

## CHAPTER 2

---

# FasL-Independent Activation of Fas

Faustino Mollinedo and Consuelo Gajate

### Abstract

**F**as death receptor (also named CD95 or APO-1) is physiologically activated through binding to its cognate ligand, FasL. Fas/FasL interaction induces oligomerization and aggregation of Fas receptor, leading eventually to apoptosis after protein-protein interactions with adaptor and effector proteins. However, recent evidences demonstrate that either oligomerization of the receptor in trimers, as well as Fas aggregation in large clusters do not require its interaction with FasL. Activation of Fas through its translocation into membrane rafts, forming Fas caps, can be rendered independently of FasL. This FasL-independent cocapping of Fas in membrane rafts generates high local concentrations of Fas, providing scaffolds for coupling adaptor and effector proteins involved in Fas signaling. Thus, Fas receptor can be modulated either extracellularly, via FasL, or intracellularly independently of its ligand. Unraveling the molecular mechanism involved in FasL-independent activation of Fas will raise putative novel therapeutic interventions, especially in disorders where apoptosis is deficient such as cancer and autoimmune diseases, avoiding in this way the deleterious side effects that preclude the use of systemic activation of the Fas receptor by its ligand.

### Abbreviations

The abbreviations used are: DED, death effector domain; DEF, death effector filament; DISC, death-inducing signaling complex; ET-18-OCH<sub>3</sub>, 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (edelfosine); FADD, Fas-associated death domain-containing protein; FasL, Fas ligand; JNK, c-Jun NH<sub>2</sub>-terminal kinase; lpr, lymphoproliferation; MKK7, mitogen-activated protein kinase kinase 7; TNF, tumor necrosis factor.

### Introduction

In May 1989 Dr. Shin Yonehara et al<sup>1,2</sup> reported an IgM monoclonal antibody that could kill several human cell lines, and termed Fas (ES7-associated cell surface antigen) the cell surface protein recognized by the antibody. In July of the same year, Dr. Peter H. Krammer and his associates reported a mouse monoclonal antibody, named anti-APO-1 antibody which promoted apoptosis in human leukemic cells and activated lymphocytes.<sup>3</sup> In 1991 Dr. Shigekazu Nagata and his associates succeeded in cloning the membrane protein recognized by the killing antibody, the Fas antigen,<sup>4</sup> that turned out to be identical to the APO-1 protein identified later in Krammer's group.<sup>5</sup> Then Nagata's group cloned the corresponding physiological ligand of the Fas death receptor, named Fas ligand (FasL).<sup>6-8</sup> These findings identified the Fas/FasL system as the major regulator of apoptosis at the cell membrane in mammalian cells through a

receptor/ligand interaction. Subsequent studies led to a detailed characterization of the initial events triggered after binding of Fas to its cognate ligand. However, the notion that Fas requires interaction with its ligand to trigger an apoptotic response has been recently challenged. Current evidence indicates that Fas can be activated in a FasL-independent manner, suggesting that Fas can also receive orders from within the cell. Thus, cells could dictate its own demise through activation of Fas death receptor without the external influence of its ligand. Unraveling the signaling pathways and molecules involved in the intracellular activation of Fas, independently of FasL, can provide the basis for novel therapeutic strategies and for the development of new compounds able to modulate apoptosis.

### **Fas Death Receptor Functions through Oligomerization and a Cascade of Protein-Protein Interactions**

The Fas death receptor (also called CD95 or APO-1), a major member of the tumor necrosis factor (TNF)/nerve growth factor receptor family<sup>4,9</sup> transmits apoptosis signals initiated by the interaction with its membrane-bound or soluble natural ligand FasL or by agonistic anti-Fas antibodies.<sup>1,3</sup> FasL belongs to the TNF family<sup>6</sup> and can be found as a 40-kDa membrane-bound or a 26-kDa soluble cytokine.<sup>10</sup>

Mature Fas (Fig. 1) is a 45-kDa type I transmembrane receptor of 319 amino acids with a single transmembrane domain of 17 amino acids (from Leu-158 to Val-174), an N-terminal cysteine-rich extracellular domain (18 cysteine residues in 157 amino acids) and a C-terminal cytoplasmic domain of 145 amino acids that is relatively abundant in charged amino acids (24 basic and 19 acidic amino acids). The cytoplasmic portion of Fas contains a domain of about 85 amino acids termed "death domain", which is homologous to other death receptors and plays a crucial role in transmitting the death signal from the cell's surface to intracellular pathways.<sup>4,9,11</sup> Unlike the intracellular regions of other transmembrane receptors involved in signal transduction, the death domain does not possess enzymatic activity, but mediates signaling through protein-protein interactions. The death domain has the propensity to self-associate and form large aggregates in solution. The tertiary structure of the Fas death domain, revealed by NMR spectroscopy,<sup>12</sup> consists of six antiparallel, amphipathic  $\alpha$  helices.<sup>12,13</sup> Helices  $\alpha 1$  and  $\alpha 2$  are centrally located and flanked on each side by  $\alpha 3/\alpha 4$  and  $\alpha 5/\alpha 6$ . This leads to an unusual topology in which the loops connecting  $\alpha 1/\alpha 2$  and  $\alpha 4/\alpha 5$  cross over each other.<sup>12,13</sup> The presence of a high number of charged amino acids in the surface of the death domain is probably responsible for mediating the interactions between death domains. Stimulation of Fas by FasL results in receptor aggregation,<sup>14,15</sup> previously assembled in trimers,<sup>16,17</sup> and recruitment of the adaptor molecule Fas-associated death domain-containing protein (FADD)<sup>18</sup> through interaction between its own death domain and the clustered receptor death domains. FADD also contains a "death effector domain" (DED) that binds to an analogous domain repeated in tandem within the zymogen form of caspase-8.<sup>19</sup> Upon recruitment by FADD, procaspase-8 oligomerization drives its activation through self-cleavage, activating downstream effector caspases and leading to apoptosis.<sup>20</sup> Thus, activation of Fas results in receptor aggregation and formation of the so-called "death-inducing signaling complex" (DISC),<sup>21</sup> containing trimerized Fas, FADD and procaspase-8 (Fig. 2).

Mice carrying the lymphoproliferation (lpr) point mutation which converts Ile-225 to Asn-225 in the cytoplasmic region of the mouse Fas antigen, are characterized by a deficient Fas antigen that leads to a lymphoproliferation syndrome showing lymphadenopathy and a systemic lupus erythematosus-like autoimmune disease.<sup>22</sup> The corresponding mutation in human Fas (V238N) leads to inhibition of apoptosis,<sup>11</sup> together with a dramatic inhibition in Fas death domain self-association and binding to FADD,<sup>12</sup> suggesting that this point mutation alters the protein structure of the death domain. These data suggest that the intracellular portion of the Fas molecule is critical for death receptor oligomerization required for apoptotic

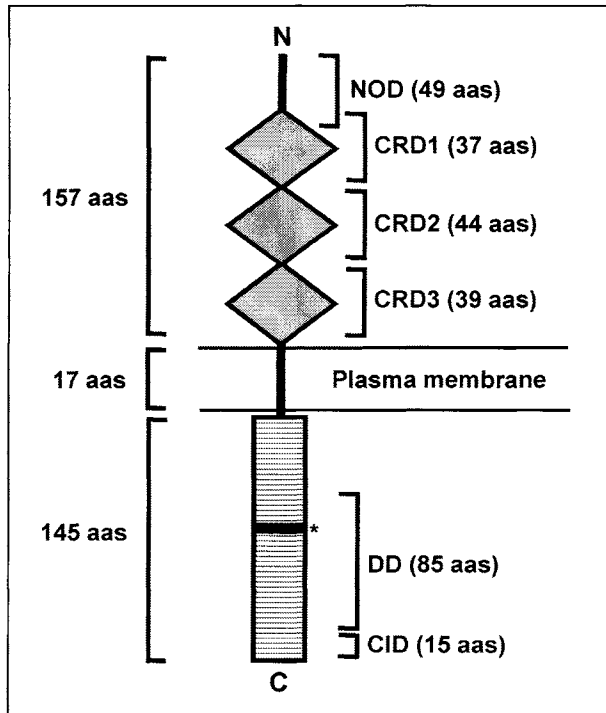


Figure 1. Schematic diagram of the human Fas death receptor. Mature human Fas protein consists of 319 amino acids (aas) with an N-terminal extracellular domain of 157 aas, a short transmembrane region (17 aas) and a C-terminal cytoplasmic domain of 145 aas. Relevant domains for Fas oligomerization and apoptotic activity are shown. An N-terminal extracellular oligomerization domain (NOD) of 49 aas (Arg-1 to Pro-49) responsible for FasL-independent oligomerization of the receptor. Three cysteine-rich domains (CRD1 -Gln31 to Val-67, CRD2 -Pro-68 to Cys-111-, and CRD3 -Arg-112 to Lys150-) containing 4, 6 and 8 Cys residues in each domain respectively. A cytoplasmic death domain (DD) of 85 aas (Ser-214 to Ile -298) is crucial for oligomerization and apoptotic signaling. The last 15 amino acids (Asp-305 to Val-319) of the Fas amino acid sequence represent a C-terminal inhibitory domain (CID). The corresponding *lpr* point mutation in human Fas (Val238N) is also shown (\*). Domains and membrane are not to scale. Amino acid numbering is according to Itoh et al<sup>4</sup> for the mature human Fas sequence.

activity. Current evidence indicates that the molecular ordering of the initial events in physiological Fas-mediated signaling include four successive steps.<sup>23</sup> (a) FasL-induced formation of Fas microaggregates at the cell surface, (b) recruitment of FADD to form a DISC in an actin filament-dependent manner, (c) formation of large Fas surface clusters positively regulated by DISC-generated caspase-8, (d) actin filament-dependent internalization of activated Fas through an endosomal pathway.

### Fas Oligomerization without Interaction with Its Ligand FasL

An early view of the molecular events leading to Fas activation considered that, upon binding to homotrimers of FasL, the Fas receptor homo-oligomerized through the intracellular death domains resulting in its trimerization. Due to the propensity of the intracellular Fas death domains to associate with one another, FasL ligation led to the clustering of Fas death domains and Fas aggregation in trimers promoting the recruitment of FADD and procaspase-8 that together with the receptor formed the DISC and triggered apoptosis.<sup>21</sup>

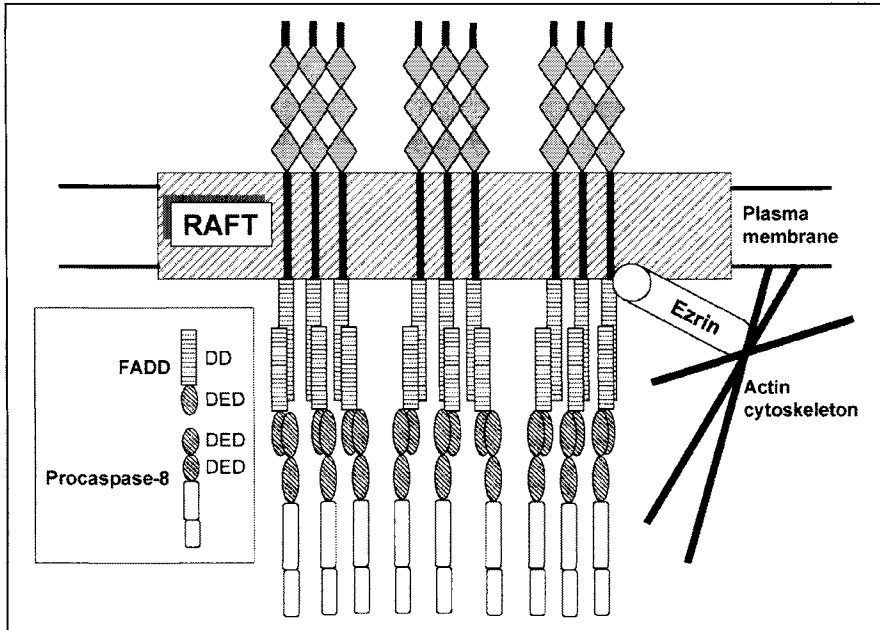


Figure 2. Schematic representation of a tentative model for activation of Fas receptor through its aggregation in membrane rafts. Fas molecules are brought together and concentrate in membrane rafts facilitating the formation of DISCs, following protein-protein interactions between Fas-FADD through their respective death domains (DD), and FADD-procaspase-8 through their respective death effector domains (DED). Actin cytoskeleton through ezrin could be involved in the clustering of Fas in rafts.

However, experimental evidences accumulated in the last five years have modified our view of how Fas-signaling activation takes place. Recent evidence suggests that Fas receptor is in trimer status before FasL binding.<sup>16,17</sup> Preassociated Fas complexes were found in living cells by means of fluorescence resonance energy transfer between variants of green fluorescent protein.<sup>17</sup> A FasL- and death domain-independent oligomerization domain in the extracellular region of the Fas receptor, mapping to the NH<sub>2</sub>-terminal 49 amino acids, mediates homo- and hetero-oligomerization of the death receptor<sup>16</sup> (Fig. 1). Thus, Fas in unstimulated cells seems to be constitutively oligomerized and ligand binding is suggested to induce conformational changes of oligomerized subunits relative to each other, bringing together intracellular domains that are separated in the basal state. However, apoptosis can be triggered in the absence of FasL by overexpressing the Fas cytoplasmic domain or a Fas receptor lacking the NH<sub>2</sub>-terminal 42 amino acids,<sup>16</sup> suggesting that the extracellular oligomerization domain of Fas is not required to initiate signaling and that self-association of the death domain is necessary and sufficient to induce cell death and occurs in the absence of an intact extracellular oligomerization domain. Thus, two major oligomerization domains seem to be present in the Fas receptor (Fig. 1), one mapping to the extracellular region of the receptor, likely related to the regulation of the nonsignaling state, and another one, involved in apoptotic signaling, mapping to the intracytoplasmic region, the death domain.<sup>16</sup>

The intracellular death domains of death receptors show a high tendency to self-associate<sup>24,25</sup> and when overexpressed by gene transfer in eukaryotic cells trigger signaling for cytotoxicity.<sup>25</sup> These findings indicate that the Fas receptor plays an active role in its own clustering and

suggest the existence of cellular mechanisms that restrict its self-association, thus preventing constitutive signaling. Altogether, these data show that Fas oligomerization can be rendered in the absence of FasL.

### Fas-Mediated Apoptosis Does Not Require the Participation of FasL

It has been proposed that doxorubicin-induced apoptosis in human T-leukemic cells is mediated by FasL expression with subsequent autocrine and/or paracrine induction of cell death through binding of FasL to the membrane Fas receptor.<sup>26</sup> This led to postulate in 1996 that Fas/FasL interactions could account for chemotherapy-associated apoptosis.<sup>26</sup> In addition to doxorubicin, additional anticancer drugs, such as methotrexate or bleomycin, were reported to promote induction of FasL expression and upregulation of membrane FasL, leading to autocrine or paracrine Fas/FasL-dependent apoptosis.<sup>26-29</sup> Activation-induced cell death (AICD) in T cells as well as cell death promoted by different antitumor drugs have been reported to be mediated by Fas/FasL interaction.<sup>30,31</sup> Cell lines resistant to Fas were reported to be insensitive to anticancer drug-induced apoptosis, and drug-induced cell death was prevented by Fas neutralizing antibodies.<sup>26</sup> However, the above notion involving Fas/FasL interactions in chemotherapy-induced apoptosis became rapidly controversial as several research groups did not obtain the same results, and thereby did not reach the same conclusions as above, using even similar experimental conditions that included same cell types and chemotherapeutic drugs.<sup>32-38</sup> Furthermore, a number of reports showed that blockade of Fas/FasL interactions did not prevent apoptosis induced by doxorubicin and additional cytotoxic drugs.<sup>32-38</sup> Although many cytotoxic drugs have been reported to act independently of the Fas system,<sup>34-36,39,40</sup> we and others detected FasL-independent activation of Fas in the mechanism of action of a number of antitumor drugs, including the antitumor ether lipid 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (ET-18-OCH<sub>3</sub>, edelfosine), cisplatin (CDDP), etoposide (VP16), and vinblastine.<sup>38,41</sup> Cells deficient in Fas were resistant to the proapoptotic action of ET-18-OCH<sub>3</sub>, but became sensitive to the antitumor ether lipid when transfected with Fas.<sup>38</sup> The presence or absence of Fas cell surface expression in cancer target cells correlated with their sensitivity or resistance, respectively, to the proapoptotic activity of ET-18-OCH<sub>3</sub>.<sup>38</sup> Down-regulation of FADD by transient transfection of an antisense FADD construct inhibited tumor cell sensitivity to cisplatin, etoposide or vinblastine, whereas overexpression of FADD sensitized tumor cells to drug-induced cell death.<sup>41</sup> Transfection of cells with FADD dominant negative decreased apoptosis induced both by cisplatin or antitumor ether lipids,<sup>41,42</sup> and transient transfection with either MC159 or E8, two viral proteins that inhibit apoptosis at the level of FADD and procaspase-8, respectively,<sup>43</sup> protected cells from cisplatin-induced cytotoxicity.<sup>41</sup> Nevertheless, incubation with blocking anti-Fas antibodies (such as ZB4 and SM1/23 antibodies) or with the soluble Fas-IgG chimera fusion protein to block the interaction of Fas with FasL failed to inhibit drug-induced apoptosis, and drug-mediated induction of FasL expression was not always detected in distinct tumor cells.<sup>38,41</sup> Thus, these data suggest that, at least, some anticancer drugs induce cell death through a Fas/FADD pathway in a FasL-independent manner, and can partially explain the previous contradictory results on the involvement of Fas signaling in the action of anticancer drugs.

On the other hand, the unique mechanism of action of the antitumor ether lipid ET-18-OCH<sub>3</sub> demonstrated the intracellular activation of Fas independently of FasL.<sup>38,44,45</sup> This antitumor ether lipid mediates apoptosis in cancer cells through Fas activation once the drug is inside the cell, and normal cells are spared because they are unable to incorporate significant amounts of the drug. Fas-expressing cells that do not take up ET-18-OCH<sub>3</sub> from the culture medium are unaffected by the ether lipid when this latter is added exogenously, but they undergo rapid apoptosis following microinjection of ET-18-OCH<sub>3</sub> into the target cell.<sup>38</sup>

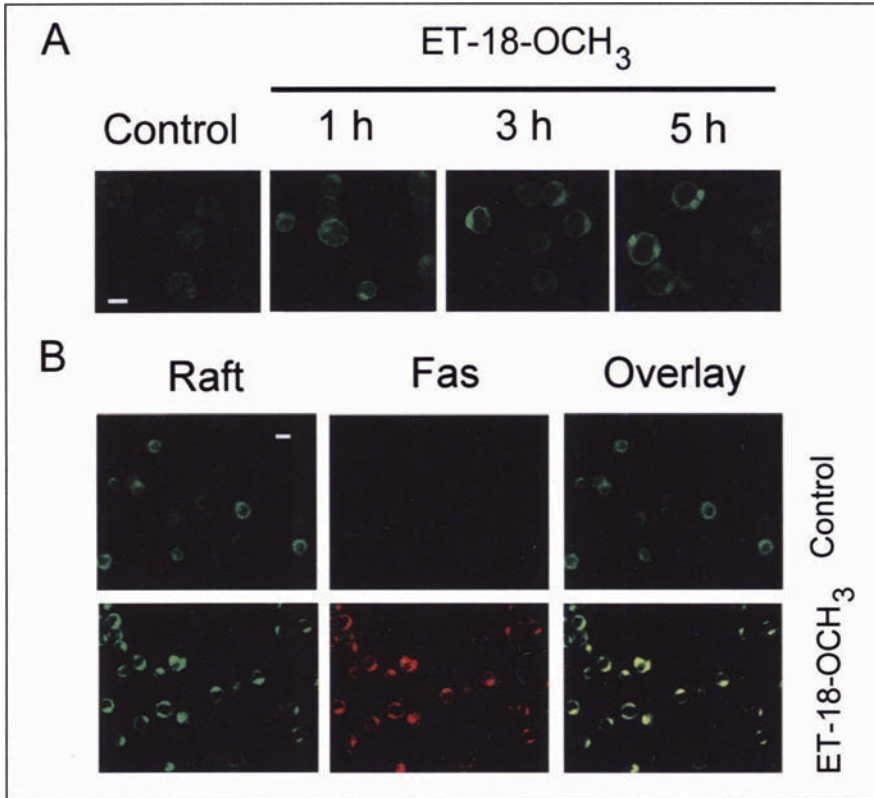


Figure 3. Cocapping of membrane rafts and Fas in human leukemic Jurkat cells treated with the antitumor ether lipid ET-18-OCH<sub>3</sub>. A) Time-course of the effect of ET-18-OCH<sub>3</sub> on aggregation of membrane rafts. T-leukemic Jurkat cells were either untreated (Control) or treated with 5  $\mu$ g/mL ET-18-OCH<sub>3</sub> for the times indicated. Cells were then stained with fluorescein isothiocyanate-labeled cholera toxin B subunit (FITC-CTx) used as a raft marker, and analyzed by confocal microscopy. Bar, 7  $\mu$ m. B) Colocalization of membrane rafts (Raft) and Fas in ET-18-OCH<sub>3</sub>-treated Jurkat cells. Cells were either untreated (Control) or treated with ET-18-OCH<sub>3</sub> for 3 hours, and processed for confocal microscopy using FITC-CTx (green fluorescence for lipid rafts) and anti-Fas monoclonal antibody, followed by CY3-conjugated anti-mouse antibody (red fluorescence for Fas). Areas of colocalization between membrane rafts and Fas in the overlay panels are yellow. Bar, 10  $\mu$ m. (From C. Gajate and F. Mollinedo. *Blood* 2001; 98:3860-3863. © American Society of Hematology, used with permission, ref. 46.)

### FasL-Independent Translocation and Capping of Fas into Membrane Rafts

While investigating the mechanism of action of the antitumor ether lipid ET-18-OCH<sub>3</sub> we found that this drug induced apoptosis in leukemic cells through translocation and capping of Fas into membrane rafts (Fig. 3), independently of FasL.<sup>46</sup> This was assessed by both confocal microscopy and isolation of membrane rafts through sucrose gradient centrifugation.<sup>46</sup> Raft disruption inhibited both ET-18-OCH<sub>3</sub>-induced Fas capping and apoptosis.<sup>46</sup> Thus, these data involved for the first time membrane rafts in Fas-mediated apoptosis and cancer chemotherapy. Subsequent studies also found that Fas was translocated into lipid rafts following activation with FasL, as well as recruitment of FADD and procaspase-8 to the rafts forming

the so-called DISC.<sup>47,48</sup> However, the translocation and capping of Fas into membrane rafts following ET-18-OCH<sub>3</sub> treatment was independent of FasL.<sup>38,46</sup> In a recent study, resveratrol, a polyphenol found mainly in grape skin with antitumor chemopreventive properties, has also been found to redistribute Fas in rafts independently of FasL.<sup>49</sup>

Membrane rafts are membrane microdomains consisting of dynamic assemblies of cholesterol and sphingolipids.<sup>50-54</sup> The presence of saturated hydrocarbon chains in sphingolipids allows for cholesterol to be tightly intercalated, leading to the presence of distinct liquid-ordered phases, membrane rafts, dispersed in the liquid-disordered matrix, and thereby more fluid, lipid bilayer.<sup>55</sup> One key property of membrane rafts is that they can include or exclude proteins to varying degrees. Membrane rafts may serve as foci for recruitment and concentration of signaling molecules at the plasma membrane, and thus they have been implicated in signal transduction from cell surface receptors.<sup>51</sup>

We hypothesize that accumulation of Fas into aggregates of stabilized membrane lipid domains from a highly dispersed distribution may represent a general mode of regulating Fas activation. Thus, membrane rafts could serve, in addition to generate high local concentration of Fas, as platforms for coupling adaptor and effector proteins required for Fas signaling (Fig. 2). This is of particular importance in Fas-mediated signal transduction as most of the initial signaling events depend largely on protein-protein interactions. Furthermore, this could facilitate and amplify signaling processes by local assembly of various cross-interacting signaling molecules (Gajate et al, in preparation).

Interestingly the lipid molecule ceramide has been involved in the clustering of Fas into ceramide-rich rafts.<sup>56,57</sup> However, ceramide acts as a mediator of the clustering process not as an initiator of the process, amplifying the primary Fas signaling events. Thus, C<sub>16</sub>-ceramide did not trigger Fas clustering in the absence of a stimulatory anti-Fas antibody or FasL.<sup>57</sup> It is suggested<sup>56-60</sup> that Fas-FasL complexes enter initially into small membrane rafts and induce a weak formation of the DISC leading to caspase 8 activation. This rather weak caspase 8 activation then would generate ceramide through sphingomyelinase translocation and activation to the small lipid rafts. Due to the high amount of sphingomyelin present in rafts (as much as 70% of all cellular sphingomyelin may be found in rafts<sup>61</sup>), the generated ceramide could induce coalescence of elementary rafts<sup>62</sup> leading to the formation of big patches containing Fas-FasL complexes that would further lead to enhanced DISC formation, thereby potentiating Fas signaling. Thus, sphingomyelinase and ceramide serve to amplify the signaling of Fas at the membrane level after the initial Fas-FasL interaction.

However, the current view supports the notion that Fas/FasL interaction, although can enhance cell death,<sup>63,64</sup> is not essential in drug-induced apoptosis, and a growing number of agents and experimental conditions can induce Fas activation without the participation of FasL (Table 1). As recent evidence indicates that Fas clustering can occur without the participation of FasL, it could be suggested that either the different treatments exerting Fas clustering promote sphingomyelinase-dependent ceramide generation or induce physical changes in the plasma membrane similar to those elicited by ceramide inducing coalescence of rafts leading to large raft platforms and subsequent capping. Thus, activation of Fas by FasL or in a ligand-independent fashion leads to a visible aggregation of Fas under the microscope as compared with untreated cells, supporting that the Fas aggregation observed<sup>138,41,65-69</sup> is in a macroscopic level.

### FasL-Independent Activation of Fas by Unrelated Agents

In the last few years a number of reports have evidenced that Fas can be activated under conditions that preclude interaction with FasL. Table 1 lists the agents reported so far to cause FasL-independent activation and clustering of Fas. These data suggest a common mechanism whereby divergent stimuli can activate membrane-associated events that target the Fas apoptotic

**Table 1. FasL-independent activation of Fas by different agents**

Experimental Evidence			
Inducer	FasL-Independent Activation of Fas	Fas Clustering/ Capping	Cocapping of Fas and Rafts
Camptothecin	ref. 103		
CDDP	ref. 41	ref. 41	
Curcumin	ref. 71	ref. 71	
ET-18-OCH <sub>3</sub>	ref. 38, 46	ref. 38, 46	ref. 46
Glutamine deprivation-mediated cell shrinkage	ref. 104	ref. 104	
HCV core protein	ref. 105	ref. 105	
JNK activation (via MKK7)	ref. 69	ref. 69	
Resveratrol	ref. 49	ref. 49	ref. 49
TGF- $\beta$ 1	ref. 106		
TK/GCV	ref. 70	ref. 70	
Ultraviolet light	ref. 65, 67, 107	ref. 65, 67, 107	
Vanadate	ref. 72	ref. 72	
Vinblastine	ref. 41	ref. 41	
VP16	ref. 41	ref. 41	

Fas clustering or aggregation was visualized by immunofluorescence confocal microscopy or assessed by immunoprecipitating Fas using limiting antibody concentrations.<sup>65</sup> Co-capping of Fas and rafts was visualized by immunofluorescence confocal microscopy.<sup>46</sup> CDDP, cisplatin. HCV, hepatitis C virus. TK/GCV, herpes simplex thymidine kinase/ganciclovir. VP16, etoposide. TGF- $\beta$ 1, transforming growth factor- $\beta$ 1.

pathway in a manner that precludes its natural ligand FasL. This FasL-independent activation of Fas is blocked by dominant negative-FADD, antisense FADD, caspase-8 inhibitors, or by MC159 and E8, that inhibit the FADD/caspase-8 pathway, involving FADD and caspase-8 in this process.<sup>41,49,69-72</sup> On these grounds it is clear that this Fas clustering induces apoptosis in a FADD-mediated way, thus suggesting that Fas clustering promotes FADD recruitment and DISC formation, independently of FasL (Fig. 2).

Formation of platforms where a large amount of signaling molecules are brought together increases DISC formation and therefore potentiates Fas signaling. Because activation of caspase-8 is induced by proximity,<sup>73</sup> its concentration in lipid rafts will favor caspase-8 activation, triggering the downstream apoptotic signaling. It can be envisaged that the intrinsic enzymatic activity of caspase-8, upon approximation of additional procaspase-8 molecules mediated by the adapter FADD molecules, attains a sufficient concentration to activate the apoptosis pathway. Using chimeras of caspase-8 with either CD8 or Tac, Martin and coworkers<sup>74</sup> found that oligomerization at the cell membrane powerfully induces caspase-8 autoactivation and apoptosis. On these grounds, it can be envisaged that these oligomerization processes would be facilitated enormously in the large Fas aggregates formed during stimulation, leading to activation of caspase-8 and the ensuing generation of downstream apoptotic signals.

Thus, Fas clustering could be an efficient way to elicit apoptosis through recruitment of the DED-containing proteins FADD and caspase-8 into Fas caps (Fig. 2). In addition, it has also been demonstrated that FADD and caspase-8 coalesce into what appear to be perinuclear "death effector filaments" (DEFs) inducing receptor-independent apoptotic signals and apoptosis.<sup>75,76</sup> Overexpression of either FADD or caspase-8 induces apoptosis through the



formation of unique filament structures that contain the death effector domains of these proteins,<sup>75,76</sup> being named accordingly “death effector filaments” (DEFs). Thus, formation of death effector filaments leads to intracellular assemblies of apoptosis-signaling complexes that can initiate or amplify apoptotic stimuli independently of receptors at the plasma membrane. Cycloheximide has been shown to induce cell death in human leukemic Jurkat and CEM C7 T-cell lines in a FADD-dependent and receptor-independent manner through DEF formation.<sup>68</sup> Also, a number of antitumor drugs, including microtubule-disrupting agents, may induce apoptosis via caspase-8 activation independently of the Fas/FasL system.<sup>77</sup>

As stated above the initial events in Fas signaling are largely dependent on the local concentration of the three major components of DISC, either Fas, FADD and caspase-8, and in fact, oligomerization of each one are able to mount an apoptotic response. Thus, formation of Fas caps leads to the recruitment of these molecules in a limited space, increasing the probability of interactions among them, and thereby promoting a strong apoptotic response.

### How Are Fas Clusters Formed from Internal Signals?

As shown in Table 1, FasL-independent activation is mediated by Fas clustering, and recent evidence shows cocapping of Fas in membrane rafts.<sup>46,49</sup> How are these Fas clusters generated? As FasL is not strictly required, signals from inside the cell must regulate this process. The formation of Fas clusters as well as the recruitment of Fas into membrane rafts in a FasL-independent manner could involve intracellular processes, changes in the physicochemical properties of cell membranes or both.

Vanadate-elicited Fas aggregation and Fas-FADD association, as well as caspase-8 activation, were dependent on c-Jun NH<sub>2</sub>-terminal kinase (JNK) activation,<sup>72</sup> as assessed by the use of the selective JNK inhibitor D-JNKI1.<sup>78</sup> These results highlight a major role for JNK in the signaling mechanisms leading to FasL-independent Fas activation. In fact, selective JNK activation by overexpressing the mitogen-activated protein kinase kinase 7 (MKK7) induced cell death mediated by FADD and Fas activation, independently of FasL.<sup>69</sup> Persistent JNK activation led to clustering of Fas.<sup>69</sup> Other inducers of FasL-independent Fas capping lead to a rapid and persistent activation of JNK, such as the antitumor ether lipid ET-18-OCH<sub>3</sub>,<sup>79</sup> and vanadate.<sup>72</sup> These data suggest that persistent JNK activation could be at least one of the signaling events leading to Fas clustering. In this regard, ceramide, which also favors Fas aggregation, induces apoptosis through sustained JNK activation.<sup>80</sup> However, the molecular events between JNK activation and Fas clustering remain to be elucidated. Persistent JNK activation is linked to cell death induced by different agents and stress conditions,<sup>79,81-84</sup> and its critical role in apoptosis is also supported by the lack of cell death on hippocampal neurons in JNK3-deficient and in JNK1/JNK2 double knockout mice.<sup>85,86</sup> In addition JNK-mediated apoptosis could involve mitochondria as JNK can translocate into mitochondria promoting phosphorylation and inactivation of anti-apoptotic Bcl-2 and Bcl-x<sub>L</sub>.<sup>87,88</sup> Furthermore, UV-induced activation of the mitochondrial-mediated death pathway is abrogated in the absence of JNK, further supporting mitochondria as the target of JNK.<sup>89</sup>

Another putative mechanism involved in Fas clustering could involve cytoskeleton, a dynamic intracellular structure that due to its continuous assembly/disassembly could be perfectly equip to translocate proteins and transmit signals.<sup>90</sup> The interactions between plasma membrane and cytoskeleton play an essential role in various cellular functions,<sup>91-93</sup> and a link between raft-mediated signaling and the interaction of actin cytoskeleton with raft membrane domains has been suggested.<sup>94</sup> Ezrin, a major protein of the so-called ERM proteins (ezrin, radixin, moesin) linking the actin cytoskeleton to the plasma membrane,<sup>95,96</sup> interacts with Fas and mediates Fas cell membrane polarization (Fig. 2) during Fas-induced apoptosis in human T lymphocytes.<sup>97</sup> In addition, disialoganglioside GD3 redistributes in membrane-associated domains colocalizing with ezrin in Fas-triggered apoptosis, and GD3 is present in ezrin

immunoprecipitates.<sup>98</sup> On the other hand, changes in cytoskeleton and microtubule disruption activate JNK,<sup>90,99,100</sup> but whether persistent JNK activation could induce reorganization of the microtubule cytoskeleton that promotes Fas clustering remains to be determined.

## Concluding Remarks

Recent evidences indicate that Fas signaling is mediated by the formation of large Fas aggregates. Under physiological conditions FasL triggers Fas aggregation in caps. Nevertheless, this capping can be also generated by nonphysiological agents without the participation of FasL (Table 1), raising the possibility for new therapeutic interventions. This is of interest due to the toxic side effects derived from the use of FasL or agonistic anti-Fas antibodies in vivo leading to a fatal hepatic damage.<sup>101,102</sup> When an agonistic anti-Fas antibody or recombinant FasL was injected into mice to activate the Fas system in vivo, the mice were quickly killed by liver failure with symptoms similar to fulminant hepatitis.<sup>101,102</sup> Thus, the FasL-independent activation of Fas offers some opportunities to find agents that can circumvent the above hepatic effects, but preserve Fas activating properties. Such a notion has found experimental support in our recent studies on the antitumor ether lipid ET-18-OCH<sub>3</sub>.<sup>45</sup> This selective antitumor compound is incorporated in significant amounts only in tumor cells, and once inside the cell promotes apoptosis through intracellular activation and capping of Fas, independently of FasL.<sup>38,44,46</sup> (Gajate et al, in preparation). Normal cells are spared because they do not take up the ether lipid.<sup>38,44,45</sup>

The increasing number of agents that promote FasL-independent activation of Fas through Fas clustering (Table 1) suggests that this process is more general than initially believed. The fact that very different experimental conditions and diverse agents, targeting distinct molecules and cellular processes, can lead eventually to an apoptotic response mediated by FasL-independent activation of Fas suggests that this process can be a general mechanism of cell death. Because most cells express Fas at their surface, we can hypothesize that when cells are committed to die, they can generate intracellular signals that trigger an efficient suicide mechanism from within the cell, via Fas activation, without receiving any order from outside through its ligand. Thus, Fas can receive efficiently orders either from outside, via FasL, or from inside the cell. Elucidation of the molecules and signaling pathways involved in this latter FasL-independent intracellular activation of Fas, through its capping into membrane rafts, is a major challenge for future research and can lead to identify new therapeutic targets.

## Acknowledgments

Work from the authors' laboratory, described in this study, was supported by grants FIS-02/1199 and FIS-01/1048 from the Fondo de Investigación Sanitaria, grant SA-087/01 from Junta de Castilla y León, and grant 1FD97-0622 from the European Commission and Comisión Interministerial de Ciencia y Tecnología.

## References

1. Yonehara S, Ishii A, Yonehara M. A cell-killing monoclonal antibody (anti-Fas) to a cell surface antigen codownregulated with the receptor of tumor necrosis factor. *J Exp Med* 1989; 169(5):1747-1756.
2. Yonehara S. To reviews on physiological and pathological roles of cell death. *Cell Struct Funct* 2003; 28(1):1-2.
3. Trauth BC, Klas C, Peters AM et al. Monoclonal antibody-mediated tumor regression by induction of apoptosis. *Science* 1989; 245(4915):301-305.
4. Itoh N, Yonehara S, Ishii A et al. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell* 1991; 66(2):233-243.

5. Oehm A, Behrmann I, Falk W et al. Purification and molecular cloning of the APO-1 cell surface antigen, a member of the tumor necrosis factor/nerve growth factor receptor superfamily. Sequence identity with the Fas antigen. *J Biol Chem* 1992; 267(15):10709-10715.
6. Suda T, Takahashi T, Golstein P et al. Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. *Cell* 1993; 75(6):1169-1178.
7. Takahashi T, Tanaka M, Brannan CI et al. Generalized lymphoproliferative disease in mice, caused by a point mutation in the Fas ligand. *Cell* 1994; 76(6):969-976.
8. Takahashi T, Tanaka M, Inazawa J et al. Human Fas ligand: Gene structure, chromosomal location and species specificity. *Int Immunol* 1994; 6(10):1567-1574.
9. Nagata S. Apoptosis by death factor. *Cell* 1997; 88(3):355-365.
10. Tanaka M, Suda T, Takahashi T et al. Expression of the functional soluble form of human fas ligand in activated lymphocytes. *Embo J* 1995; 14(6):1129-1135.
11. Itoh N, Nagata S. A novel protein domain required for apoptosis. Mutational analysis of human Fas antigen. *J Biol Chem* 1993; 268(15):10932-10937.
12. Huang B, Eberstadt M, Olejniczak ET et al. NMR structure and mutagenesis of the Fas (APO-1/CD95) death domain. *Nature* 1996; 384(6610):638-641.
13. Liang H, Fesik SW. Three-dimensional structures of proteins involved in programmed cell death. *J Mol Biol* 1997; 274(3):291-302.
14. Siegel RM, Chan FK, Chun HJ et al. The multifaceted role of Fas signaling in immune cell homeostasis and autoimmunity. *Nat Immunol* 2000; 1(6):469-474.
15. Chan FK, Chun HJ, Zheng L et al. A domain in TNF receptors that mediates ligand-independent receptor assembly and signaling. *Science* 2000; 288(5475):2351-2354.
16. Papoff G, Hausler P, Eramo A et al. Identification and characterization of a ligand-independent oligomerization domain in the extracellular region of the CD95 death receptor. *J Biol Chem* 1999; 274(53):38241-38250.
17. Siegel RM, Frederiksen JK, Zacharias DA et al. Fas preassociation required for apoptosis signaling and dominant inhibition by pathogenic mutations. *Science* 2000; 288(5475):2354-2357.
18. Chinnaiyan AM, O'Rourke K, Tewari M et al. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. *Cell* 1995; 81(4):505-512.
19. Boldin MP, Goncharov TM, Goltsev YV et al. Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. *Cell* 1996; 85(6):803-815.
20. Ashkenazi A, Dixit VM. Death receptors: Signaling and modulation. *Science* 1998; 281(5381):1305-1308.
21. Kischkel FC, Hellbardt S, Behrmann I et al. Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. *Embo J* 1995; 14(22):5579-5588.
22. Watanabe-Fukunaga R, Brannan CI, Copeland NG et al. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature* 1992; 356(6367):314-317.
23. Algeciras-Schimmich A, Shen L, Barnhart BC et al. Molecular ordering of the initial signaling events of CD95. *Mol Cell Biol* 2002; 22(1):207-220.
24. Song HY, Dunbar JD, Donner DB. Aggregation of the intracellular domain of the type 1 tumor necrosis factor receptor defined by the two-hybrid system. *J Biol Chem* 1994; 269(36):22492-22495.
25. Boldin MP, Mett IL, Varfolomeev EE et al. Self-association of the "death domains" of the p55 tumor necrosis factor (TNF) receptor and Fas/APO1 prompts signaling for TNF and Fas/APO1 effects. *J Biol Chem* 1995; 270(1):387-391.
26. Friesen C, Herr I, Krammer PH et al. Involvement of the CD95 (APO-1/FAS) receptor/ligand system in drug-induced apoptosis in leukemia cells. *Nat Med* 1996; 2(5):574-577.
27. Muller M, Strand S, Hug H et al. Drug-induced apoptosis in hepatoma cells is mediated by the CD95 (APO-1/Fas) receptor/ligand system and involves activation of wild-type p53. *J Clin Invest* 1997; 99(3):403-413.
28. Fulda S, Sieverts H, Friesen C et al. The CD95 (APO-1/Fas) system mediates drug-induced apoptosis in neuroblastoma cells. *Cancer Res* 1997; 57(17):3823-3829.
29. Poulaki V, Mitsiades CS, Mitsiades N. The role of Fas and FasL as mediators of anticancer chemotherapy. *Drug Resist Updat* 2001; 4(4):233-242.

30. Fulda S, Strauss G, Meyer E et al. Functional CD95 ligand and CD95 death-inducing signaling complex in activation-induced cell death and doxorubicin-induced apoptosis in leukemic T cells. *Blood* 2000; 95(1):301-308.
31. Nagarkatti N, Davis BA. Tamoxifen induces apoptosis in Fas+ tumor cells by upregulating the expression of Fas ligand. *Cancer Chemother Pharmacol* 2003; 51(4):284-290.
32. Villunger A, Egle A, Kos M et al. Drug-induced apoptosis is associated with enhanced Fas (Apo-1/CD95) ligand expression but occurs independently of Fas (Apo-1/CD95) signaling in human T-acute lymphatic leukemia cells. *Cancer Res* 1997; 57(16):3331-3334.
33. Gamen S, Anel A, Laserra P et al. Doxorubicin-induced apoptosis in human T-cell leukemia is mediated by caspase-3 activation in a Fas-independent way. *FEBS Lett* 1997; 417(3):360-364.
34. Eischen CM, Kortke TJ, Martins LM et al. Comparison of apoptosis in wild-type and Fas-resistant cells: Chemotherapy-induced apoptosis is not dependent on Fas/Fas ligand interactions. *Blood* 1997; 90(3):935-943.
35. Tolomeo M, Dusonchet L, Meli M et al. The CD95/CD95 ligand system is not the major effector in anticancer drug-mediated apoptosis. *Cell Death Differ* 1998; 5(9):735-742.
36. Wesselborg S, Engels IH, Rossmann E et al. Anticancer drugs induce caspase-8/FLICE activation and apoptosis in the absence of CD95 receptor/ligand interaction. *Blood* 1999; 93(9):3053-3063.
37. Ferreira CG, Tolis C, Span SW et al. Drug-induced apoptosis in lung cancer cells is not mediated by the Fas/FasL (CD95/APO1) signaling pathway. *Clin Cancer Res* 2000; 6(1):203-212.
38. Gajate C, Fonteriz RI, Cabaner C et al. Intracellular triggering of Fas, independently of FasL, as a new mechanism of antitumor ether lipid-induced apoptosis. *Int J Cancer* 2000; 85(5):674-682.
39. Fulda S, Friesen C, Los M et al. Betulinic acid triggers CD95 (APO-1/Fas)- and p53-independent apoptosis via activation of caspases in neuroectodermal tumors. *Cancer Res* 1997; 57(21):4956-4964.
40. Newton K, Strasser A. Ionizing radiation and chemotherapeutic drugs induce apoptosis in lymphocytes in the absence of Fas or FADD/MORT1 signaling. Implications for cancer therapy. *J Exp Med* 2000; 191(1):195-200.
41. Micheau O, Solary E, Hammann A et al. Fas ligand-independent, FADD-mediated activation of the Fas death pathway by anticancer drugs. *J Biol Chem* 1999; 274(12):7987-7992.
42. Matzke A, Massing U, Krug HF. Killing tumour cells by alkylphosphocholines: Evidence for involvement of CD95. *Eur J Cell Biol* 2001; 80(1):1-10.
43. Bertin J, Armstrong RC, Otilie S et al. Death effector domain-containing herpesvirus and poxvirus proteins inhibit both Fas- and TNFR1-induced apoptosis. *Proc Natl Acad Sci USA* 1997; 94(4):1172-1176.
44. Mollinedo F, Fernandez-Luna JL, Gajate C et al. Selective induction of apoptosis in cancer cells by the ether lipid ET-18-OCH<sub>3</sub> (Edelfosine): Molecular structure requirements, cellular uptake, and protection by Bcl-2 and Bcl-X(L). *Cancer Res* 1997; 57(7):1320-1328.
45. Gajate C, Mollinedo F. Biological Activities, Mechanisms of Action and Biomedical Prospect of the Antitumor Ether Phospholipid ET-18-OCH<sub>3</sub> (Edelfosine), A Proapoptotic Agent in Tumor Cells. *Curr Drug Metab* 2002; 3(5):491-525.
46. Gajate C, Mollinedo F. The antitumor ether lipid ET-18-OCH<sub>3</sub> induces apoptosis through translocation and capping of Fas/CD95 into membrane rafts in human leukemic cells. *Blood* 2001; 98(13):3860-3863.
47. Hueber AO, Bernard AM, Herincs Z et al. An essential role for membrane rafts in the initiation of Fas/CD95-triggered cell death in mouse thymocytes. *EMBO Rep* 2002; 3(2):190-196.
48. Scheel-Toellner D, Wang K, Singh R et al. The death-inducing signalling complex is recruited to lipid rafts in Fas-induced apoptosis. *Biochem Biophys Res Commun* 2002; 297(4):876-879.
49. Delmas D, Rebe C, Lacour S et al. Resveratrol-induced apoptosis is associated with Fas redistribution in the rafts and the formation of a death-inducing signaling complex in colon cancer cells. *J Biol Chem* 2003; 278(42):41482-41490.
50. Simons K, van Meer G. Lipid sorting in epithelial cells. *Biochemistry* 1988; 27(17):6197-6202.
51. Simons K, Toomre D. Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol* 2000; 1(1):31-39.
52. Ikonen E. Roles of lipid rafts in membrane transport. *Curr Opin Cell Biol* 2001; 13(4):470-477.
53. Nichols BJ, Lippincott-Schwartz J. Endocytosis without clathrin coats. *Trends Cell Biol* 2001; 11(10):406-412.

54. Manes S, del Real G, Martinez AC. Pathogens: Raft hijackers. *Nat Rev Immunol* 2003; 3(7):557-568.
55. Brown DA, London E. Structure and function of sphingolipid- and cholesterol-rich membrane rafts. *J Biol Chem* 2000; 275(23):17221-17224.
56. Grassme H, Jekle A, Riehle A et al. CD95 signaling via ceramide-rich membrane rafts. *J Biol Chem* 2001; 276(23):20589-20596.
57. Grassme H, Cremesti A, Kolesnick R et al. Ceramide-mediated clustering is required for CD95-DISC formation. *Oncogene* 2003; 22(35):5457-5470.
58. Cremesti A, Paris F, Grassme H et al. Ceramide enables fas to cap and kill. *J Biol Chem* 2001; 276(26):23954-23961.
59. Grassme H, Schwarz H, Gulbins E. Molecular mechanisms of ceramide-mediated CD95 clustering. *Biochem Biophys Res Commun* 2001; 284(4):1016-1030.
60. Kolesnick R. The therapeutic potential of modulating the ceramide/sphingomyelin pathway. *J Clin Invest* 2002; 110(1):3-8.
61. Prinetti A, Chigorno V, Prioni S et al. Changes in the lipid turnover, composition, and organization, as sphingolipid-enriched membrane domains, in rat cerebellar granule cells developing in vitro. *J Biol Chem* 2001; 276(24):21136-21145.
62. Cremesti AE, Goni FM, Kolesnick R. Role of sphingomyelinase and ceramide in modulating rafts: Do biophysical properties determine biologic outcome? *FEBS Lett* 2002; 531(1):47-53.
63. Debatin KM, Beltinger C, Bohler T et al. Regulation of apoptosis through CD95 (APO-I/Fas) receptor-ligand interaction. *Biochem Soc Trans* 1997; 25(2):405-410.
64. Cabaner C, Gajate C, Macho A et al. Induction of apoptosis in human mitogen-activated peripheral blood T- lymphocytes by the ether phospholipid ET-18-OCH3: involvement of the Fas receptor/ligand system. *Br J Pharmacol* 1999; 127(4):813-825.
65. Rehemtulla A, Hamilton CA, Chinnaiyan AM et al. Ultraviolet radiation-induced apoptosis is mediated by activation of CD-95 (Fas/APO-1). *J Biol Chem* 1997; 272(41):25783-25786.
66. Bennett M, Macdonald K, Chan SW et al. Cell surface trafficking of Fas: A rapid mechanism of p53-mediated apoptosis. *Science* 1998; 282(5387):290-293.
67. Aragane Y, Kulms D, Metze D et al. Ultraviolet light induces apoptosis via direct activation of CD95 (Fas/APO-1) independently of its ligand CD95L. *J Cell Biol* 1998; 140(1):171-182.
68. Tang D, Lahti JM, Grenet J et al. Cycloheximide-induced T-cell death is mediated by a Fas-associated death domain-dependent mechanism. *J Biol Chem* 1999; 274(11):7245-7252.
69. Chen Y, Lai MZ. c-Jun NH2-terminal kinase activation leads to a FADD-dependent but Fas ligand-independent cell death in Jurkat T cells. *J Biol Chem* 2001; 276(11):8350-8357.
70. Beltinger C, Fulda S, Kammertoens T et al. Herpes simplex virus thymidine kinase/ganciclovir-induced apoptosis involves ligand-independent death receptor aggregation and activation of caspases. *Proc Natl Acad Sci USA* 1999; 96(15):8699-8704.
71. Bush JA, Cheung Jr KJ, Li G. Curcumin induces apoptosis in human melanoma cells through a Fas receptor/caspase-8 pathway independent of p53. *Exp Cell Res* 2001; 271(2):305-314.
72. Luo J, Sun Y, Lin H et al. Activation of JNK by vanadate induces a Fas-associated death domain (FADD)-dependent death of cerebellar granule progenitors in vitro. *J Biol Chem* 2003; 278(7):4542-4551.
73. Muzio M, Stockwell BR, Stennicke HR et al. An induced proximity model for caspase-8 activation. *J Biol Chem* 1998; 273(5):2926-2930.
74. Martin DA, Siegel RM, Zheng L et al. Membrane oligomerization and cleavage activates the caspase-8 (FLICE/MACHalpha1) death signal. *J Biol Chem* 1998; 273(8):4345-4349.
75. Siegel RM, Martin DA, Zheng L et al. Death-effector filaments: Novel cytoplasmic structures that recruit caspases and trigger apoptosis. *J Cell Biol* 1998; 141(5):1243-1253.
76. Perez D, White E. E1B 19K inhibits Fas-mediated apoptosis through FADD-dependent sequestration of FLICE. *J Cell Biol* 1998; 141(5):1255-1266.
77. Goncalves A, Braguer D, Carles G et al. Caspase-8 activation independent of CD95/CD95-L interaction during paclitaxel-induced apoptosis in human colon cancer cells (HT29-D4). *Biochem Pharmacol* 2000; 60(11):1579-1584.
78. Bonny C, Oberson A, Negri S et al. Cell-permeable peptide inhibitors of JNK: novel blockers of beta-cell death. *Diabetes* 2001; 50(1):77-82.

79. Gajate C, Santos-Beneit A, Modolell M et al. Involvement of c-Jun NH2-terminal kinase activation and c-Jun in the induction of apoptosis by the ether phospholipid 1-O-octadecyl-2-O-methyl-rac-glycero-3-phosphocholine. *Mol Pharmacol* 1998; 53(4):602-612.
80. Verheij M, Bose R, Lin XH et al. Requirement for ceramide-initiated SAPK/JNK signalling in stress- induced apoptosis. *Nature* 1996; 380(6569):75-79.
81. Chen YR, Wang X, Templeton D et al. The role of c-Jun N-terminal kinase (JNK) in apoptosis induced by ultraviolet C and gamma radiation. Duration of JNK activation may determine cell death and proliferation. *J Biol Chem* 1996; 271(50):31929-31936.
82. Chen YR, Zhou G, Tan TH. c-Jun N-terminal kinase mediates apoptotic signaling induced by N-(4- hydroxyphenyl)retinamide. *Mol Pharmacol* 1999; 56(6):1271-1279.
83. Gajate C, An F, Mollinedo F. Rapid and Selective Apoptosis in Human Leukemic Cells Induced by Aplidine through a Fas/CD95- and Mitochondrial-mediated Mechanism. *Clin Cancer Res* 2003; 9(4):1535-1545.
84. Gajate C, An F, Mollinedo F. Differential cytostatic and apoptotic effects of ecteinascidin-743 in cancer cells. Transcription-dependent cell cycle arrest and transcription-independent JNK and mitochondrial mediated apoptosis. *J Biol Chem* 2002; 277(44):41580-41589.
85. Yang DD, Kuan CY, Whitmarsh AJ et al. Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the Jnk3 gene. *Nature* 1997; 389(6653):865-870.
86. Kuan CY, Yang DD, Samanta Roy DR et al. The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development. *Neuron* 1999; 22(4):667-676.
87. 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(12):8469-8478.
88. Kharbanda S, Saxena S, Yoshida K et al. Translocation of SAPK/JNK to mitochondria and interaction with Bcl-x(L) in response to DNA damage. *J Biol Chem* 2000; 275(1):322-327.
89. Tournier C, Hess P, Yang DD et al. Requirement of JNK for stress-induced activation of the cytochrome c- mediated death pathway. *Science* 2000; 288(5467):870-874.
90. Mollinedo F, Gajate C. Microtubules, microtubule-interfering agents and apoptosis. *Apoptosis* 2003; 8(5):413-450.
91. Luna EJ, Hitt AL. Cytoskeleton—plasma membrane interactions. *Science* 1992; 258(5084):955-964.
92. Tsukita S, Oishi K, Sato N et al. ERM family members as molecular linkers between the cell surface glycoprotein CD44 and actin-based cytoskeletons. *J Cell Biol* 1994; 126(2):391-401.
93. Dransfield DT, Bradford AJ, Smith J et al. Ezrin is a cyclic AMP-dependent protein kinase anchoring protein. *Embo J* 1997; 16(1):35-43.
94. Harder T, Simons K. Clusters of glycolipid and glycosylphosphatidylinositol-anchored proteins in lymphoid cells: Accumulation of actin regulated by local tyrosine phosphorylation. *Eur J Immunol* 1999; 29(2):556-562.
95. Bretscher A. Regulation of cortical structure by the ezrin-radixin-moesin protein family. *Curr Opin Cell Biol* 1999; 11(1):109-116.
96. Mangeat P, Roy C, Martin M. ERM proteins in cell adhesion and membrane dynamics. *Trends Cell Biol* 1999; 9(5):187-192.
97. Parlato S, Giammarioli AM, Logozzi M et al. CD95 (APO-1/Fas) linkage to the actin cytoskeleton through ezrin in human T lymphocytes: A novel regulatory mechanism of the CD95 apoptotic pathway. *EMBO J* 2000; 19(19):5123-5134.
98. Giammarioli AM, Garofalo T, Sorice M et al. GD3 glycosphingolipid contributes to Fas-mediated apoptosis via association with ezrin cytoskeletal protein. *FEBS Lett* 2001; 506(1):45-50.
99. Yujiri T, Fanger GR, Garrington TP et al. MEK kinase 1 (MEKK1) transduces c-Jun NH2-terminal kinase activation in response to changes in the microtubule cytoskeleton. *J Biol Chem* 1999; 274(18):12605-12610.
100. Gajate C, Barasoin I, Andreu JM et al. Induction of apoptosis in leukemic cells by the reversible microtubule- disrupting agent 2-methoxy-5-(2',3',4'-trimethoxyphenyl)-2,4,6- cycloheptatrien-1 -one: Protection by Bcl-2 and Bcl-X(L) and cell cycle arrest. *Cancer Res* 2000; 60(10):2651-2659.
101. Ogasawara J, Watanabe-Fukunaga R, Adachi M et al. Lethal effect of the anti-Fas antibody in mice. *Nature* 1993; 364(6440):806-809.

102. Tanaka M, Suda T, Yatomi T et al. Lethal effect of recombinant human Fas ligand in mice pre-treated with *Propionibacterium acnes*. *J Immunol* 1997; 158(5):2303-2309.
103. Shao RG, Cao CX, Nieves-Neira W et al. Activation of the Fas pathway independently of Fas ligand during apoptosis induced by camptothecin in p53 mutant human colon carcinoma cells. *Oncogene* 2001; 20(15):1852-1859.
104. Fumarola C, Zerbini A, Guidotti GG. Glutamine deprivation-mediated cell shrinkage induces ligand-independent CD95 receptor signaling and apoptosis. *Cell Death Differ* 2001; 8(10):1004-1013.
105. Moorman JP, Prayther D, McVay D et al. The C-terminal region of hepatitis C core protein is required for Fas-ligand independent apoptosis in Jurkat cells by facilitating Fas oligomerization. *Virology* 2003; 312(2):320-329.
106. Kim SG, Jong HS, Kim TY et al. Transforming Growth Factor- $\beta$ 1 Induces Apoptosis through Fas Ligand-independent Activation of the Fas Death Pathway in Human Gastric SNU-620 Carcinoma Cells. *Mol Biol Cell* 2003.
107. Zhuang S, Kochevar IE. Ultraviolet A radiation induces rapid apoptosis of human leukemia cells by Fas ligand-independent activation of the Fas death pathways. *Photochem Photobiol* 2003; 78(1):61-67.

Fas Signaling

Wajant, H. (Ed.)

2006, VIII, 160 p. 32 illus., 4 illus. in color., Hardcover

ISBN: 978-0-387-32172-1