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Review

Systems biology of death receptor networks: live and let die

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The extrinsic apoptotic pathway is initiated by death receptor activation. Death receptor activation leads to the formation of death receptor signaling platforms, resulting in the demolition of the cell. Despite the fact that death receptor-mediated apoptosis has been studied to a high level of detail, its quantitative regulation until recently has been poorly understood. This situation has dramatically changed in the last years. Creation of mathematical models of death receptor signaling led to an enormous progress in the quantitative understanding of the network regulation and provided fascinating insights into the mechanisms of apoptosis control. In the following sections, the models of the death receptor signaling and their biological implications will be addressed. Central attention will be given to the models of CD95/Fas/APO-1, an exemplified member of the death receptor signaling pathways. The CD95 death-inducing signaling complex (DISC) and regulation of CD95 DISC activity by its key inhibitor c-FLIP, have been vigorously investigated by modeling approaches, and therefore will be the major topic here. Furthermore, the non-linear dynamics of the DISC, positive feedback loops and bistability as well as stoichiometric switches in extrinsic apoptosis will be discussed. Collectively, this review gives a comprehensive view how the mathematical modeling supported by quantitative experimental approaches has provided a new understanding of the death receptor signaling network.

Facts

- Death receptor activation leads to the formation of death receptor signaling platforms, which activate caspase cascade resulting in the demolition of the cell.
- Quantitative regulation of death receptor network until recently has been poorly understood.
- Within the last decade, a number of mathematical models of death receptor signaling were created that changed the understanding of the extrinsic apoptosis.

Open Questions

- Which system properties determine death receptor-mediated apoptotic cell death?
- What are the initial conditions leading a cell into apoptosis?
- What is the role of DISC dynamics in apoptosis initiation?

The extrinsic apoptotic pathway is initiated by signals emanating from the cell-surface death receptors triggered by death ligands.[1] The binding of death ligands results in the formation of the death receptor signaling platforms and subsequent apoptosis initiation.[2,3] In the recent years, remarkable progress has been made in decoding the death receptor network. The major players of the death receptor signaling pathways have been extensively characterized: death receptors and their activating platforms, apoptosis initiation mechanisms and their inhibition.[4] Nevertheless, despite the fact that the death receptor network has been well described, its understanding in quantitative terms has been largely missing.[5] Indeed, a number of questions have remained unsolved: What are system properties that determine death receptor-mediated apoptotic cell death? What are the initial conditions leading a cell into apoptosis? Activation of which critical nodes, for example, groups of proteins, defines apoptosis initiation? Can the cell be a 'little bit dead'? To answer these questions by addressing the death receptor network on a quantitative level, the emerging field of systems biology was implemented.[6–8] Systems biology combines mathematical modeling with powerful experimental methodology, providing a quantitative assessment of the signaling pathways.[5,9] The application of systems biology to the analysis of death receptor networks resulted in new

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fascinating insights into the network regulation.[6–8,10–11] It turned out that as in the famous 'live and let die' movie, the cell also controls cell death in a very precise but also dynamic way, and the same 'agent' can both kill the cells or let them live, depending on the circumstances, for example, the concentrations of the molecules in the pathway. Below we shall discuss in detail how systems biology studies provide new understanding of the death receptor networks and comment on the identified non-linear responses, stoichiometric switches, feedback loops and bistability behavior in death receptor system defining life or death of the cell.

Signaling of CD95 as a Prototypic Death Receptor

The death receptor family belongs to the tumor necrosis factor (TNF) superfamily.[1,4] All members of the death receptor family are characterized by the presence of an intracellular ∼80 amino-acid long motif, which is termed the death domain playing a key role in the formation of the death receptor platforms.[2] The major death receptors characterized to date include: CD95 (also named Fas/APO-1), TNF-R1, TRAIL receptor 1 (TRAIL-R1), TRAIL-R2, DR3 and DR6.[2]

The CD95-mediated apoptotic pathway is one of the best-studied death receptor signaling pathways.[3] Stimulation of CD95 with CD95 ligand (CD95L) or with agonistic anti-CD95 antibodies such as anti-APO-1 induces apoptosis in sensitive cells.[4] The central apoptosis-initiating platform in CD95 signaling is the CD95 death-inducing signaling complex (DISC) comprising CD95, the adaptor protein FADD, procaspase-8, procaspase-10 and cellular FLICE-like inhibitory proteins (c-FLIP;[4] Figure 1). All interactions at the DISC are based on the so-called homotypic contacts. FADD is recruited to CD95 via death domain interactions, whereas procaspase-8/10 and c-FLIP are recruited to the DISC via death effector domain (DED) interactions (Figure 1). Several isoforms of the DED proteins were found at the DISC: two isoforms of procaspase-8, procaspase-8a (p55) and procaspase-8b (p53); three isoforms of procaspase-10 and c-FLIP isoforms, including long (L), short (S) and Raji (R)[12] (Figure 1).

A number of studies has demonstrated that procaspase-8 is activated at the DISC via formation of the procaspase-8 homodimers[13] (Figures 1 and 2a). Furthermore, recently a new paradigm in the activation of caspase-8 at the DISC was unraveled: it was shown that procaspase-8 is activated via the formation of DED chains at the DISC.[14–16] These chains comprise procaspase-8 molecules interacting via their DEDs, thereby bringing the caspase-like domains into the close proximity to each other (Figure 1). The DED chain enables the formation of the procaspase-8 homodimers, which is, in turn, a prerequisite for procaspase-8 activation. After formation of the active homodimer, procaspase-8a/b (p55/p53) undergoes processing with 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[17,18] (Figure 1). Active caspase-8 heterotetramers $p10_2$-$p18_2$ initiate the apoptotic cascade by cleavage and activation of the effector caspases, which in turn leads to the demolition of the cell.

The activation of procaspase-8 at the DISC is inhibited by c-FLIP proteins that are also recruited to the DISC via DED interactions. Short c-FLIP isoforms, c-FLIP$_S$ and c-FLIP$_R$, comprise only tandem DEDs, whereas the long isoform, c-FLIP$_L$, also has the C-terminal caspase-like domain that lacks caspase activity[12] (Figure 1). Short c-FLIP isoforms could block caspase-8 activation upon overexpression, whereas the long isoform, c-FLIP$_L$, can both block and accelerate caspase-8 activation.[19,20] The inhibition occurs in the presence of high concentrations of c-FLIP$_L$ at the DISC, whereas acceleration of caspase-8 activation at the DISC
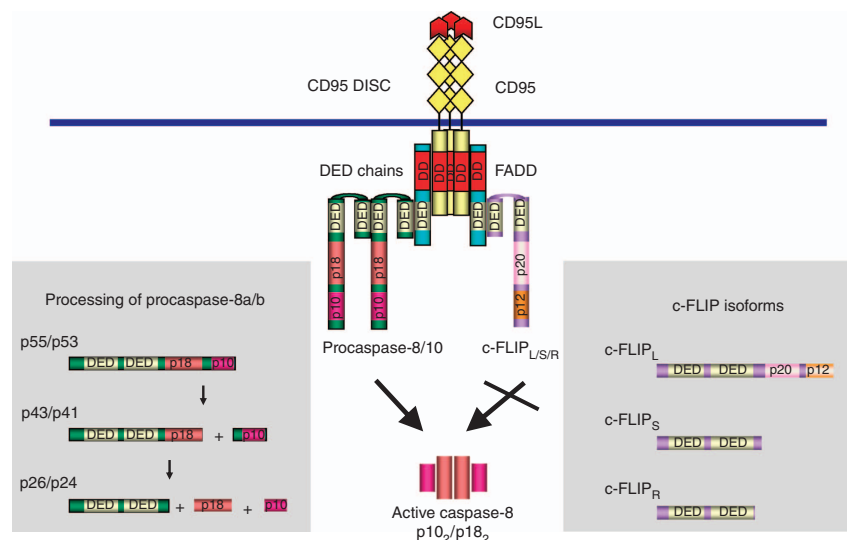

Figure 1 The scheme of the CD95 DISC. CD95L stimulation leads to the induction of the CD95 death-inducing signaling complex (DISC) comprising CD95, adaptor protein FADD, procaspase-8/10 and c-FLIP. All interactions at the DISC are based on the so-called homotypic contacts. FADD is recruited to CD95 via death domain (DD) interactions, whereas procaspase-8/10 and c-FLIP are recruited to the DISC via death effector domain (DED) interactions. Procaspase-8 is activated in the DED chains via the formation of procaspase-8 homodimers. Left side: two isoforms of procaspase-8: procaspase-8a (p55) and procaspase-8b (p53) as well as their processing to p43/p41, p26/p24, p18 and p10 are shown. Right side: three isoforms of c-FLIP are depicted: c-FLIP$_L$, c-FLIP$_R$ and c-FLIP$_S$
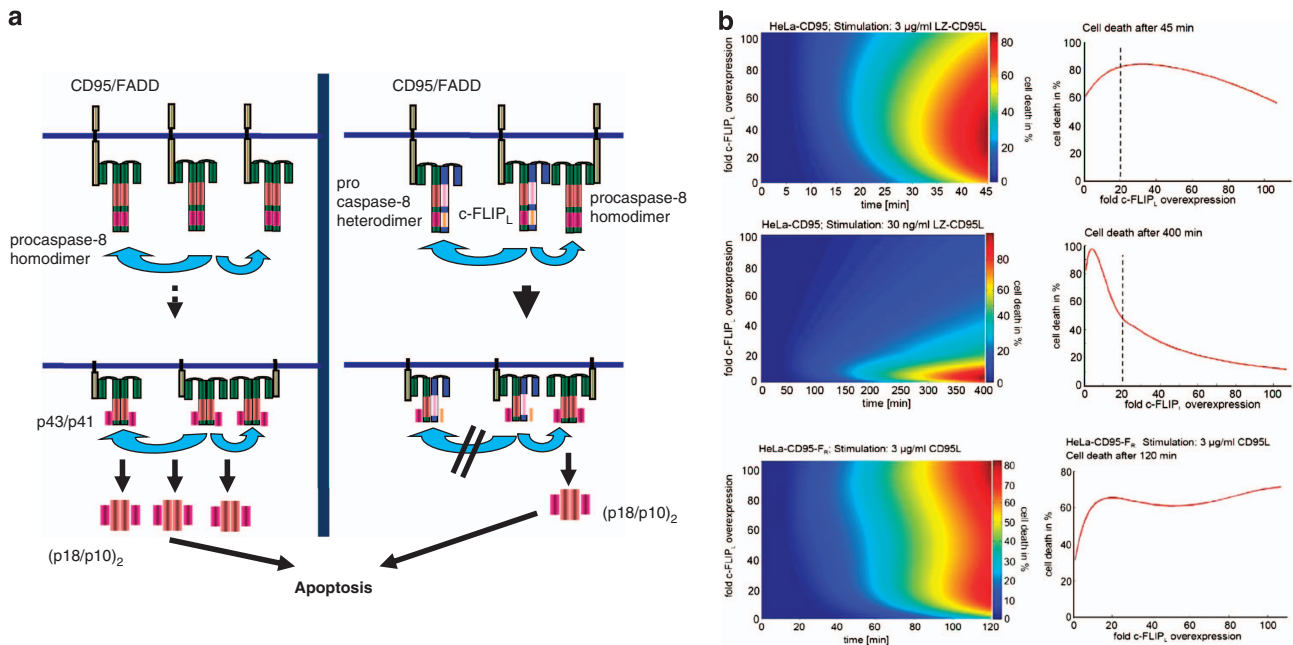
Figure 2 Modeling pro-apoptotic properties of c-FLIP$_L$. (**a**) The scheme of formation of procaspase-8/procaspase-8 homodimer (left) and heterodimer procaspase-8/c-FLIP$_L$ (right) at the DISC. Both scenarios could lead to caspase-8 processing to p43/p41 and active caspase-8 heterotetramer. (**b**) Predictions of the model for cell death upon different stimulation strength and different levels of c-FLIP$_L$. The lower panel demonstrates concentration-dependent effects of c-FLIP$_L$ on the apoptosis induction upon high c-FLIP$_R$ overexpression. Adapted from Fricker et al.[19]

takes place upon lower concentrations of c-FLIP$_L$. The latter has been reported to be mediated by the formation of catalytically active procaspase-8/c-FLIP$_L$ heterodimers in which the procaspase-8-active loop is stabilized by c-FLIP$_L$, thereby increasing the catalytic activity of procaspase-8[21–23] (Figure 2a).

Downstream from CD95 DISC, the apoptotic signal transduction is controlled at several levels. Two types of cells have been described.[4] Type I cells are characterized by high numbers of the CD95 DISCs, and, correspondingly, high amounts of active caspase-8, that leads to the activation of caspase-3 and apoptosis induction. In type II cells, a lower number of CD95 DISCs is formed and the apoptotic signal is mediated via cleavage of Bid by procaspase-8 and transloca-tion of truncated Bid to mitochondria. At the mitochondria, truncated Bid was suggested to interact with the mitochon-drial-specific phospholipid cardiolipin, which promotes BAX or BAK oligomerization.[24] The latter leads to the disruption of the integrity of the outer mitochondrial membrane, which is followed by mitochondrial outer membrane polarization (MOMP), cytochrome C release from mitochondria and subsequent apoptosome formation followed by caspase-9 and then caspase-3 activation[25] (the mitochondrial branch of the apoptotic pathway is reviewed in detail in this issue by Rehm and colleagues). Apoptosis induction in type II cells could be blocked by anti-apoptotic Bcl-2 family members. Activation of initiator caspase-9 and effector caspase-3 and -7 could be inhibited by X-linked inhibitors of apoptosis (XIAPs).[26,27] Interestingly, the action of XIAPs could be blocked by the SMAC proteins, which are released from mitochondria in the course of apoptosis.[27] Thus, a complex network of pro- and anti-apoptotic regulators controls death receptor-induced apoptosis.

Importantly, we and others have shown that CD95 is not only a potent apoptosis inducer but is also capable of activating multiple non-apoptotic pathways controlled by NF-κB, Erk1/2, p38, JNK and AKT.[2,4,28] Furthermore, recent publications indicate that CD95-induced NF-κB and MAPK activation are also important for phagocyte attraction and generation of the 'find me' signal via the secretion of cytokines, making these pathways responsible for the proper execution of apoptosis as well.[29]

The First Models of CD95 Signaling has Demonstrated that C-FLIP is the Key Molecule Defining the Live or Let Die Decision

Several mathematical formalisms have been used to model death receptor signaling.[5,9] So far, Ordinary Differential Equations (ODEs) and Boolean modeling have been used the most. ODEs use the change in molecular concentrations over time, which allows to describe kinetics of a particular signaling pathway. Boolean models are more abstract as they use bimodal switches (on/off) to describe the behavior of the systems and do not require the knowledge of the kinetic parameters. Hence, Boolean models could not be applied for studies of the dynamic changes occurring within the signaling network. However, they are extremely valuable in character-izing large signaling networks.

The first theoretical model of apoptosis by Fussenegger et al.[30] has been build using literature data only. The model was generated using ad hoc parameters therefore, its predictive power was limited. It was followed by ODE-based mathematical model of CD95 signaling generated by Bentele et al.[31] The strong advantage of this model that it was trained against experimental data generated with quantitative

western blot data in type I B-lymphoblastoid cells SKW6.4.[31] The topology of the model included five major subsystems representing key regulatory nodes in CD95 apoptosis: CD95 DISC, effector caspases, mitochondria, XIAPs and degradation, the latter was approximated by PARP cleavage. Altogether, the model comprised 41 components presented in 32 reactions. The model had a strong predictive power, for example, it could well predict the timing of caspase activation.[31] Importantly, the major finding of the model was the identification of the DISC as a crucial node of CD95-mediated cell death. Furthermore, the model predicted that CD95-mediated apoptosis is characterized by the threshold behavior.[15,31] Notably, it turned out that this behavior is entirely based on the amounts of the core DISC proteins and their affinities.[31] The model predicted the threshold mechanism as follows: a low stimulation strength would lead to a low number of activated CD95 that could recruit adaptor protein FADD. FADD associated to CD95 could bind both procaspase-8 and its inhibitor c-FLIP. However, the affinity of c-FLIP to FADD is higher than that of procaspase-8. Consequently, all active sites at the DISC, for example, FADDs, would be occupied by c-FLIP upon low number of active receptors. In this scenario, procaspase-8 could not bind to the DISC and initiate apoptosis. Thus, the amount of c-FLIP bound to FADD would be the rate-limiting step of caspase-8 activation at the DISC. This prediction has been confirmed by vigorous experimental data: a decrease of the amount of c-FLIP led to a decrease of the threshold, for example, the cells were undergoing CD95-induced apoptosis upon stimulation with lower amounts of CD95L.[31] Furthermore, we have found that upon low CD95 stimulation strength the amount of c-FLIP at the DISC was increased compared with high stimulation strength.[32] These experiments ultimately confirmed the threshold mechanism suggested by the model and strongly supported the role of c-FLIP as a guardian of the threshold of extrinsic apoptosis.

The question which arises next is: how can cells undergo apoptosis if the affinity of c-FLIP to the DISC is higher than the affinity of procaspase-8? This would naturally mean that c-FLIP would always be recruited to the DISC first and would not allow procaspase-8 recruitment and subsequent activation. Interestingly, the amount of c-FLIP proteins in a number of the cells is significantly lower than the amount of procaspase-8, which was quantitatively measured, in particular, in SKW6.4 and HeLa cell lines.[15,19] Thus, upon high stimulation strength, c-FLIP could not block all DISCs any longer because of the increased number of active receptors and correspondingly higher number of procaspase-8 compared with c-FLIP in the cell. Hence, the amount of c-FLIP proteins in the cell defines the threshold behavior in the CD95-mediated apoptosis.[31]

Another prominent feature of c-FLIP proteins is a high turnover rate that allows them to modulate extrinsic apoptosis pathways in the dynamic manner.[12,33] Indeed, c-FLIP proteins are characterized by a very short half-life time and they quickly undergo proteosomal degradation.[33] This property of c-FLIP has been studied using a mathematical model of the dynamic turnover and stability of c-FLIP isoforms by Toivonen et al.[34] This model was developed on the basis of the model by Bentele et al.[31] The c-FLIP degradation rates as

a first-order reaction have been added to the model. In this study, changes in the level of c-FLIP at the single-cell level were taken into account and Monte Carlo simulations to investigate apoptotic response within cell populations considering intracellular variations of the c-FLIP level were applied.[34] It was shown that amount of c-FLIP in the cell at the moment of death receptor stimulation defines timing of cell death.[34]

Hence, as a result of these modeling works, it became evident that the amount of c-FLIP proteins is crucial in defining the sensitivity to apoptosis in the death receptor network.

The Expression Level of c-FLIP Might Define its Functions: Lessons from the DISC Models

The model by Bentele et al. only considered anti-apoptotic action of c-FLIP proteins, which is not entirely correct, taken that there are three c-FLIP isoforms, c-FLIP$_L$, c-FLIP$_S$ and c-FLIP$_R$, described to have different action. Indeed, one of the controversies that existed in CD95 signaling was the role of the long isoform of c-FLIP, c-FLIP$_L$. c-FLIP$_L$ has been assigned both pro- or anti-apoptotic roles in the pathway.[20,35] The pro-apoptotic effects of c-FLIP$_L$ as mentioned above are mediated by the formation of catalytically active procaspase-8/c-FLIP$_L$ heterodimers in which the procaspase-8 active loop is stabilized by c-FLIP$_L$, thereby increasing the catalytic activity of procaspase-8 (Figure 2a).

To define the conditions when c-FLIP$_L$ has a pro-apoptotic action, an ODE-based model of the DISC was developed by Fricker et al.[19] The model was validated using quantitative western blot and single-cell analysis based on experiments in HeLa cells overexpressing CD95 (HeLa-CD95 cells). The topology of the model comprised the generation of homo- and hetero-dimers of c-FLIP$_{L/S}$ and procaspase-8 at the DISC. In accordance with the literature, only procaspase-8 homodimers and procaspase-8-c-FLIP$_L$ heterodimers were considered as species leading to apoptosis (Figure 2a). Dimers formed by procaspase-8 and the short isoform of c-FLIP, c-FLIP$_S$, as well as between different c-FLIP isoforms were considered inactive in terms of apoptosis induction. Consistent with this, the optimal conditions for the proapoptotic effect of c-FLIP$_L$ would be achieved upon the concentration range of c-FLIP$_L$ leading to the optimal amount of c-FLIP$_L$-procaspase-8 heterodimers formed at the DISC (Figure 2a). Remarkably, the model predicted that this 'ideal case' scenario occurs under conditions when the concentration of c-FLIP$_L$ is 20 times higher than the endogeneous one[19] (Figure 2b).

Another valuable outcome of this model was the prediction that the apoptosis-promoting effect of c-FLIP$_L$ would be stronger upon a very low rate of caspase-8 activation.[19] To generate an experimental setup with a very-low rate of caspase-8 activation, short c-FLIP isoforms were overexpressed in HeLa-CD95 cells.[19] c-FLIP$_{S/R}$ were considered to occupy most of procaspase-8-binding sites at the DISC, thereby creating a limited amount of binding sites for procaspase-8. This naturally leads to a very-low rate of caspase-8 activation up to complete inhibition. It has to be noted that this scenario very much reflects cancer cells that often highly overexpress c-FLIP proteins and, therefore, are resistant toward death receptor-mediated apoptosis.

Consistent with the model predictions, the 20 times increase of c-FLIP$_L$ levels in these HeLa-CD95 cells overexpressing short c-FLIP isoforms, led to a significant sensitization toward CD95-mediated apoptosis[19] (Figure 2b, lower panel). Apparently, the overexpression of short c-FLIP isoforms has created the optimal amount of procaspase-8-c-FLIP$_L$ heterodimers at the DISC resulting in apoptosis sensitization. This also shows that short c-FLIP isoforms can have the pro-apoptotic role upon favorable stoichiometric conditions. Thus, the model by Fricker et al. unraveled the specific concentration range of c-FLIP$_L$ and c-FLIP$_S$ at the DISC leading to apoptosis acceleration (Figure 3). This work has also shown that the level of expression of a protein might define its cellular function contributing to the generic understanding of the origin of cell type specificity.

The model by Fricker et al. could uncover the concentration range of the c-FLIP$_L$ isoform leading to its proapoptotic effect.[19] This occurs upon intermediate levels of its overexpression, which is about 20 times higher than its endogeneous level (Figures 2b and 3). Naturally, high overexpression of c-FLIP$_L$ would lead to inhibition of procaspase-8 activation by outnumbering the procaspase-8 at the DISC and thereby preventing the formation of proapoptotic procaspase-8 homodimers and procaspase-8-c-FLIP$_L$ heterodimers leading to apoptosis (Figure 3). The inhibiting function of c-FLIP$_L$ upon high overexpression was supported by experimental data from a number of groups.[12,19] Hence, c-FLIP$_L$ has a very complicated action, it could promote or block CD95-induced apoptosis depending upon its concentration at the DISC. The model by Fricker et al. allowed to identify these stoichiometric switches in terms of exact numbers of c-FLIP proteins in the cell leading to the different systems behavior (Figure 3).

Interestingly, the concentration-specific action of c-FLIP$_L$ also takes place upon induction of CD95-mediated NF-κB activation.[36] Here, we have shown that the generation of the cleavage product p43-FLIP at the DISC is an essential step for this signal.[36,37] p43-FLIP is generated at the CD95 DISC by procaspase-8 activity in the non-linear manner arising from the competitive binding of procaspase-8 and c-FLIP to the DISC. The model of the CD95-mediated NF-κB activation generated by Neumann et al. could incorporate this non-linear behavior and describe DISC dynamics of CD95-mediated apoptosis and NF-κB activation.[36] This ODE model supported by experimental data generated in HeLa-CD95 cells demonstrated three modes of c-FLIP$_L$ action (Figure 4a). These three scenarios included low, intermediate and high levels of c-FLIP$_L$ at the DISC. Low levels of c-FLIP$_L$ disabled NF-κB as no p43-FLIP could be formed. Intermediate levels of c-FLIP$_L$ at the DISC led to p43-FLIP generation and the transduction of the NF-κB signal. High concentrations of c-FLIP$_L$ at the DISC blocked procaspase-8 activity resulting in the inhibition of p43-FLIP cleavage product generation and subsequently NF-κB activity (Figure 4a). Interestingly, similar to predictions of the model by Fricker et al. only intermediate levels of c-FLIP$_L$ at the DISC resulted in the proactive function of c-FLIP$_L$, for example, promotion of CD95-mediated NF-κB activation.

The non-linearity of p43-FLIP generation by procaspase-8 at the DISC (Figure 4a) translates into the non-linearity of CD95-mediated apoptosis/NF-κB activation shown in the phase diagram (Figure 4b). Here, an important discovery has originated from in silico analysis. It was shown that the ratio between c-FLIP and procaspase-8 determines the regions of the predominant NF-κB and/or apoptosis induction in a highly non-linear manner (Figure 4b).

Thus, systems biology studies of the DISC have demonstrated the dual role of c-FLIP proteins. Coming back to 'live and let die' c-FLIP proteins have a role similar to 'secret agents': they can both kill or protect the cells from apoptosis depending upon the circumstances, which in this particular case are the concentrations of the molecules in the pathway.
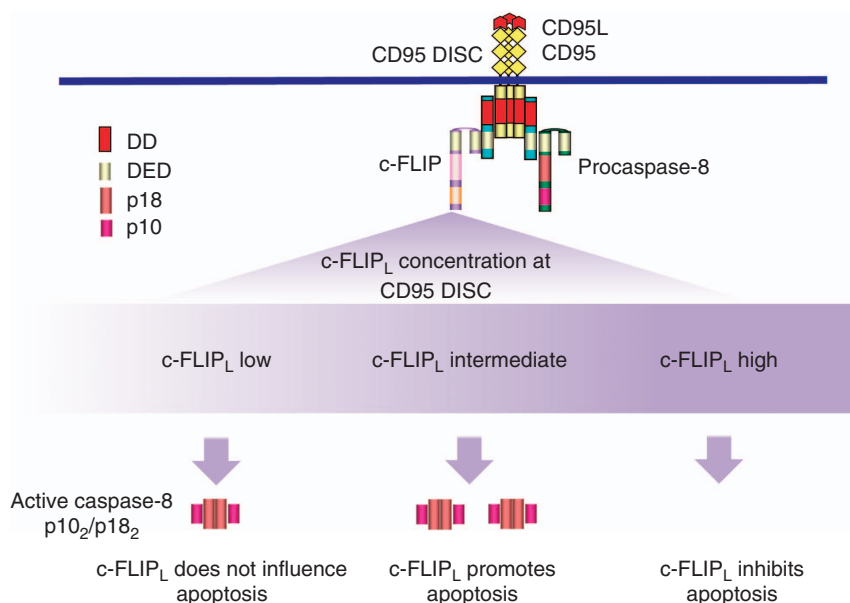

Figure 3 Concentration-dependent effects of c-FLIP$_L$ on apoptosis induction. The diagram presenting concentration-dependent effects of c-FLIP$_L$ on the apoptosis induction. Generation of active caspase-8 heterotetramers p10$_2$-p18$_2$ is demonstrated as an indication of apoptosis
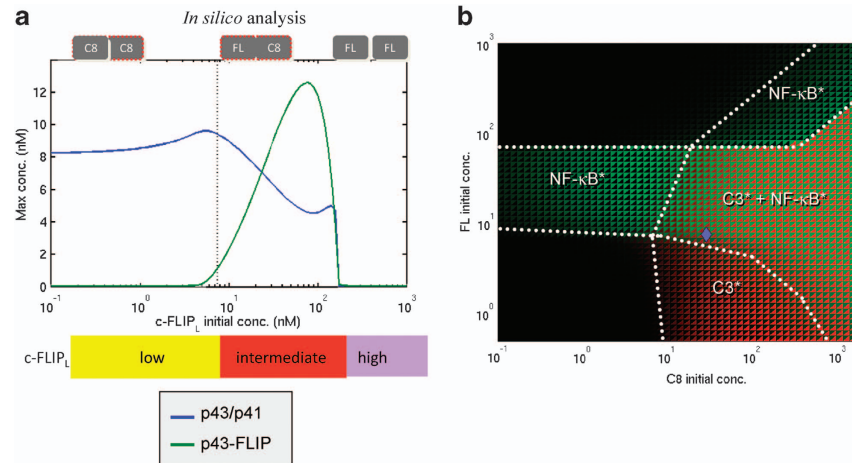
Figure 4 Non-linear dynamics of CD95 signaling. (**a**) The non-linear dynamics of the generation of p43-FLIP and p43/p41 caspase-8, leading to the induction of NF-κB and apoptosis, respectively. (**b**) Non-linearity of life/death decisions at CD95 depending on the amounts of caspase-8 and c-FLIP. NF-κB activity is shown in green, caspase-3 activation as an indication of apoptosis is shown in red. Adapted from Neumann et al.[36]

Dynamics of 'Chains of Death': c-FLIP is not the Only Factor Determining Life and Death

Until recently, most modeling studies of the DISC have concentrated on the key role of c-FLIP in mediating life/death decisions at CD95.[5] However, recent findings have demonstrated that c-FLIP is not the major factor defining apoptosis initiation. It was shown independently by two groups (Professor MacFarlane with coworkers and our group), that the generation of DED chains at the CD95 and TRAIL-R DISCs forms the platform for procaspase-8 activation and is another important constraint for DISC-mediated apoptosis initiation.[14,15]

In order to understand DED chain dynamics at the DISC, a mathematical model based on agent-based modeling was generated by Schleich et al.[15] The cell was modeled as a three-dimensional grid, and the recruitment probabilities of FADD, procaspase-8 and c-FLIP to the DISC were estimated proportional to the distance of these molecules to the receptors. The model could describe well procaspase-8 processing in the chains. Interestingly, in order to re-model experimental data, we had to introduce the parameter of the restricted chain length.[15] Obviously, a non-restricted chain length could lead to cell death upon spontaneous activation of one death receptor via the formation of one rather long DED chain with a high number of procaspase-8 molecules. Therefore, the assumption of the model upon restricted chain length nicely fitted to the biological knowledge. The restriction of the chain length indicates, however, the existence of the unknown mechanism of the chain termination, which might result from some yet undiscovered structural constrains in the receptor vicinity. Another possibility for the chain termination might result from the DED protein numbers. Indeed, upon high stimulation strength, the majority of procaspase-8 molecules might be recruited into the chains. This might create the natural limitation of the chain length based on the procaspase-8 number in the cell.[15] In terms of dynamics, the model predicted that the shorter chains are formed upon high stimulation strength, whereas low stimulation strength would

lead to longer chains. Indeed, in order to efficiently kill the cell upon low stimulation, a higher number of the active caspase-8 molecules is required, which in turn could be achieved by increasing the chain length. These predictions of the model were confirmed by experimental data using quantitative western blot and mass spectrometry analysis.[15] Thus, the study by Schleich et al. shows that the CD95 DISC stoichiometry is very dynamic and strongly depends on the stimulation strength. These dynamics also preclude that one universal DISC stoichiometry exists, rather CD95 DISCs comprise DED chains of different length and composition defined by the amount of molecules in the cell and the stimulation strength.

Undoubtedly, the discovery of DED chains provides a new challenge for the DISC studies as many mechanisms have to be revised. The same is true for mathematical models of the DISC. Indeed, previous DISC models did not consider DED chains upon model construction and were based solely on competition binding mechanisms, between procaspase-8 and c-FLIP for binding to FADD at the DISC. Nevertheless, the in silico simulations of these models were confirmed quite well by experimental data. This likely reflects the fact that the procaspase-8/c-FLIP ratio is crucial for caspase-8 activation also in the context of the chains. The further development of computational studies of DED chains at the DISC should provide further insights into dynamics of apoptosis initiation. In addition, a number of questions has to be addressed in terms of molecular architecture of the chains. In particular, the role of c-FLIP has to be determined in the context of chains: what is the exact inhibitory mechanism of c-FLIP, whether c-FLIP-binding terminates the DED chain or promotes chain elongation. The other challenging questions are: whether DED chains are stable, how one can define their structure considering different isoforms/cleavage products of procaspase-8, procaspase-10 and c-FLIP; how affinities of different DED proteins in the chain and their composition stoichiometry influence the switch between life and death.

Modeling Extrinsic Pathways Mediated by Other Death Receptors and Downstream from the DISC

Modeling of the extrinsic apoptotic pathway downstream from the DISC for CD95 and other death receptors, in particular, TRAIL-R1/2, has provided further insights into death receptor biology. Here important knowledge came from the combination of single-cell analysis with mathematical modeling[6,7,38] (reviewed in detail in this issue by Gaudet and co-workers[39]). The development of the FRET-based caspase activity probes[38] and localization-based caspase activity probes[40] allowed to elegantly monitor caspase activation at the single-cell level. Using FRET-based caspase activity probes, it was demonstrated that in type II cells there is a lag time after death receptor stimulation, which was termed pre-MOMP delay.[41,42] In this time, no activation of the effector caspases could be detected at the single-cell level; however, directly after the MOMP, a very fast processing of the FRET probe occurs, indicating that effector caspases are only active after MOMP. The same caspase activity probes allowed to shed light on the mechanism of cell-to-cell variability upon TRAIL induction.[43] A remarkable outcome of this study was that differences in cell death timing are based primarily on variations of protein expression levels of the cells.

TRAIL signaling provides another level of complexity compared with CD95L signaling because TRAIL binds to two death receptors TRAIL-R1 and TRAIL-R2 as well as to several decoy receptors.[44] The kinetic modeling of different TRAIL receptor complexes consisting of either hetero- or homotrimers was the subject of another modeling study by Szegezdi et al.[44] that found how to selectively enhance apoptosis by modulating the composition of receptor complexes. The crosstalk of TRAIL apoptotic signaling with non-apoptotic signaling pathways was the subject of a number of modeling studies that revealed the synergistic effects leading to more effective cell death.[45–47] This is a very important aspect, as enhancement of TRAIL killing and overcoming TRAIL resistance in cancer cells could potentially have a major role in the development of contemporary anti-cancer therapies.

The modeling studies also addressed the mechanisms of bistability and the positive feedback loops in extrinsic apoptosis. The study by Eissing et al. developed a minimal model of caspase-8- and caspase-3-positive feedback in type I cells and has demonstrated that caspase activation in extrinsic apoptosis is characterized by a bistability behavior defined by the inhibitors of caspase activation.[48] Interestingly, bistability has also been found as a feature of ligand/receptor interactions in the theoretical modeling studies of death receptor oligomerization reactions by Ho et al.[49] Furthermore, this study unraveled that depending on local receptor densities the bistability can be characterized by reversible or irreversible behavior.[49] The hidden positive feedback loop in caspase activation has been discovered by the model of Legewie et al.[50] This model has investigated the interplay of caspases-3, -9 and XIAP and uncovered that activated caspase-3 sequesters XIAP from caspase-9, which, in turn, enhances caspase-9 activity and subsequently caspase-3 activation. An important contribution to the analysis of the positive feedback loop of caspase-8, -3 and -6 has been made in the study of Würstle et al.[51] The authors have demonstrated

that caspase-8 dimerization/dissociation has a key role in the control of caspase activation events and avoiding of spontaneous apoptosis.

A central contribution to the field was the analysis of type I and type II signaling by Aldridge et al.[52] The original report on type I and type II signaling has demonstrated that type I cells are characterized by high amount of caspase-8 formed at the DISC resulting in caspase-8-mediated caspase-3 activation and independence of the following apoptosis execution from the MOMP-dependent caspase activation cascade.[53] Later, Aldridge et al. have also analyzed the downstream modulators in type I and type II cells and have shown that the ratio of caspase-3 to XIAP has a central role in defining type I/type II.[52] These seemingly contradictory statements in fact fit together quiet well. The caspase-3/XIAP ratio defines whether apoptosis could be executed without mitochondria activation. Indeed, upon high amounts of active caspase-3 activated by caspase-8 apoptosis could occur without MOMP. However, when the XIAP levels in the cell are high, only MOMP would allow to overcome apoptotic threshold, as the latter would lead to SMAC and cytochtome c release from mitochondria resulting in the higher amounts of active caspase-3 in the cell and subsequent apoptosis. Aldridge et al. have elegantly classified all cell lines using a phase diagram (separatrix) built on the ratio between caspase-3 and XIAP. Importantly, the prototypic type I cell line SKW6.4 appeared at the part of the diagram representing the lowest number of XIAP.[52] The type II cells lines, in particular, colon carcinoma HCT116 cells, were located at the part of the separatrix corresponding to the higher amounts of XIAP, and, therefore, requiring the mitochondria activation to undergo apoptosis. Furthermore, Aldridge et al. have demonstrated that the deletion of XIAP in HCT116 cells turns them into type I cells that are independent from the mitochondria activation. Jost et al. have observed a similar switch from a type II to a type I phenotype in the hepatocytes of Bid$^{-/-}$ XIAP$^{-/-}$ mice.[54] Another interesting conclusion from the Aldridge study was that type I cells do not necessarily need high levels of caspase-8 formed at the DISC but are rather capable of undergoing apoptosis without mitochondrial involvement. This again shows that there is no contradiction between the two studies but the study by Aldridge et al. rather represents a more systematic insight into type I/type II problem.

Posttranslational modifications, such as ubiquitinylation, phosphorylation, cleavage, glycosylation and others, have a key role in death receptor signaling control.[2] Cleavage as a central modification of apoptotic signaling has been addressed by most of the models created to date.[5] Degradation of c-FLIP by ubiquitinylation was investigated in a mathematical model of the dynamic turnover and stability of c-FLIP isoforms by Toivonen et al.[34] Bagci et al.[55] created a model of the bistabilty switch between apoptosis and survival based on the regulation of nitric oxide species and inhibition of caspase-8 and -3 by nitric oxide species. The crosstalk between phosphorylation and ubiquitinylation is a highly relevant topic in contemporary biomedical research. In particular, the switch between apoptosis, necroptosis and survival signaling is highly dependent on this crosstalk.[56] Not surprisingly this question is currently in the center of systems biology studies. Finally, a remarkable progress has recently

been achieved in decoding the network of ubiquitin-ligases and deubiquitinylation enzymes controlling the ubiquitinylation status of death receptor platform components.[57,58] Therefore, the next challenge in modeling will be to incorporate ubiquitinylation control into the existing models of death receptor signaling.

An important contribution to the understanding of the extrinsic apoptotic pathway was the development of Boolean models of death receptor signaling, which allowed to understand the mechanism of the crosstalk between TNF-R and CD95 stimulation in hepatocytes on a larger scale.[59] Indeed, the model by Schlatter et al. included 86 nodes and 125 reactions. The model predicted the cellular response upon stimulation with several stimuli: CD95L, TNF-α, UV-B irradiation, interleukin-1β, insulin, and was validated by experiments in mouse hepatocytes and Jurkat T cells. This large model can reproduce well the apoptotic behavior, which was fascinating. Furthermore, the model has discovered high connectivity of different nodes of the apoptotic network and importance of the feedback loops. Importantly, a number of new ways to regulate the death receptor network were made. Furthermore, the application of Boolean models by Calzone et al. allowed to establish the mechanism of crosstalk between apoptosis, necroptosis and NF-κB signaling pathways, which is yet another challenging question in modern death receptor biology.[60]

Conclusions and Outlook

Systems biology of the extrinsic signaling pathway has developed a number of powerful models, which describe cell death in silico with an impressive level of detail. Death receptor-induced apoptosis can now be described in quantitative terms by the amounts of pro- and anti-apoptotic regulators in the apoptotic complexes, kinetic rates of cell death and the global quantitative view on the regulation of the network. Bistability switches, positive feedback loops in caspase activation and non-linear dynamics of apoptosis responses have been identified and thoroughly characterized by computational approaches. The models could perfectly well describe the regulation of the most important steps of death receptor activation and how subtle changes in the concentrations of apoptotic inhibitors can lead to a change in the outcome between life and death. This progress has to be further implemented for studying diseases connected to defects in the death receptor network. Identification of the most sensitive nodes in the death receptor pathways could allow to develop targeted therapies and new drugs that would allow to interfere with these diseases. There are already first impressive efforts in this direction, which so far mostly have addressed the regulation of the intrinsic apoptotic pathway.[61–63] The future development of similar studies of the extrinsic pathway and connection of these models to personalized medicine could provide new horizons in systems medicine.

Conflict of Interest

The author declares no conflict of interest.

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