A mathematical model of caspase function in apoptosis

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Caspases (cysteine-containing aspartate-specific proteases) are at the core of the cell's suicide machinery. These enzymes, once activated, dismantle the cell by selectively cleaving key proteins after aspartate residues. The events culminating in caspase activation are the subject of intense study because of their role in cancer, and neurodegenerative and autoimmune disorders. Here we present a mechanistic mathematical model, formulated on the basis of newly emerging information, describing key elements of receptor-mediated and stress-induced caspase activation. We have used mass-conservation principles in conjunction with kinetic rate laws to formulate ordinary differential equations that describe the temporal evolution of caspase activation. Qualitative strategies for the prevention of caspase activation are simulated and compared with experimental data. We show that model predictions are consistent with available information. Thus, the model could aid in better understanding caspase activation and identifying therapeutic approaches promoting or retarding apoptotic cell death.

Keywords: caspases, apoptosis, mechanistic mathematical models

Programmed cell death or apoptosis plays a critical role in nature. It is a process by which the cell commits suicide when malfunctions arise from cell stress, cell damage, or conflicting cell division signals[1]. Cell suicide is also required, for instance, during normal embryonic development as tissues are formed. However, maintaining the balance between cell death and survival is key. Many cancers, for example, are difficult to eradicate because they fail to respond to apoptotic signals. Conversely, neurodegenerative disorders such as Parkinson's, Alzheimer's, and Huntington's diseases are characterized by excessive apoptotic activity in certain classes of neurons[2,3]. Autoimmune disorders and central immune system phenomena such as elimination of self-reactive lymphocytes, destruction of virus-infected T cells, and elimination of active immune cells after successful immune response are also strongly linked with caspase activity[4]. Central to apoptotic cell death is a family of proteases termed caspases (cysteine-containing aspartate-specific proteases).

A more complete picture of the intriguing role these unique enzymes play in apoptosis is beginning to emerge, and has recently been outlined[2–7]. Under normal circumstances caspases are present as inactive proteins termed zymogens or procaspases, which must be activated. However, once activated they seek out and dismantle key protein targets by making selective cuts after aspartate residues. The activation of these powerful enzymes (whose inactive forms are constitutively expressed) is regulated at a number of points. Hence, a cascade of events must occur before a cell is irreversibly committed to apoptotic death. In response to stress, damage, or a signal to undergo programmed cell death, a family of caspases termed initiator caspases is activated. Active initiator caspases (caspase-8,9 among others) then activate a second group of caspases termed executioner or effector caspases (caspase-3,6,7). Once activated, executioner caspases seek out and cleave their respective protein targets, thereby dismantling the cell.

The objective here is to compile central elements of this recent portrait of caspase activation into a corresponding mathematical description. Because the bulk of our current understanding is qualitative, this model describing the events required to initiate apoptosis is more of an exploratory foundation than a finalized quantitative exercise. We maintain, however, that formulating such a mathematical model based upon current qualitative understanding is a fruitful undertaking. First, modeling offers a rigorous framework for storing and examining current knowledge. Caspase activation involves a number of important players and influences both cancer and neurodegeneration. Thus, a framework that accounts for the various interactions could be of great value in designing therapeutic approaches that restore the healthy balance between cell survival and cell death. Second, as new details emerge and more quantitative information becomes available, the model can be updated. Moreover, the current framework could easily be embedded in larger models of cell growth. Therefore, the model represents a flexible yet rigorous method to store, visualize, and interact with current and newly emerging biological information.

Initially we summarize the salient features of the model. The mathematical derivation of the kinetics and mass balances is presented in the Experimental Protocol. Second, we simulate several qualitative strategies to prevent activation or inhibit the activity of effector caspases. We then compare predictions with experimental observations to validate our model structure.

Results

The model describes, mechanistically, the activation of initiator caspases, such as caspase 8 and 9, by receptor-mediated and stress-induced mechanisms, respectively. These initiator caspases, in turn, activate effector caspases.

The model framework is shown in Figure 1. The FAS surface death receptor (R) activates procaspase-8 (c_{8z}) (Fig. 1). FAS (also called CD95 or Apo1) is a member of the tumor necrosis factor (TNF) gene superfamily that is defined by cysteine-rich extracellular domains[8] and a homologous cytoplasmic sequence termed the death domain (DD)[9,10]. The cell receives and processes a suicide order through this receptor by binding an extracellular death ligand (L) causing the death domains of several FAS receptors to cluster[11] at the specific rate r_b. In the clustered conformation, an adapter protein named FADD (F)

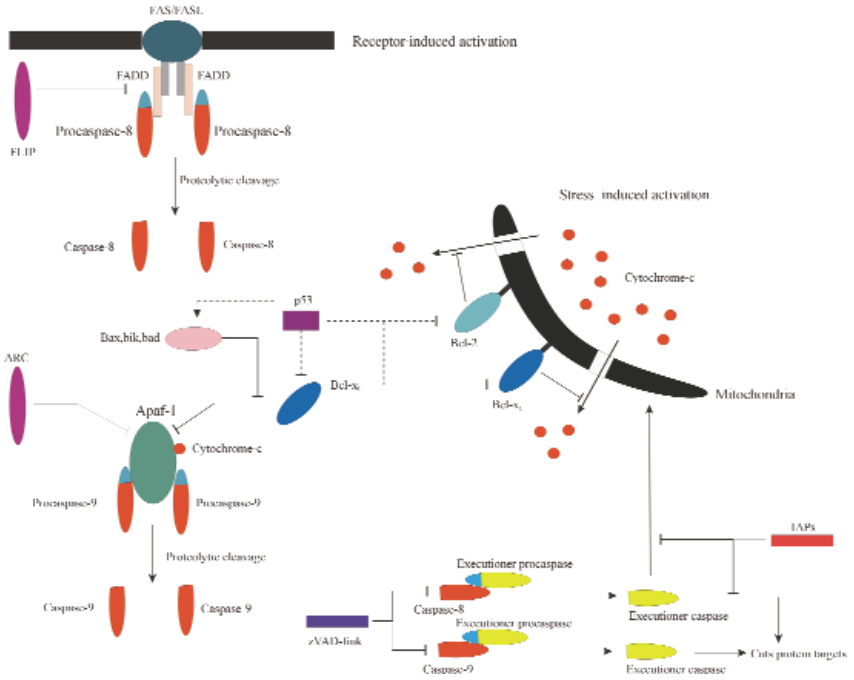

Figure 1. Executioner caspase activation cascade. Executioner caspase can be activated by initiator caspase-8 and/or 9, which also must be activated. Procaspase-8 is activated by the FAS/FADD death receptor complex, whereas, procaspase-9 is activated by the Apaf-1/cytochrome-c complex. All symbols defined in text.

(Fas-associated death domain, also called Mort 1) binds to the FAS death domain through a complementary death domain of its own[12,13] at the specific rate r_F. Once FADD is in place, procaspase-8, which is constitutively expressed at the specific rate Ω_{8z}, can bind to a domain on FADD termed the death effector domain (DED)[14]. This brings two molecules of procaspase-8 into close proximity, which allows them to activate one another by proteolytic cleavage[15,16] at the specific rate r_{8za1}. Once activated, caspase-8 (c_{8a}) activates executioner-level caspases, for example caspase-3 (c_{Ea}), by proteolytic cleavage at the specific rate r_{8Ea}.

Stress-related factors also induce caspase activation in the absence of an external death signal. Procaspase-9 (expressed constitutively at the specific rate Ω_{9z}; ref. 17) is activated following the release of mitochondrial cytochrome *c* (C_c) into the cytosol. Cytochrome *c*, along with apoptotic protease-activating factor 1 (Apaf-1; A1) are required for procaspase-9 (c_{9z}) activation. Apaf-1 is thought to bind cytosolic cytochrome *c*, forming a complex that in turn binds procaspase-9, allowing activation to occur, probably by a mechanism similar to FAS activation of procaspase-8 (ref. 18). The specific rate of formation of the Apaf-1–Cytochrome *c* complex is denoted as r_{A1}. The activation of procaspase-9 by the Apaf-1–cytochrome *c* complex occurs at the specific rate r_{9za1}. Once activated, caspase-9 (c_{9a}) can activate executioner caspases at the specific rate r_{9Ea}. Several apoptotic agents, such as UV irradiation, staurosporine, ceramide, overexpression of proapoptotic Bcl-2 family members (see below), nutritional stress, and amino acid limitation[19–22], can cause cytochrome *c* to be released leading to procaspase-9 activation. Active initiator caspases in turn activate executioner caspases that influence the loss of mitochondrial membrane potential[7] and eventually cause the release of cytochrome *c*, thereby forming a positive activation loop.

The activation and activity of initiator and executioner caspases can be arrested at several points. First, a family of proteins, termed inhibitors of apoptosis (IAPs), inhibit executioner caspases directly[23]. There is debate, however, if this is their only role, because IAPs when overexpressed prevent activation of effector caspases[24,25]. We assume IAPs irreversibly convert active executioner caspases to an inactive form at the specific rate r_{IAP}. Second, a set of FADD-like interleukin-1 beta converting enzyme (ICE) proteins termed FLIPs (I_8) (also called Casper, I-FLICE, FLAME, and CASH) contain a death effector domain similar to FADD and may compete with procaspase-8 for FADD binding sites[26,27]. Third, the protein, the apoptosis repressor with caspase recruitment domain (ARC; I_9) could inhibit procaspase-9 activation by competing for Apaf-1 binding sites[28]. Fourth, decoy substrates such as zVAD-fmk (I_{Ea}) covalently bind to initiator caspases, thereby preventing the activation of executioner caspases[29].

The Bcl-2 family of proteins plays a dual role in apoptosis, and contains both antiapoptotic proteins such as Bcl-2 and Bcl-x_L and proapoptotic proteins such as Bax, Bad, and Bik (refs 5,30). The antiapoptotic proteins play several key roles restraining the activation of caspase-9. They inhibit the release of cytochrome *c* from the mitochondria and compete with procaspase-9 for Apaf-1 binding sites[18,19]. Bcl-2 (b_2) and Bcl-x_L (b_x) show identical activities with respect to preventing cytochrome *c* release and Apaf-1 binding[5,31]; however, Bcl-2 is localized to the outer mitochondrial membrane whereas only a fraction of Bcl-x_L is membrane-bound[5,32]. Thus, we assume as a first approximation that the fraction of Bcl-2 that binds Apaf-1 is small compared with Bcl-x_L. Effectiveness of the antiapoptotic Bcl-2 family members may be blunted by proapoptotic proteins such as Bax or Bik, denoted as b_e. We assume b_e reversibly binds antiapoptotic Bcl-2 family members, thereby neutralizing their protective abilities. The ratio of anti- versus proapoptotic Bcl-2 family members, for example Bcl-x_L to Bax, is tightly controlled by *p53* (a gene involved in cell cycle regulation). The *p53* gene induces Bax and represses Bcl-x_L expression under stress conditions, thereby promoting stress-induced apoptosis[33]. Mutations in *p53* alter the balance between anti- and proapoptotic Bcl-2 family members. The influence of *p53* is accounted for in the mass balances around the Bcl-2 family members by adjusting their specific expression rates.

Simulation. The model equations were solved using the ODE15s routine of Matlab 5.3 (The Mathworks Inc; Natick, MA). The model was encoded using the Simulink 2.0 package of Matlab 5.3. The model code, a graphical user interface (GUI) to run the model, and the parameter file used in this study are available at http://www.cems.umn.edu.

Receptor-mediated caspase activation. The simulation of receptor-mediated executioner caspase activation is shown in Figure 2A. The receptor-mediated death signal is received at time zero. Simulation of the wild-type cascade is consistent with the mechanism summarized above. Before the death signal is received, the fraction of active initiator and executioner caspase is well below 0.5% (low frequency of self-activation). When the death signal is received, procaspase-8, activated by the FAS–FADD complex (~50% activation within 2 h of death signal receipt), activates executioner caspases within minutes. Subsequently, as the fraction of active executioner caspase increases, the mitochondria releases cytochrome *c*, which, when bound to Apaf-1, catalyzes procaspase-9 activation. The initiation of procaspase-9 activation occurs roughly 20–30 min after that of procaspase-8 (lag time required for the release of cytochrome *c* from the mitochondria).

Disruption of FADD's ability to bind to the clustered FAS/FASL death domains (shown in Fig. 2B) decreases active caspase-8 (complete binding disruption results in <0.1% active caspase-8 upon receipt of the death signal). Less active caspase-8 naturally implies a decrease in active executioner caspase. This prediction is consistent with a number of investigations[12,34,35]. However, the obvious route to limit FADD binding, that is, knocking out the FADD gene, may not

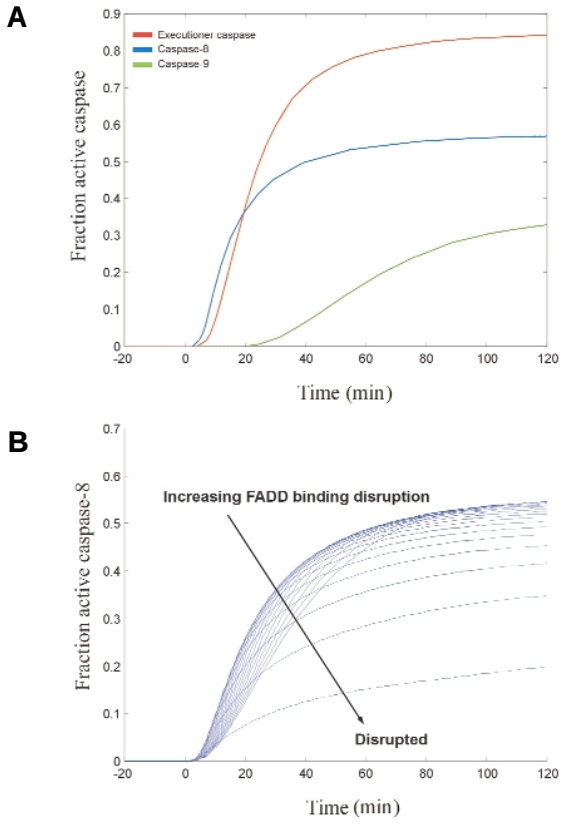

Figure 2. Receptor mediated caspase activation. (A) Wild-type fraction of active caspase versus time. (B) Fraction of active caspase-8 versus time for FADD disrupted cells. A death signal is intercepted by the FAS receptor at time 0 hr and procaspase-8 is activated; in turn activating executioner caspase within minutes. Procaspase-9 is activated roughly 20-30 min after procaspase-8. Disruption of FADD's ability to bind to the clustered FAS death domains results in decreased active caspase-8.

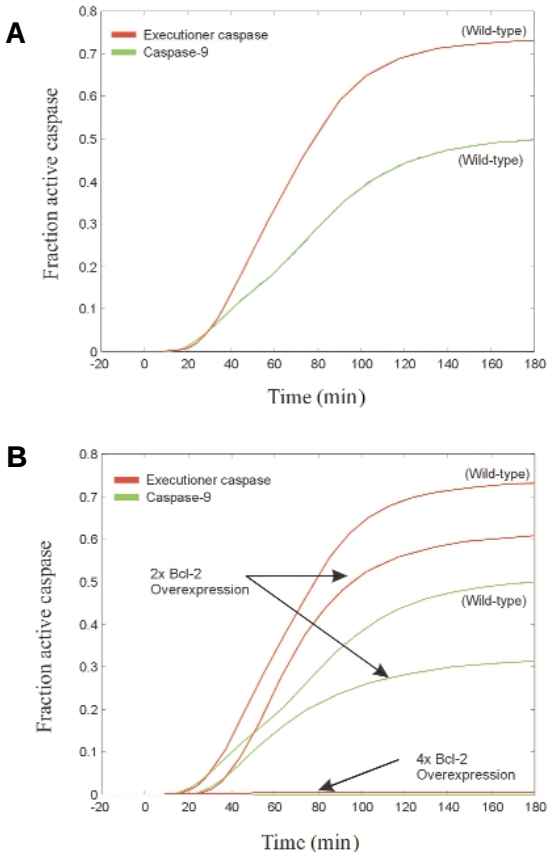

Figure 3. Stress induced caspase activation. (A) Fraction active of caspase-9 and executioner caspase versus time for wild-type cells. (B) Fraction of active initiator and executioner caspases versus time for different levels of Bcl-2 overexpression (maximum 4-fold). Bcl-2 blocks the release of cytochrome-c from the mitochondria, thereby, down-regulating procaspase-9 activation which in turn down-regulates executioner caspase activation.

be the most appropriate. Yeh and colleagues[35] found that FADD knockout mice did not survive beyond day 11.5 of embryogenesis (mice exhibited signs of cardiac failure and abdominal hemorrhaging), indicating FADD may have other critical signaling functions in embryonic development. To achieve decreased FADD binding, in the absence of side effects, the death domains of the FAS receptor could be disrupted, but such an approach is not practical. Perhaps a more realistic alternative would be the oral or intravenous introduction of decoy substrates or proteins such as ARC (see below).

Stress-induced caspase activation. Simulation of stress-induced caspase activation is shown in Figure 3A. The stress death signal is received at time zero. Within approximately 10 min the mitochondria begins to release cytochrome *c*, which then binds to Apaf-1 and catalyzes procaspase-9 activation. Within 1 h, approximately 35–40% of executioner caspase is active. At 2 h, 70% of executioner caspase is active. The predicted time scale of activation is roughly equivalent tothat observed by Kluck et al. where it was shown in cell-free incubation of *Xenopus* egg mitochondria that DEVD-specific protease activity was first observed after a lag of approximately 2–3 h (ref. 19). The fraction of activated executioner caspase is equal in stress and receptor-mediated activation routes; however, the fraction of caspase-9 that is activated (about 10%) is slightly higher in the stress-induced cells. The lag time between receipt of the death signal and activation of executioner caspases is greater in stress-induced than in receptor-induced activation. This might be expected in that the concentration of cytochrome *c* in the cytosol is very small at the receipt of the death signal; it is only after cytochrome *c* begins to leak from the mitochondria in significant amounts that caspase-9 activation can get underway.

Overexpression of Bcl-2 is predicted to block the release of mitochondrial cytochrome *c*, thereby blocking the activation of procaspase-9 (Fig. 3B). This finding is consistent with the observations of Yang et al. in which Bcl-2 was overexpressed in human acute myeloid leukemia cells[18]. Kluck et al. have shown a similar role for Bcl-2 in a cell-free system composed of *Xenopus* egg extracts[19]. There exists a critical overexpression level (predicted with current parameter set to be greater than fourfold) that is required for Bcl-2 to completely block cytochrome *c* release. Below this threshold, cytochrome *c* release is inhibited but not blocked.

The interaction between procaspase-9 and the Apaf-1–cytochrome *c* complex can be disrupted by means of overexpression of Bcl-x_L (or other antiapoptotic Bcl-2 family members), whereby antiapoptotic forces competitively bind to Apaf-1, decreasing the activation of executioner caspases (Fig. 4B). However, the competitive effect of Bcl-x_L can be blunted and even reversed by overexpression of proapoptotic agents (Fig. 4C). Enhanced concentrations of proapoptotic Bcl-2 family members, such as Bik, bind antiapoptotic Bcl-2 family members (e.g. Bcl-x_L) and remove their inhibitory effect, allowing the activation of caspase-9 by the Apaf-1 complex to proceed. Thus, the ratio of anti- versus proapoptotic Bcl-2 family members determines, even after cytochrome *c* is released from the mitochondria, whether or not executioner caspases will be activated. Mutations in the cell cycle-regulatory protein p53 decrease the active fraction of both initiator and executioner caspas-

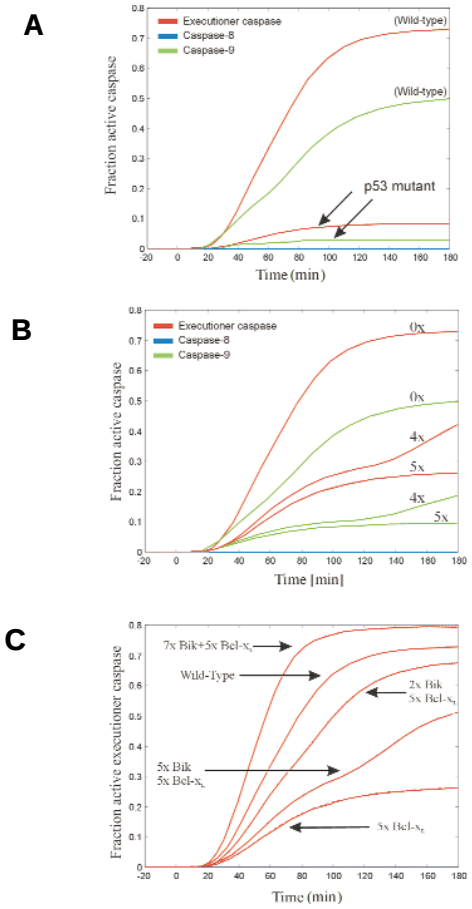

Figure 4. Deactivating stress induced caspase activation. (A) Fraction of active initiator and executioner caspase versus time for wild-type and p53 mutant cells. (B) Fraction of active executioner caspase versus time for different levels of Bcl-x_L overexpression. (C) Fraction of active executioner caspase for mixed Bcl-x_L/Bik overexpression. Mutation of the p53 cell cycle gene reduces sensitivity to stress induced death signals, making the cell cancerous in the process. Upregulation of Bcl-x_L decreases initiator and executioner caspase activation by competitively binding to Apaf-1 (cytochrome-c is released from the mitochondria). However, this effect can be reversed by upregulation of proapoptotic Bcl-2 family members.

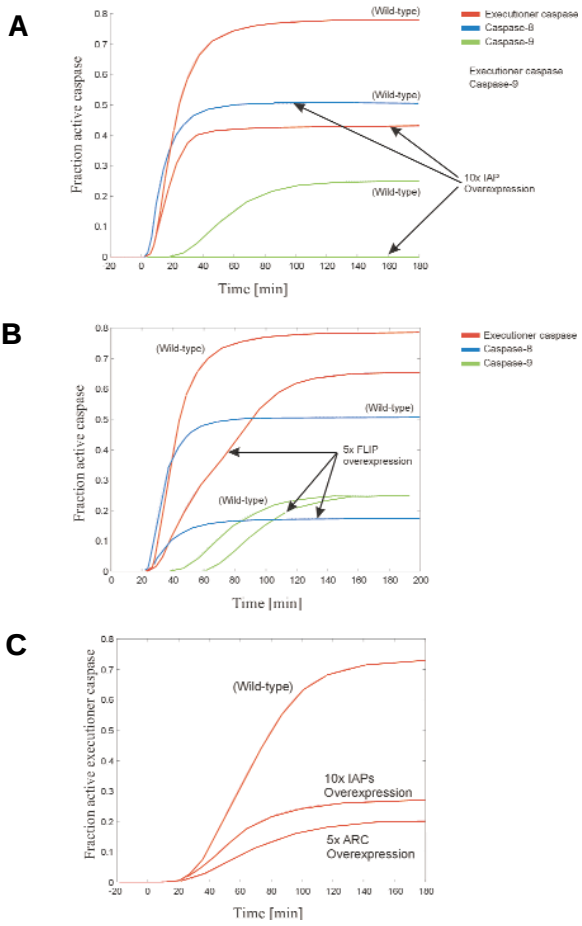

Figure 5. The effect of decoy proteins and IAPs. (A & B) Increased concentration of decoy proteins and IAPs for receptor mediated caspase activation. Fraction of active initiator and executioner caspases versus time. (C) Increased concentration of decoy proteins and IAPs for stress induced caspase activation. Fraction of active executioner caspase versus time. Increased decoy concentration reduces the active fraction of initiator caspase whereas IAPs overexpression reduces the fraction of active executioner caspase.

es (Fig. 4A). However, this is not a feasible antiapoptotic therapeutic avenue, as *p53* mutation is a consistent marker of cancer.

Overexpression of decoys and IAPs. Overexpression of decoy proteins ARC, FLIP, and the artificial polypeptide zVAD-fmk block executioner caspase activation by interfering with the activation and/or function of initiator caspases (Fig. 5). The last line of defense against executioner caspase activity stems from IAPs. Simulation of mild IAP overexpression is shown in Figure 5. The activity of executioner caspase is negatively influenced by IAPs overexpression to similar degrees in both receptor- and stress-mediated cases, consistent with a number of investigations[23–25]. Moreover, IAP overexpression only completely blocks effector caspase activity if above a certain threshold. Thus, as a therapeutic mechanism one might expect IAP overexpression to slow rather than halt cell death. This portrait is consistent with recent findings where inhibition of caspase-1 activity slowed the progression of Huntington's disease in a mouse model system[36].

A combination of stress- and receptor-mediated caspase activation. The different strategies proposed to block or accelerate executioner caspase activation are directed toward a specific activation route; however, there are many such routes. Which therapies or combination of therapies will be effective when both receptor- and stress-mediated routes are active? We show in Table 1 that no single therapy, with the exception of IAPs overexpression and/or the overexpression of multiple decoy proteins, can block executioner caspase activation. In fact, the bulk of strategies are predicted to be ineffective, because they target only a single activation route. For example, overexpression of antiapoptotic Bcl-2 family members in the presence of both activation routes will not block caspase activation because receptor-mediated activation is free to proceed. Conversely, disruption of FADD's ability to bind the clustered death domains of the FAS receptor also does not block executioner caspase activation when both routes are operating. Only strategies that independently target both stress- and receptor-mediated apoptosis can block executioner caspase activation. Thus, for example, enhancing the activity of the decoy protein ARC (by various means) is a feasible strategy to block executioner caspase activation, whereasa overexpression of Bcl-2 and ARC is not. The number of redundant feedback mechanisms downregulating stress-induced as opposed to receptor-induced apoptosis may indicate that receptor-mediated executioner activation is a primary death route and that external death signals have high priority.

Strategies to accelerate executioner caspase activation need not involve multiple changes, as only one initiation route is required to start the cascade. For example, to initiate executioner caspase activation in *p53* mutant cells, one might overexpress the death ligand and the FAS receptor. Conversely, to initiate executioner caspase activa-

Table 1. Predicted effects of combined therapies based on simultaneous receptor- and stress-induced caspase activation[a]

	Overexpression						Disruption or mutation	
	Bcl-2	Bcl-x_L	Bik/Bax /Bad	ARC	FLIPs	IAPs	FADD	p53
Bcl-2	–	–	–	–	+	+	+	–
Bcl-x_L	–	–	–	–	+	+	+	–
Bik/Bad/ Bax	–	–	–	–	–	+	–	–
ARC	–	–	–	–	+	+	+	–
FLIPs	+	+	–	+	–	+	–	+
IAPs	+	+	+	+	+	+	+	+
FADD	+	+	–	+	–	+	–	+
p53	–	–	–	–	–	+	+	–

[a]Elements that are off the diagonal denote combinations of potential therapies; diagonal elements denote single therapies. For example, the left uppermost element denotes Bcl-2 overexpression alone, whereas the right uppermost element denotes Bcl-2 overexpression in combination with *p53* mutation. Plus signs (+) denote therapies that decrease executioner caspase activation, minus (–) signs the opposite. No single therapy, excluding IAPs overexpression, results in disruption of executioner caspase activation when both activation routes are active. Only therapy combinations that independently attack each activation route can retard executioner caspase activation.

tion in receptor-deficient cells, one could overexpress proapoptotic Bcl-2 family members or place the cells under nutritional stress. From the aforementioned, we can conclude that it is easier to initiate apoptosis than it is to block it.

Discussion

We found that our model-simulated strategies to block executioner procaspase activation were consistent with experimental observations. Bcl-2 overexpression, above a critical threshold, was an effective weapon to block the activation of procaspase-9. However, this does not guarantee that the cell will not undergo apoptosis by caspase-8 activation of executioner caspases. Likewise, Bcl-x_L overexpression negatively influences procaspase-9 activation, but its impact can be reversed by proapoptotic forces. Disruption of FADD's ability to bind to the clustered death domains of the FAS/FASL complex is an effective tool for blocking caspase-8 activation, however, it will not prevent apoptosis by the caspase-9 channel when cytochrome *c* is released in response to other stimuli. As a last line of defense, the activity of executioner caspases can be directly inhibited by overexpression of IAPs. Thus, in cases where both death routes are active, only strategies that selectively address each are effective at blocking executioner caspase activation (excluding IAPs overexpression, which blocks executioner caspase activity directly). Conversely, initiation of apoptosis requires only one activation route.

The large number of redundant control mechanisms regulating stress-induced caspase activation might indicate that this is a secondary death pathway when compared to receptor-mediated routes. Indeed, receptor-mediated caspase activation is not as well controlled and therefore might be a "death override" of sorts that will operate whenever an external death signal is received, with no regard for the physiological state of the cell. Thus, in neurodegenerative or retinodegenerative disorders, when seemingly healthy neurons or retinal cells commit suicide, the model points to therapeutic strategies that retard receptor-mediated activation. For example, overexpression of the IAP-like protein p35 prevented apoptosis-caused retinal degeneration in *Drosophila* mutants[37]. The reverse argument can be made for cancerous cells; we must enhance receptor-mediated suicide pathways without causing hypersensitivity to external death signals.

Our understanding of the complex and subtle role of apoptosis in cancer and neurodegenerative disorders can be greatly enhanced by building models. Combating cancer by upregulating receptor-mediated apoptosis brings with it the risk of hypersensitivity to death signals. Conversely, Alzheimer's might be transformed into cancer if hypersensitive apoptosis is overly restrained. Thus, cancer and neurodegenerative diseases require analysis beyond simple plus/minus engineering. Since they are tightly coupled to death pathways, strategies to combat these diseases in the absence of consideration of this interplay run the risk of upsetting the delicate balance between survival and death. The mathematical model of caspase activation presented here allows grouping of diverse disease families based on the role of apoptosis in their etiology. The primary impact of such a tool is to provide a quantitative means of formulating and testing therapeutic strategies to restore the healthy balance between cell survival and cell death.

Table 2. Nomenclature used in derivations

Abbreviation	Meaning
$[c_{kz}]$	Concentration of procaspase-k (μmol gdw^{-1})
$[c_{ka}]$	Concentration of active caspase-k (μmol gdw^{-1})
[xz]	Concentration of executioner procaspase [μmol gdw^{-1}]
[xa]	Concentration of active executioner caspase (μmol gdw^{-1})
[R]	Concentration of FAS receptor (μmol gdw^{-1})
[L]	Concentration of FAS ligand (μmol L^{-1})
[RL]	Concentration of FAS–FAS ligand complex (μmol gdw^{-1})
[F]	Concentration of FADD protein (μmol gdw)
$[RL.F_2]$	Concentration of FAS–FASL–FADD complex (μmol gdw^{-1})
[A1]	Concentration of Apaf-1 protein (μmol gdw^{-1})
$[C_c]$	Concentration of cytosolic cytochrome *c* (μmol gdw^{-1})
$[A1.C_c]$	Concentration of Apaf-1–cytochrome *c* complex (μmol gdw^{-1}]
$[b_2]$	Concentration of Bcl-2 (μmol gdw^{-1})
$[b_x]$	Concentration of Bcl-x_L (μmol gdw^{-1}]

Experimental protocol

We present here the derivation of kinetics and mass balances used to develop our model.

Rate of FAS death domain clustering, r_b. The clustering of the FAS death domains results from the binding of the trimeric FAS ligand L to free FAS surface receptors R. Although we treat the binding of L as reversible, the clustering is assumed to be irreversible; hence, the clustering proceeds by means of the elementary steps

$$R + L \underset{}{\overset{S}{\rightleftharpoons}} \tilde{R}L$$
$$\tilde{R}L \xrightarrow{T} RL \qquad (1)$$

where $\tilde{R}$ and RL denote the unclustered/clustered FAS/FASL complex, respectively. The site balance around the FAS receptor is given by

$$[R]_T = [R]_F + [\tilde{R}L] + [RL] \qquad (2)$$

where $[R]_T$ and $[R]_F$ denote the total and free-ligand concentration, respectively. Assuming step T in equation (1) to be rate controlling implies that the specific rate of death domain clustering, r_b, is given by

$$r_b = \frac{k_t([R]_T - [RL])[L]}{K_S^{-1} + [L]} \qquad (3)$$

where [L] and K_S denote the free death ligand concentration and the binding equilibrium constant, respectively.

Rate of FADD binding, r_F. The specific rate of FADD binding to the clustered FAS death domains, denoted as r_F, is assumed to occur by means of the elementary reactions

$$RL + F \underset{}{\overset{A}{\rightleftharpoons}} RL.F$$
$$RL.F + F \underset{}{\overset{B}{\rightleftharpoons}} RL.F_2 \qquad (4)$$

where F denotes the FADD protein and $RL.F_n$ denotes the clustered receptor with n bound FADD adapter proteins. The site balance around clustered receptor is given by

$$[RL]_T = [RL]_F + [RL.F] + [RL.F_2] \tag{5}$$

Assuming the first FADD addition is rate controlling yields

$$r_F = \frac{k_a[F][RL]_T}{(1 + K_A[F] + K_A K_B[F]^2)} - \frac{[RL.F_2]}{K_A K_B[F]} \tag{6}$$

where K_A, K_B denote the equilibrium constants for the first and second FADD additions, respectively.

Rate of formation of the Apaf-1–cytochrome *c* complex, r_A. The Apaf-1–cytochrome *c* complex (A1.C_c) catalyzes the activation of stress-induced initiator caspases, such as procaspase-9. The formation of this key complex is inhibited by antiapoptotic Bcl-2 family members, for example Bcl-x_L (denoted as b_x), by means of competition for binding sites on Apaf-1 (A1). Proapoptotic Bcl-2 family members, denoted as b_e, can bind to antiapoptotic family members and remove their inhibitory effect (described by the last elementary reaction). The formation of A1.C_c is governed by the elementary reactions

$$\begin{aligned} C_c + A1 &\overset{H}{\rightleftarrows} A1.C_c \\ A1 + b_x &\overset{I}{\rightleftarrows} A1.b_x \\ b_x + b_e &\overset{J}{\rightleftarrows} b_x.b_e \end{aligned} \tag{7}$$

The site balance around Apaf-1 is given by

$$[A1]_T = [A1]_F + [A1.C_c] + [A1.b_x] \tag{8}$$

Assuming cytochrome *c* binding to be rate controlling yields

$$r_{A1} = \frac{k_h[C_c][A1]_T}{(1 + K_H[C_c] + K_I[b_x])} - k_{-h}[A1.C_c] \tag{9}$$

where K_H and K_I denote the equilibrium constant(s) governing the binding of cytochrome *c* and antiapoptotic Bcl_2 family members to Apaf-1, respectively, and $[b_x]$ denotes free Bcl-x_L. From the balance around total Bcl-x_L we can determine the free b_x concentration in terms of the total

$$[b_x] = \frac{[b_x]_T}{1 + K_J[b_e]} \tag{10}$$

where $[b_e]$ and K_J denote the free activator (assumed approximately equal to total) and the activator-binding equilibrium constant, respectively.

Rate of initiator procaspase activation, r_{Tkza}. The activation of initiator procaspases (e.g., procaspase-8 and -9) is catalyzed by the RL.F_2 and A1.C_c complexes, respectively. It is assumed that two procaspase-k proteins reversibly bind to their respective activating complex and are brought into close contact, thereby resulting in proteolytic cleavage. The binding of procaspase-k competes with an inactive decoy protein denoted as I_k (decoy binding is considered reversible). The elementary reactions describing procaspase-k activation are then

$$\begin{aligned} c_{kz} + x &\overset{C,K}{\rightleftarrows} x.c_{kz} \\ x.c_{kz} + c_{kz} &\overset{D,L}{\rightleftarrows} x.(c_{kz})_2 \\ x.(c_{kz})_2 &\xrightarrow{E,M} x + 2c_{kz} \\ I_k + x &\overset{F,N}{\rightleftarrows} x.I_k \\ I_k + x.c_{kz} &\overset{G,O}{\rightleftarrows} I_k.x.c_{kz} \end{aligned} \tag{11}$$

where k = 8,9 and x denotes the RL.F_2 complex in the case of procaspase-8 and the A1.C_c complex for procaspase-9. The site balance around complex x is given by

$$[x]_T = [x]_F + [x.c_{kz}] + [x.(c_{kz})_2] + [x.I_k] + [I_k.x.c_{kz}] \tag{12}$$

Assuming the proteolytic cleavage to be rate limiting yields the activation rate

$$r_{kza1} = \frac{k_a[c_{kz}]^2[RL.F_2]_T}{((K_C K_D)^{-1} + K_D^{-1}[c_{kz}] + [c_{kz}]^2 + K_F(K_C K_D)^{-1}[I_k] + K_G K_D^{-1}[c_{kz}][I_k])} \tag{13}$$

and

$$r_{kza2} = \frac{k_a[c_{kz}]^2[A1.C_c]_T}{((K_K K_L)^{-1} + K_L^{-1}[c_{kz}] + [c_{kz}]^2 + K_N(K_K K_L)^{-1}[I_k] + K_O K_L^{-1}[c_{kz}][I_k])} \tag{14}$$

where K_j denotes the respective equilibrium constant and where the subscript j stands for all the subscripts in the equation, and $[I_k]$ denotes the free decoy concentration (assumed to be approximately equal to the total decoy concentration). Additionally, two molecules of free procaspase-k can activate each other by proteolytic cleavage at the specific rate

$$r_{kza2} = k_{kza2}[c_{kz}]^2 \tag{15}$$

The total specific rate of procaspase-k activation is then

$$r_{Tkza} = r_{kza1} + r_{kza2} \tag{16}$$

Rate of executioner caspase activation, r_{kEa}. Executioner caspases, such as caspase-3, can be activated by either of the initiator caspases. The activation of executioner caspases can be inhibited decoy proteins. Thus, the elementary reactions governing executioner caspase activation are

$$\begin{aligned} c_{ka} + c_{Ez} &\overset{P}{\rightleftarrows} c_{ka}.c_{Ez} \\ c_{ka}.c_{Ez} &\xrightarrow{Q} c_{ka} + c_{Ea} \\ c_{ka} + I_{Ea} &\overset{R}{\rightleftarrows} c_{ka}.I_{Ea} \end{aligned} \tag{17}$$

where k = 8,9. The last elementary step describes the binding of decoy proteins to active initiator caspase, thereby inhibiting executioner caspase activation. The site balance around active initiator caspase is given by

$$[c_{ka}]_T = [c_{ka}]_F + [c_{ka}.c_{Ez}] + [c_{ka}.I_{Ea}] \tag{18}$$

Assuming the activation step to be rate limiting yields

$$r_{k3a} = \frac{k_q[c_{Ez}][c_{ka}]_T}{(K_p^{-1} + [c_{Ez}] + K_R K_p^{-1}[I_{Ea}])} \tag{19}$$

where K_q denotes the respective equilibrium constants and where the subscript q stands for all the subscripts in the equation, and $[I_{Ea}]$ denotes the free concentration of decoy protein (assumed to be approximately equal to the total concentration).

Rate of executioner caspase inactivation, r_{IAP}. Executioner caspases can be directly inhibited by IAPs. We assume that IAPs reversibly bind to the active executioner caspase, thereby, inactivating it

$$c_{Ea} + IAPs \overset{U}{\rightleftarrows} c_{Ea}.IAPs \tag{20}$$

The site balance around active executioner caspase (ignoring the binding of protein targets) is given by

$$[c_{Ea}]_T \simeq [c_{Ea}]_F + [c_{Ea}.IAPs] \tag{21}$$

As a first approximation, we neglect the back reaction; hence, the rate of inactivation of executioner caspase is given by

$$r_{IAP} \simeq \frac{k_u[IAPs][c_{Ea}]_T}{(1+K_U[IAPs])} \tag{22}$$

where K_U and *[IAPs]* denotes the binding equilibrium constant and the free concentration of IAPs (assumed to be approximately equal to the total concentration).

Rate of mitochondrial cytochrome *c* release, r_C. We assume that the specific rate of cytochrome *c* release can be described by

$$r_C = \alpha_{CE}\upsilon(c_{Ea}, b_2) + \sum_j \alpha_{Cj}\upsilon(j, b_2) \tag{23}$$

where α_{CE} and α_{Cj} denote the specific rate of cytochrome *c* efflux caused by executioner caspase and chemical/nutritional factor j, respectively. The term $\nu(c_{Ea},b_2)$ describes the interaction of executioner caspase and antiapoptotic members of the Bcl-2 family that protect the mitochondria. We assume that $\nu(c_{Ea},b_2)$ is directly proportional to the ratio of executioner caspase to b_x and obeys

$$0 \le \upsilon(c_{Ea}, b_2) \le 1 \tag{24}$$

One would expect, generally, that if the ratio of executioner caspase to b_x is greater than some threshold (ε_{Eb2}), cytochrome *c* would be released; conversely, if the ratio is less than or equal to the threshold, no cytochrome *c* is released. These arguments lead to

$$\upsilon(c_{Ea}, b_2) = \begin{cases} 1 & \dfrac{[c_{Ea}]}{[b_2]} > \varepsilon_{Eb_2} \\ 0 & \dfrac{[c_{Ea}]}{[b_2]} \le \varepsilon_{Eb_2} \end{cases} \tag{25}$$

Similar arguments apply to $\nu(j,b_2)$.

Model mass balances. The model balances are given by:

$$\frac{d[RL]_T}{dt} = r_b - \mu[RL]_T$$
$$\frac{d[R]_T}{dt} = \Omega_R - \mu[R]_T$$
$$\frac{d[F]}{dt} = \Omega_F - 2r_F - \mu[F]$$
$$\frac{d[RL.F_2]_T}{dt} = r_F - \mu[RL.F_2]_T$$
$$\frac{d[C_c]}{dt} = r_C - r_{A1} - \mu[C_c]$$
$$\frac{d[A1]_T}{dt} = \Omega_{A1} - \mu[A1]_T$$
$$\frac{d[A1.C_c]}{dt} = r_{A1} - \mu[A1.C_c]$$
$$\frac{d[c_{k2}]}{dt} = \Omega_k - 2r_{Tkza} - \mu[c_{k2}]$$
$$\frac{d[c_{ka}]}{dt} = 2r_{Tkza} - \mu[c_{ka}]$$
$$\frac{d[c_{Ez}]}{dt} = \Omega_{Ez} - \sum_{w=8}^{9} r_{wEa} - \mu[c_{Ez}]$$
$$\frac{d[c_{Ea}]}{dt} = \sum_{w=8}^{9} r_{wEa} - \mu[c_{Ez}] - r_{IAP}$$
$$\frac{d[b_j]}{dt} = \Omega_{b_j} - \mu[b_j]$$
$$\frac{d[I_w]}{dt} = \Omega_{I_w} - \mu[I_w]$$

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