WW-L, Gentle IE, Nachbur U, Anderton H, Vaux DL, Silke J (2010) RIPK1 is not essential for TNFR1-induced activation of NF-κB. Cell Death Differ 17:482–487

Yan N, Shi Y (2005) Mechanisms of apoptosis through structural biology. Annu Rev Cell Dev Biol 21:35–56

Yang ZR (2005) Prediction of caspase cleavage sites using Bayesian bio-basis function neural networks. Bioinformatics 21:1831–1837

Yu J, Zhang L (2005) The transcriptional targets of p53 in apoptosis control. Biochem Biophys Res Commun 331:851–858

Yu JW, Jeffrey PD, Shi Y (2009) Mechanism of procaspase-8 activation by c-FLIPL. Proc Natl Acad Sci USA 106:8169–8174

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