Cell death and life in cancer: mathematical modeling of cell fate decisions

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Abstract Tumor development is characterized by a compromised balance between cell life and death decision mechanisms, which are tightly regulated in normal cells. Understanding this process provides insights for developing new treatments for fighting with cancer. We present a study of a mathematical model describing cellular choice between survival and two alternative cell death modalities: apoptosis and necrosis. The model is implemented in discrete modeling formalism and allows to predict probabilities of having a particular cellular phenotype in response to engagement of cell death receptors. Using an original parameter sensitivity analysis developed for discrete dynamic systems, we determine variables that appear to be critical in the cellular fate decision and discuss how they are exploited by existing cancer therapies.

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1 Introduction

Evading various programmed cell death modalities is considered as one of the major hallmarks of cancer cells [1]. A better understanding of the pro-death or prosurvival roles of the genes associated with various cancers, and their interactions with other pathways would set a ground for re-establishing a lost death phenotype and identifying potential drug targets.

Recent progress in studying the mechanisms of cell life/death decisions revealed its astounding complexity. Among many, one can mention three difficulties on the way to characterize, describe and create strict mathematical descriptions of these mechanisms.

First, the signaling network allowing a cell to react to an external stress (such as damage of DNA, nutrient and oxygen deprivation, toxic environment) is assembled from highly redundant pathways which are able to compensate each other in one way or another. For example, there exist at least seven distinct and parallel survival pathways associated with action of AKT protein [2]. Disruption of one of these pathways in a potential cell death-inducing cancer therapy can be in principle compensated by the others. Thus, understanding and modeling the survival response in its full complexity is a daunting task.

Second, cellular death is an extremely complex phenotype that cannot merely be described as a simple disaggregation of cellular components driven by purely thermodynamical laws. Several distinct modes of cell death were identified in the last decade [3], such as necrosis, apoptosis and autophagy. Importantly, all these cell death modalities are controlled by cellular biochemical mechanisms, activated in response to diverse types of stress: roughly speaking, a cell is usually pre-programmed to die in a certain manner, sending appropriate signals to its surroundings so as to limit tissue toxicity and allow recycling of its components. Necrosis is a type of cell death usually associated with a lack of important cellular resource such as ATP, which makes functioning of many biochemical pathways impossible. This is why it was long thought of as an uncontrolled and purely thermodynamics-driven degradation of cellular structures. However, recent research showed that necrosis can be triggered by specific signals through the activation of tightly regulated pathways, and can even proceed without ATP depletion [3]. By contrast, apoptosis as a form of cellular suicide was, from the very beginning, described as a mode of cell death requiring energy for the permeabilization of mitochondrial membranes and cleavage of intracellular structures. Autophagy remains a relatively poorly understood cell death mechanism, which seems to serve both as a survival or a death modality. Upon certain stress conditions, and until this stress is relieved, cellular components such as damaged proteins or organelles are digested and recycled into reusable metabolites, and metabolism is reoriented so as to spare vital functions. Long lasting, non-relievable stress was described as triggering autophagic cell death, through unaffordable cellular self-digestion. However, no experimental evidence ever unambiguously demonstrated that such cell death is directly executed by autophagy in vivo, but in the special case of the involution of Drosophila melanogaster salivary glands [3].
The third difficulty can be attributed not directly to the complexity of the biochemical mechanisms but rather to our capabilities of apprehending the design principles used by biological evolution. Inspired by engineering practices, we tend to investigate complex systems by splitting them into relatively independent modules and associating well-characterized non-overlapping functions to each molecular detail. Applying such reductionist approaches to biology comes with a caveat. Most cellular molecular machineries cannot be naturally dissected or associated with well-defined functions, and sets of overlapping functions can be distributed among groups of molecular players.

Not having the ambition to deal with the whole complexity of cell fate decisions in vivo, we decided to concentrate on modeling the outcome of a classical and rather well-defined experiment of inducing cell death: adding to a cell culture specific ligands (Tumor Necrosis Factor, TNF, or other members of its family such as FASL). These so-called death ligands can engage death receptors and trigger apoptosis or necrosis, or activate pro-survival mechanisms [5]. The net outcome of such experiments depends on many circumstances: cell type, dose of the ligand, duration of the treatment, specific mutations in cell genomes, etc. Moreover, it is believed that the outcome can have intrinsic stochastic nature governed by cellular decision making mechanisms and intrinsic molecular noise [6]. Trying to characterize the biochemical response of a cell to this relatively simple kind of perturbation allows to understand certain cell fate decision mechanisms.

In this paper, we briefly describe and carefully analyze a mathematical model of cell fate decision between survival and two alternative modes of cell death: apoptosis and necrosis. The model was created and introduced in [4]. Here propose the principles for wiring and parametrizing a biological diagram that describes this cellular switch. In addition to [4], here, by applying a novel sensitivity analysis specifically developed for discrete modeling, we identify fragile sites of the cell fate decision mechanism. In conclusion, we compare our analysis with our current knowledge of cellular decision making fragilities utilized by cancer and cancer therapies.

2 Mathematical model of cell fate decision

In [4] we summarized the current knowledge on the interactions between cell fate decision mechanisms in a simplistic wiring diagram (see Fig. 1) where a node represents either a protein (TNF, FADD, FASL, TNFR, CASP8, cFLIP, BCL2, BAX, IKK, NFκB, CYT_C, SMAC, XIAP, CASP3), a state of protein (RIP1ub, RIP1K), a small molecule (ROS, ATP), a molecular complex (Apoptosome, C2_TNF, DISC_FAS), a group of molecular entities sharing the same function (BAX can thus represent either of BAX and BAK, cIAP either cIAP1 or cIAP2, and BCL2 any of the BH1-4 BCL2 family members,), a molecular process (Mitochondria permeabilization transition, MPT, Mitochondrial outer membrane permeabilization, MOMP) or a phenotype (Survival, Apoptosis, Non-apoptotic cell death, NonACD). Each directed and
signed edge represents an influence of one molecular entity on another, either positive (arrowed edge) or negative (headed edge).

The phenotype nodes on the diagram are simple interpretations of the following molecular conditions: 1) activated NFκB is read as survival state; 2) lack of ATP is read as nonapoptotic cell death state; 3) activated CASP3 is read as apoptotic cell death. Absence of any of such conditions is interpreted as a "naive" cell state, corresponding to the fourth cellular phenotype.

After extensive examination of the biological literature we converted the diagram into a logical mathematical model of cell fate decisions triggered by activation of cell death receptors. The wiring diagram and the logical rules defining the model are shown on Fig. 1.

By applying a technique adapted to discrete formalism [7], we reduced this model to a 11-dimensional network, thus enabling a complete analysis of the asynchronous dynamics (see [4] for details). This analysis identified 27 stable logical states and no cyclic attractors. Moreover, it showed that the distribution of the stable logical states in the discrete 22-dimensional space of internal model variables (without considering input and output variables) forms four compact clusters, each corresponding to a particular cellular phenotype. Three of these clusters can be attributed to a particular cell fate (survival, apoptosis, necrosis) while the forth represents a “naive” survival state, where no death receptors are induced.

3 Computing phenotype probabilities

As we have already mentioned, the cellular fate decision machinery is characterized by stochastic response, i.e. given a stimuli, the cell can reach several final states, corresponding to different phenotypes, with different probabilities. The role of mathematical modeling in this case can be to predict these probabilities as absolute values that can be matched to an experiment, or at least to predict the relative changes of the probabilities after introducing some perturbations to the system.

We have implemented this idea for the mathematical model of cell fate decisions described above in the following manner.

In order to describe our results, let us introduce the notion of asynchronous state transition graph. On this graph, each node represents a state of the system which in this case can be encoded by a n-dimensional vector of 0s and 1s (n being the dimension of the system). A directed edge exists between two states x and y if there exists an index $i \in \{1, \ldots, n\}$ such that $y_i = f_i(x) \neq x_i$ and $y_j = x_j$ for $j \neq i$ (here, $f_i$ denotes the logical rule of variable $x_i$, see Fig. 1 for a complete list of the model logical rules). In principle, the state transition graph could be defined independently and without the biological diagram, however, this would require a tremendous amount of empirical knowledge about the set of all permissible transitions between the cell states which is not available. Hence, the biological diagram with associated logical rules is used as a compact representation and a tool to generate the state transition graph. Detailed instructions on this procedure can be found in [8, 9].
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Fig. 1 Biological diagram of molecular interactions involved in cell fate decisions derived from the biological literature. The diagram is roughly divided by dashed lines into three modules corresponding to three submechanisms of cell fate decisions. Notations: 1) Proteins: TNF, FADD, FASL, TNFR, CASP8, cIAP, cFLIP, BCL2, BAX, IKK, NFκB, CYT_C, SMAC, XIAP, CASP3; 2) States of proteins: RIP1ub (ubiquitinated form of RIP1), RIP1K (kinase function of RIP1); 3) Small molecules: ATP, ROS (Reactive oxygen species); 4) Molecular complexes: Apoptosome, C2\_TNF, DISC\_FAS; 5) Molecular processes: MPT (Mitochondria permeabilization transition), MOMP (Mitochondrial outer membrane permeabilization); 6) Phenotypes: Survival, Apoptosis, NonACD (Non-apoptotic cell death). Below the table of logical rules defining the discrete mathematical model is provided.
The set of all possible states provides a discrete phase space of the system. The state transition graph contains all possible ways of the systems dynamics (trajectories). In other words, it is the multidimensional epigenetic landscape of the cell fate decision system. Note that the state transition graph is assumed to be rather sparse compared to the fully connected graph where any two state transitions would be possible. Hence, on this landscape, one can determine bifurcating states, points of no return, etc.

The state transition graph allows to address the following question: Starting from a distinguished state of a cell, what is the probability to arrive to each of the stable states? In biological terms: Which proportions of a population of resting cells exposed to death ligand will eventually display each of the different phenotypes - cell fate?

To answer the question, we converted the state transition graph into a Markov process of random walk on a graph, following the method described in [9]. To do that, we associated to each transition between two states a probability (called transition probability). By applying classical algorithms to the transition probability matrix (strongly connected decomposition and topological sort), we obtained an absorbing discrete Markov chain, and then analyzed it with classical techniques [10].

One of the critical points in such type of analysis lies in the choice of the transition probabilities. Once again, defining these probabilities directly from some empirical observations is impossible at present time. Hence, these probabilities should be derived from the logical model with the use of some additional assumptions.

The simplest assumption is to consider all transitions firing from a given state as equiprobable. Biological interpretation of such an assumption is not simple. In a way, we consider a “generic” cell in which all possible system trajectories take place with equal probabilities (without dominance, i.e. any preferable route). One can argue that in any particular concrete cell, this would not be true anymore and that the generic cell is not representative of anything real observed in any biological experiment. Having in mind this difficulty, we avoid direct interpretation of absolute values of probabilities, concentrating rather on relative changes of them in response to some system modifications such as removing a node or fixing a node’s activity. It happens that such a “generic” cell model is already capable of reproducing a number of known experimental facts.

When the state transition graph is parametrized by transition probabilities, one can use standard techniques to compute the probability of hitting a given stable state, considering that a random walk starts from a given initial state. Then this probability is associated with a probability of observing a particular phenotype in given experimental conditions. For doing this, it is convenient to define a unique initial state, which we choose to represent the “physiological state”, the one representing un-induced cells growing in a plate. In the model of Fig. [1] it is the state in which all elements are inactive except ATP, FADD and cIAP. This is a stable state, which looses its stability when TNF variable is changed from 0 to 1 and the dynamical system starts to evolve in time.

Using this approach, we performed a series of in silico experiments in which the probability of arriving to stable states was computed for the initial (“wild-type”)
model, or for a series of modified ("mutant") model. Typical model modifications consisted in fixing some nodes' activities to 0 or to 1. For our cell fate decision model, the results are provided in Fig. 2. In [4] this table was systematically compared with the experimental data of the cell death phenotype modifications observed in various mutant experimental systems, including cell cultures and mice. The model was able to qualitatively recapitulate all of them and to suggest some new yet unexplored experimentally mutant phenotypes. The most interesting in this setting would be to consider synthetic interactions between individual mutants, when several nodes on the diagram are affected by a mutation simultaneously.

Fig. 2 Changes in the phenotype probabilities from the random walk on the state transition graph, starting from the initial physiological state. Various "mutant" modifications of the dynamical system are tested here. Here "A" denotes Apoptosis, "N" denotes Necrosis and "S" denotes Survival, "0" denotes Naive state. "O.e." stands for overexpression of a protein, "antiox" corresponds to blunting the capacity of NFκB to prevent ROS formation, "z-VAD-fmk" simulates the effect of caspase inhibitor z-VAD-fmk.

4 Identification of fragile points of the cell fate decision machinery

Changing distribution of transition probabilities on the asynchronous state transition graph can drastically change the probabilistic outcome of a computational experi-
ment. At the same time, the probabilities for a random walk to converge to some attractor depend also on the structure of the state transition graph which is determined solely from the discrete model. In order to understand what are the critical determinants of a cellular choice, we applied a novel strategy of discrete model analysis consisting in parametrizing the state transition graph by changing relative importance of certain variables. In a certain sense, this strategy corresponds to a sensitivity analysis, commonly applied for continuous models based on ordinary differential equations and chemical kinetics approach [11].

First of all, we postulate that our “reference” parametrization corresponds to the equal probabilities of any possible transition from a state. As mentioned earlier, this corresponds to a “generic” cell model, where the relative speeds of all biochemical processes are assumed equal. Mathematically, considering the dynamics as a Markov process, all transitions from a given state $x$ to any of its asynchronous successor are assigned equal probabilities (if $x$ has $r$ successors, these probabilities are equal to $1/r$). We will modify this default parametrization by systematically changing relative speeds of certain elements. This will lead to some re-parametrization of the state transition graph and consequent changes in the probabilities to reach attractors.

The key idea of priority classes [12, 13] consists in grouping variables of a discrete model into classes according to the speeds of the underlying processes governing their turnover rates. For instance, in the case of genetic regulatory networks, a natural grouping consists in putting de novo protein synthesis (transcription + translation) in a slow transition class in comparison with other processes such as post-translational protein modifications (phosphorylation, ubiquitination, ...) or complex formation. Following this idea, we can regroup nodes into priority classes to which some priority ratios $w$ are assigned. Said differently, each variable $x_i$ is assigned a priority value $w_i$. For a given node, a value $w_i > 1$ corresponds to a higher than default priority, and a value $w_i < 1$ to a lower than default priority. The ratio $w_i$ can be interpreted as a global turnover rate of the component represented by this node: those that are produced (activated) and degraded (deactivated) fast will have a large $w_i$.

Consider a state $x$, with $r$ asynchronous successors. By definition, between $x$ and each of its successors, one and only one variable can be updated. Let $y$ denote one of the successors of $x$, and $i$ be the index of the corresponding updated variable. With the uniform assumption described before, the probability of the transition ($x \rightarrow y$) is independent of $i$ and is equal to $1/r$. With priority classes, this probability is now weighted by $w_i$, making the transition more probable if component $i$ belongs to a “fast” class ($w_i$ greater than one) and less probable if it belongs to a “slow” class ($w_i$ less than one). Obviously, for computing the actual transition probabilities $p_{x \rightarrow y}$, a normalization should be applied so that:

$$\sum_{y \text{ succ. of } x} p_{x \rightarrow y} = 1.$$
Once the new values of the transition probabilities have been computed, the same treatments as before can be applied, leading to new values for the probabilities to reach the different phenotypes, starting from a given initial condition.

This general method may be applied in two different ways. First, one may use it to compute more realistic probabilities, that could be compared to actual experimental results (the probability to reach an attractor being compared with the proportion of cells exhibiting the corresponding phenotype). However, such calculations would need a complete classification of the relative speeds of all biochemical mechanisms involved in the model. Given the number and heterogeneity of these mechanisms, it is still difficult to obtain such classification. Instead, we used the method as a sensitivity analysis tool, in order to detect which variables are more critical than others in the decision-making process. Using the reduced model evoked earlier (see [4]), we considered each variable independently, and successively boosted it or slowed it down by some multiplicative factor. More precisely, to detect the sensitivity of the network with respect to the turnover of variable $x_i$, we performed the calculations for different values of $w_i$, the other weights $w_j$ being kept at one (the reference value).

By comparing the probabilities to reach the three phenotypes - survival, apoptosis and necrosis - with those of the initial model, one can detect whether the system’s response is sensitive or not to the turnover rate of variable $x_i$. We performed such experiments for the nine inner variables of the reduced model. Figure 3 presents the results we obtained.

![Fig. 3](image)

**Fig. 3.** Testing the effect of varying node turnovers on the resulting phenotypic probabilities. The absciss on the graphs shows the value of $w$ priority value, where $w = 1$ corresponds to the probabilities computed for the default wild-type model (see Fig. 2). The colors are those adopted in [4]: orange corresponds to apoptosis, purple to necrosis and green to survival.

The plots reveal several interesting properties. First, the most sensitive components, which correspond to the curves with the highest amplitude, are RIP1, NFkB
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and CASP8. This reinforces the idea that these three components play a crucial role in the decision process. This seems reasonable, especially for RIP1 and CASP8, as they occupy an upstream position in the regulatory graph. Interestingly, CASP3 turnover does not seem to be so important, although CASP3 is a marker of apoptosis. This confirms that even though CASP3 is essential for the existence of apoptosis in the model (its removal completely suppress apoptotic outcome, see Fig. [4]), its turnover rate does not appear to be important in the dynamics of the decision process (once it goes from 0 to 1, most of the decision has already been made). Remarkably, the turnovers of MOMP and MPT, both contributing to the permeabilization of mitochondrial membrane, have different effects: MOMP seems to affect mainly the decision between survival and necrosis, while MPT plays a role in the switch between apoptosis and necrosis.

The sensitivity analysis that is presented here is an extension of the results proposed in [4]. In contrast with the all-or-none perturbations evoked in the previous part (where a node is fixed to 0 or 1), here we consider finer perturbations by modifying the turnover rates of the model’s variables. A next step would be to consider the relative strengths of the model’s interactions, instead of the model’s variables. Such an approach is currently investigated.

5 Comparison with the fragilities exploited by cancer and its treatment

Deregulations of the signalling pathways studied here can lead to drastic and serious consequences. Hanahan and Weinberg proposed that escape of apoptosis, together with other alterations of cellular physiology, represents a necessary event in cancer promotion and progression [1]. As a result, somatic mutations leading to impaired apoptosis are expected to be associated with cancer. In the cell fate model presented here, most nodes can be classified as pro-apoptotic or anti-apoptotic according to the results of “mutant” model simulations, which are correlated with experimental results found in the literature. Genes classified as pro-apoptotic in our model include caspases-8 and -3, APAF1 as part of the apoptosome complex, cytochrome c (Cyt_c), BAX, and SMAC. Anti-apoptotic genes encompass BCL2, cIAP1/2, XIAP, cFLIP, and different genes involved in the NFkB pathway, including NFKB1, RELA, IKBKG and IKBKB (not explicit in the model). Genetic alterations leading to loss of activity of pro-apoptotic genes or to increased activity of anti-apoptotic genes have been associated with various cancers. Thus, we can cross-list the alterations of these genes deduced from the model with what is reported in the literature and verify their role and implications in cancer.

For instance, concerning pro-apoptotic genes, frameshift mutations in the ORF of the BAX gene are reported in > 50% of colorectal tumours of the micro-satellite mutator phenotype [14]. Expression of CASP8 is reduced in ≈24% of tumours from patients with Ewing’s sarcoma [15]. Caspase-8 was suggested in several studies to
function as a tumour suppressor in neuroblastomas [16] and in lung cancer [17] (see Fig. 4).

On the other hand, constitutive activation of anti-apoptotic genes is often observed in cancer cells. The most striking example is the over-expression of the BCL2 oncogene in almost all follicular lymphomas, which can result from a t(14;18) translocation that positions BCL2 in close proximity to enhancer elements of the immunoglobulin heavy-chain locus [18]. As for the survival pathway, elevated NFκB activity, resulting from different genetic alterations or expression of the v-rel viral NFκB isoform, is detected in multiple cancers, including lymphomas and breast cancers [19]. An amplification of the genomic region 11q22 that spans over the cIAP1 and cIAP2 genes is associated with lung cancers [20], cervical cancer resistance to radiotherapy [21], and oesophageal squamous cell carcinomas [22] (see Fig. 4).

Some of the components of the cell fate decision machinery are considered currently for the use in cancer treatment in pre-clinical or clinical trials. To give some examples, SMAC mimetics directly target dysregulated, neoplastic cells that over-express IAPs or under-express SMAC [23]. BCL-2 inhibitors, most notably BAX mimetics, are currently passing clinical trials (for example, see [24]).

In our sensitivity analysis, the variables NFκB and CASP8 appear among the most "vulnerable" components of the cell fate decision machinery, which could explain why the gene products they represent are fragile points used by cancer.

BCL-2 does not show up as a much sensitive node in the model. However, its direct target, MPT is a fragile site, accordingly to our analysis. Also analysis of our model shows that RIP1 is a powerful and sensitive switch able to reverse phenotype probabilities. Until so far we are not aware about possible targeting of RIP1 functions in cancer treatment, which can be explained by still relatively poor characterization of its substrates and difficulties connected with targeting specific RIP1 activities.

6 Conclusion

Mathematical models provide a way to test biological hypotheses in silico. They recapitulate consistent heterogeneous published results and assemble disseminated information into a coherent picture using an appropriate mathematical formalism (discrete, continuous, stochastic, hybrid, etc.), depending on the questions and the available data. Then, modeling consists of constantly challenging the obtained model with available published data or experimental results (mutants or drug treatments, in our case). After several refinement rounds, a model becomes particularly useful when it can provide counter-intuitive insights or suggest novel promising experiments.

Here, we have conceived a mathematical model of cell fate decision, based on a logical formalization of well-characterized molecular interactions. Former mathematical models only considered two cellular fates, apoptosis and cell survival [25].
In contrast, we include a non-apoptotic modality of cell death, mainly necrosis, involving RIP1, ROS and mitochondria functions.

By analyzing properties of the state asynchronous transition graphs associated with the discrete model, we implemented a procedure to simulate the process of stochastic cellular decision making in response to activation of death receptors. These simulations were able to predict relative changes for probabilities of cellular phenotypes in response to some system perturbations such as a knock-out of a gene or treatment with a drug. These predictions happened to be fully compatible with published data from mouse experiments, and provided new predictions to be tested.

Moreover, on this model we have tested a novel strategy of discrete model analysis, consisting in finding fragile or most sensitive places of the cell fate decision machinery. Changing the cellular parameters determining choices made at these fragile sites affect the probabilities for a cell to reach a particular cellular phenotype. We found out that this type of analysis can explain some of the common fragilities associated with tumorigenesis and also with currently employed cancer treatment strategies.

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