Cellular Automata Modeling of FASL-Initiated Apoptosis

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Two strategies for fighting cancer by modulating FASL-induced apoptosis were modeled by 2D-cellular automata. Our models predict that cancer cells can be killed by maximizing the apoptosis via joint suppression of FLIP and IAP inhibitors by siRNA and SMAC proteins, respectively. It was also predicted that the presumed feedback loop CASP3 \rightarrow CASP9 \rightarrow IAP in the intrinsic pathway accelerates the apoptosis, but does not change significantly the concentration of DFF40, the protein that decomposes DNA. The alternative strategy of preventing the killing of the immune system’s T-cells, via minimizing their tumor-induced FAS-L apoptosis by overexpression of FLIP and IAP, was also shown to be promising with a predicted considerable synergy action of the two inhibitors. Dual suppression or overexpression of apoptosis inhibitors emerges thus as promising approach in the fight against cancer. Our modeling has also brought some light on the process of turning type-I cells into type-II ones, which emerges as compensatory mechanism in case of damaged or silenced FASL pathway by preserving about the same self-death level at only 10–12% lower performance rate.

1. Introduction. – Apoptosis is a process of programmed cell death, which is the most common mechanism by which the body eliminates damaged or unneeded cells [1]. The abnormal functioning of apoptosis is associated with a multitude of diseases [2], and is the critical aspect of cancer development and tumor-cell survival [3]. Such health implications underscore the pharmacological potential of designing strategies to manipulate apoptosis [4–11]. Apoptosis is initiated via two types of signaling pathways, the components of which are potential anticancer therapeutic targets. The intrinsic pathways are activated by developmental signals or severe cell stress, while the extrinsic pathways are triggered by pro-apoptotic ligands, such as FAS ligand (FASL), which binds to the pro-apoptotic death receptor FAS (CD95). The FASL/FAS binding induces recruitment of the FAS-associated death domain (FADD) protein and the initiator caspases 8 or 10 as procaspases, forming a death-inducing signaling complex (DISC). Formation of the DISC brings procaspase molecules into close proximity of one another, enabling their autocatalytic activation and release into the cytoplasm where they activate effector caspases (CASP3, CASP6, and CASP7). The latter split the heterodimer DFF (DNA Fragmentation Factor), and the released DFF40 fragments DNA, while CASP6 cleaves proteins vital for cell survival. The caspase
cascade is regulated by c-FLIP (FADD-like apoptosis regulator) proteins and the IAP (Inhibitor of Apoptosis) protein family, XIAP being known as the most potent inhibitor (Fig. 1).

The FAS protein plays a critical role for the immune system by killing pathogen-infected cells [12] and preventing autoimmunity and tumors. Recent studies [13] have revealed important details of the role of T-cells in the immune response to fight cancerous and HIV-infected cells. The latter induce apoptosis in T-cells and kill them by overexpressing FASL, while preventing their own destruction by the same apoptotic mechanism. Based upon these recent findings, the present study developed a dynamic model simulating two strategies against cancer. The classical approach aims to kill cancerous cells, a goal that we simulated by strong suppression of apoptosis inhibitors FLIP and IAP by siRNA and SMAC, respectively, following the recent study of Wilson et al. [14] and Cheung et al. [15]. The opposite strategy of maximizing the inhibition of FLIP and IAP was applied to simulate blocking the apoptotic process in T-cells that is caused by a tumor counterattack [13]. Such a strategy to restore the potency of the immune system could also be of interest in the fight against HIV infection.

2. Results and Discussion. – We simulated the strategy of blocking the apoptosis in the immunological response of T-cells by maximizing the inhibitor action of FLIP [16–22] and IAP [23][24], either individually or jointly [14]. IAPs are characterized by the presence of between one and three specific domains called baculoviral repeats (BIRs), which are directly involved in their caspase-inhibitory activity. FLIP acts by attaching to DISC, which blocks the activation of CASP8 and CASP10 (see Fig. 1). The DISC complex thus acts as a mechanistic switch to regulate apoptosis. The very process of DISC formation is a highly regulated and delicately balanced process. It includes formation of a FAS-FAS dimer and FADD association with the FAS dimer, followed by Caspase-8 and Caspase-10 recruitment and activation. The binding between FAS-FAS
dimer, and that between FAS dimer and FADD, is shown to be weak [25–27] so as to prevent accidental activation of apoptosis, and to proceed to formation of DISC complex only in the case of strong stimulus. DISC consists of a lattice of FAS dimer and FADD. The sequence of elementary steps of this complex mechanism is described in detail in the Exper. Part.

We first tested our simulation approach by comparing the apoptosis process in cancer and normal tissue (based upon microarray gene expression data taken from [28]). As shown in Fig. 2, the DFF40 protein, which starts the process of DNA decomposition, is considerably suppressed in cancer cells, relative to non-cancerous cells. The very broad range of transition probabilities for releasing DFF40 under the influence of effector caspases (CASP3 and CASP7) is consistent with our model.

The strategy for fighting cancer by helping the immune system to restore its response by blocking apoptosis in the immune system T-cells was simulated by varying the potency of FLIP and IAP inhibitors. This was modeled by varying the transition probability for each inhibitor, with an increase in the probability value correlating to higher inhibitor potency. As depicted in Fig. 3, when the two inhibitors were used jointly, the resulting concentration of DFF40 was considerably lower than the one expected from a summing up of the individual effects of each inhibitor. Thus, our model predicts a considerable synergy in the joint action of the two inhibitors.

The classical strategy to fight cancer by inducing apoptosis in cancerous cells was simulated by maximizing the DFF40 expression level. This was achieved by modulation of the FLIP and IAP inhibitors of apoptotic process performed by varying the transitional probability for their suppressors siRNA and SMAC (Fig. 1). The benefits of simultaneous suppression of the two apoptosis inhibitors, FLIP and IAP, by siRNA and SMAC, respectively, are demonstrated in Fig. 4 with the almost complete release of DFF40 resulting from the synergy of the two models of apoptosis modulation. Although FLIP is a stronger inhibitor than IAP by acting on the first part of the

![Fig. 2. The strong suppression of apoptosis in cancer cells as compared to normal cells (expression data for the FASL-induced apoptosis from [28]) is reproduced by the CA simulation within a very broad range of values of the transitional probability for releasing the DNA 'killer' DFF40.](image)
signaling cascade, its silencing cannot enable full-scale apoptosis. Only the joint suppression of both inhibitors was able to kill target cancer cell through apoptosis.

**Fig. 3.** Synergistic effect during joint suppression of the apoptotic process by the FLIP and IAP inhibitors. This finding might be of interest in developing clinical treatments preventing the killing of the immune system T-cells by cancer cells.

**Fig. 4.** Simulating the effect of FLIP and IAP inhibitor suppression by siRNA and SMAC, respectively. The values of the DFF40 steady-state concentration after 25000 model iterations enable prediction that a full-scale FASL-induced apoptosis is achievable only via joint synergistic suppression of FLIP and IAP inhibitors.

signaling cascade, its silencing cannot enable full-scale apoptosis. Only the joint suppression of both inhibitors was able to kill target cancer cell through apoptosis.
Building upon these simulation results, a more complete model of apoptosis was created by integrating the FAS-ligand-induced apoptosis pathways with the endogenous mitochondria-activated apoptosis pathways. Cells undergoing apoptosis by FASL mediated pathways are called type I, while those committing suicide by the mitochondria-mediated cascade are classified as type II [14][19]. Apoptosis of type-I cells requires high expression of caspase 8, while apoptosis of type-II cells proceeds at low levels of caspase 8. In the case of type-II cells, caspase 8 activates the Bid protein, which in turn activates the mitochondria-mediated apoptosis pathway (Fig. 5). CASP8 thus acts as a switch between type-I and type-II cells. The mode of action for the mitochondria-mediated apoptosis pathway is that mitochondria releases cytochrome C into the cytoplasm activating caspase 9, which then closes the apoptosis chain by activating caspase 3. In addition, a feedback loop from caspase 3 to caspase 9 to IAP has been hypothesized [29–31] to deactivate IAP.

As shown in Fig. 6, adding the feedback loop CASP3 → CASP9 → IAP to the mitochondria-mediated apoptosis pathway did not produce major changes in the concentration of DFF40. DFF40 was still present at sufficiently high levels for a cell to undergo apoptosis. Our model predicts that the real benefit of this feedback is that the overall signaling process speeds up the process from 7300 to 5600 iterations, due to enhanced suppression of inhibitor IAP and extra activation of CASP9.
Fig. 6 presents the chance to compare the dynamics of the intrinsic mitochondria-mediated apoptosis pathway (with a feed-back and without it) to that of the extracellular FASL-induced apoptosis. Our simulation shows that FASL is 32% faster than mitochondrial feed-forward and 12% faster than mitochondrial feed-forward + feed-back (the number of iterations for the FASL apoptosis is $5012 \pm 11.7$ vs. $5596 \pm 11.1$ vs. $7368 \pm 13.0$ for the mitochondrial apoptosis with and without feedback, resp.).

This relates apoptosis in type-I and type-II cells in the following manner. Depending on the urgency to carry out apoptosis, the cell may control the expression of caspase 8 and use low levels of this protein to activate the mitochondria-mediated pathway and high levels to activate the FASL pathway. In the event of a failure of the mitochondria-mediated apoptosis pathway due to damage, the cell could, for compensation, overexpress caspase 8 in response to a FASL stimulus. On the other hand, failures in the FASL apoptosis pathway (such as no DISC formation or mutated membrane bound FAS, etc.) could be compensated by switching on the mitochondrial pathway with feedback, which is only 12% slower at about the same DFF40 concentration. Thus, there is built-in biological redundancy to activate apoptosis where the different pathways have a similar (but not identical) signal transduction speed controlled by CASP8. This indicates the need of further modeling studies on controlling caspase 8 as toggle switch between intrinsic and extrinsic pathways.

**Experimental Part**

*General.* The term cellular automata (CA) describes dynamic systems discretely in space, time, and state. The overall system behavior is specified by rules governing local relationships. In the most common
2D version, CA models are constructed on a grid of squares called cells. The grid size may vary considerably, depending on the system. To eliminate any boundary effects, the grid is usually built on the surface of a torus. We followed the CA rules employed in biochemical enzymatic reactions [32][33] and pathways [34–36]. At each time step, each cell has a single state: empty or occupied. The cell may be occupied by an enzyme, a substrate, a product, a substrate/enzyme complex, or a product/enzyme complex. The state of a cell at a given time step depends only on its own state and the cell states in its neighborhood, all taken at the previous step. Each cell has four sites (von Neumann neighborhood), on which interactions can be simulated. The contents of a cell may break away from an occupied cell or move to join a cell that is occupied. The probabilities for moving, \( P_M \), joining, \( P_J(X) \) and \( P_J(Y) \), and breaking away, \( P_B(X) \) and \( P_B(Y) \), are \( P_M = P_J = P_B = 1 \). This means that all cells may move, join, and break apart with equal probability. The overall probability of a movement, \( P_M = 1 \), is divided into probabilities for movements onto \( k \) grid directions, where \( k = 1–4 \) is the number of unoccupied neighboring cells in the 2D-square lattice.

The only joining of two cells that has a consequence is that occurring between the specific substrate \( S(i) \) and the specific enzyme \( E(i) \). When such an encounter occurs, a substrate–enzyme complex \( SE(i) \) is formed. This complex has an assigned transitional probability, \( P(T) = 0.5 \), of changing to a new product–enzyme complex \( PE(i) \).

\[
S(i) + E(i) \xrightarrow{P(T)} SE(i) \rightarrow PE(i) \rightarrow P(i) + E(i)
\]

In such reactions, the transitional probability \( P(T) \) is regarded as a measure of enzyme activity or propensity, and may be varied within the entire range of values between 0 and 1. In this study, \( P(T) \) was varied within the entire 0 to 1 range, and, if needed, to keep it constant to optimize some other parameter, it was assumed equal to 0.5.

The rules, applied at random to all cells, represent one iteration of the modeling procedure, which determines the cells’ new states and trajectories. A cell and its four-cell environment can acquire any of the five states defined above. The initial state of the system is random and thus does not determine subsequent configurations at any iteration. After many iterations, the system reaches a relatively constant configuration (analogous to a chemical steady state), characterized by counts of cells. The model obtained by CA is statistical; many runs are performed, and the number of iterations needed to attain a steady state is averaged, and the standard deviation is determined.

The Set of Apoptosis Interactions. The following system of equations was used to simulate FASL-induced apoptosis as given in Figs. 1 and 5 (letter A is used as a prefix of a protein name to denote the protein active form):

**Attachment of Ligand**

\[ FAS + FASL \rightarrow AFAS + FASL \]

**Recruitment of DISC**

\[ AFAS + AFAS \rightarrow FASFAS \]
\[ FASFAS + FADD \rightarrow FASFADD \]

**Formation of DISC Complex**

\[ FASFADD + CASP8 \rightarrow FASFADDACASP8 \]
\[ FASFADD + CASP10 \rightarrow FASFADDACASP10 \]

**Activation of Caspsases**

\[ FASFADDACASP8 + CASP10 \rightarrow FASFADDACASP10 + ACASP8 \]
\[ FASFADDACASP8 + CASP3 \rightarrow ACASP3 \]
\[ FASFADDACASP8 + CASP6 \rightarrow ACASP6 \]
\[ FASFADDACASP8 + CASP7 \rightarrow ACASP7 \]
\[ FASFADDACASP10 + CASP3 \rightarrow ACASP3 \]
\[ FASFADDACASP10 + CASP6 \rightarrow ACASP6 \]
\[ FASFADDACASP10 + CASP7 \rightarrow ACASP7 \]
\[ ACASP3 + CASP6 \rightarrow ACASP6 \]
\[ ACASP3 + CASP7 \rightarrow ACASP7 \]
\[ ACASP8 + CASP3 \rightarrow ACASP3 \]
\[ ACASP8 + CASP6 \rightarrow ACASP6 \]
\[ ACASP8 + CASP7 \rightarrow ACASP7 \]
\[ ACASP10 + CASP3 \rightarrow ACASP3 \]
\[ ACASP10 + CASP6 \rightarrow ACASP6 \]
\[ ACASP10 + CASP7 \rightarrow ACASP7 \]
ACASP3 + CASP7 → ACASP7 + ACASP3
ACASP7 + CASP6 → ACASP6 + ACASP7

Activation of Apoptosis
DFF45DFF40 + ACASP3 → DFF45ACASP3 + DFF40
DFF45DFF45 + ACASP7 → DFF45ACASP7 + DFF40

Inhibition of Apoptosis
FLIP + FASFADDACASP8 → FLIPFASFADDACASP8
FLIP + FASFADDACASP10 → FLIPFASFADDACASP10
AIAP + ACASP3 → 1APCAS3
AIAP + ACASP6 → 1APCAS6
AIAP + ACASP7 → 1APCAS7

Suppression of Inhibitors
siRNA + FLIP → siRNAFLIP
SMAC + IAP → IAPSMAC

Mitochondrial Apoptosis Pathway
ACASP8 + Bid → ABID + ACASP8
ABID + Mit → cytC + AMIT
ABID + Mit → SMAC + AMIT
cytC + CASP9 → ACASP9 + cytC
ACASP9 + CASP3 → ACASP3 + ACASP9
ACASP3 + CASP9 → ACASP9 + ACASP3
ACASP9 + AIAP → ACASP9 + IAP

Parameters Used. We used the expression profiles of the apoptotic proteins [28] as a basis for input concentrations (number of CA cells): FASL, 50; FAS, 200; FADD, 125; FLIP, 125; IAP, 300; DFF, 500; CASP3, 150; CASP6, 40; CASP7, 400; CASP8, 750; CASP9, 375; CASP10, 50; Mit, 1000; Bid, 75; SMAC, 400; siRNA, 200. (The DFF and siRNA expression levels were not found in the literature, and the values presented here were assumed within the same range with the measured expression data.) The initial concentrations of the products of the above equations were assumed zero.

The modeling was performed by 100 runs for each specific case considered. The number of iterations and the cell count in each run was recorded after reaching a steady(or near-steady)-state configuration, and averaged over all runs. In the rare cases when the attainment of steady state was computationally highly demanding, we stopped the iterative process at a sufficiently large number of iterations (25,000). We used a variable-size square grid of minimum 100×100 cells embedded on a torus. The variations in the size were related to the condition of keeping comparable cell density of 60%. The basic parameter varied was that of the transitional probability $P(T)$ of apoptosis elementary steps. The preliminary parameter testing has shown that selecting $P(T) = 0.5$ for almost all pathway steps, except few which were varied within the 0 to 1 range, provides stable patterns of the pathway modeled.

Variations of Essential Probabilities. DISC consists of lattice of FAS dimer and FADD, to which CASP8 or CASP10 or FLIP can be attached. To simulate a stable and functional DISC lattice, we varied the breaking (br) and joining (j) probabilities for FAS-FAS and FAS-FADD interactions, as shown in the Table. A dependence between the joining and breaking probabilities of FAS and FADD was assumed as a condition for the formation of a stable lattice and approximately defined as $P_B(2FAS-FADD)\approx 1- P_B(FAS-FAS)$. We then selected the probability values $P_B = 0.3$ for FAS dimer dissociation, $P_T=0.8$ for joining FAS and FADD and $P_T=0.4$ for FAS-FADD bond-formation as these probabilities enabled the fastest formation of a DISC lattice to make the model consistent with existing evidence. The stability of the DISC lattice was further ensured by assuming a low probability of breaking, $P_B(2FAS-FADD) = 0.1$. This simulated the transient nature of DISC, allowing it to dissociate weakly rather than making it a permanent structure with $P_B = 0$.

Different transition probabilities of inhibition ranging from 0.05 to 0.9 were tested to simulate the effects of progression from weaker inhibition to a stronger one. A linear trend was observed with the
DFF40 concentration showing inverse relationship to the strength of inhibition. Hence, $P_T = 0.5$ was used for all inhibitory effects in order to optimize computational time.

### REFERENCES


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**Table. Variation of Probabilities Relevant to the Formation of the DISC Apoptotic Complex**

<table>
<thead>
<tr>
<th>$P_B$ (FAS-FAS)</th>
<th>$P_J$ (2FAS-FADD)</th>
<th>$P_T$ (2FAS-FADD)</th>
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<th>STD DEV</th>
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*) Optimized values given in bold.

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