A Boolean network model for invasive thyroid carcinoma

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Abstract

Thyroid cancer is a common endocrine system neoplasm characterized by being extremely heterogeneous and of unexplained incidence (idiopathic). Some subtypes of thyroid cancer are more aggressive than others and for this reason treatment needs to be differential. Nonetheless, due to its inherent variability, prognosis based on pathology and/or biochemical profiling often fails leading to a delay in proper therapeutics that increases significantly the associated mortality. The most aggressive thyroid tumors are characterized by an increase in the destruction of extracellular matrix, this is done by the matrix metalloproteinases (MMPs). The regulation of MMPs is finely tuned by several molecules, but the dynamical mechanisms which control this pathway are still unknown. Here, based on detailed molecular interaction information coming from functional tests and gene expression experiments, we develop a boolean model of the matrix metalloproteinases pathway in thyroid cancer. By observing steady state conditions perturbing the network by simulating a specific drug, we find that TNFA could be a major target of this pathway. The approach performed here could allow to understand the finely regulated process to maintain extracellular matrix homeostasis.

Introduction

Thyroid carcinomas (TCs) are the most common endocrine-related cancers. In recent years, the incidence of TCs has drastically increased, at the same time mortality rates remain largely unchanged (Davies and Welch, 2006; Veronese et al., 2015). Sub-optimal diagnostics have lead to thousands of TC-related deaths annually, in particular for the case of poorly-differentiated, anaplastic and medullary cancers whose etiology remains to be fully disclosed (Giuffrida and Gharibi, 2000; Hernández-Lemus and Mejía, 2012). A number of environmental and nutritional factors have been statistically associated to TCs. It is believed that any process leading to compensatory increases in the hormone thyrotropin will increase the risk of thyroid tumors. Apart from this, an elevated risk has been documented for women who use estrogen for gynecological reasons, especially those in pre-menopause stages (Ron et al., 1987; Hima and Sreeja, 2015).

One of the main challenges to thyroid neoplasms diagnostics and therapeutics is the enormous variability that the tumors present both at the cellular morphology and at the gene expression levels (Espinal-Enríquez et al., 2015). Tumor heterogeneity generates two main classes of problems for clinicians: first of all the determination of the specific type of tumor since fine-needle aspiration cytology is conclusive in only around 20% of the cases. Secondly, determination of the most aggressive tumor subtypes (usually anaplastic and some papillary) to decide which patients are candidates for pharmacological therapy with close follow-up and which ones should undergo invasive and costly surgical procedures (Baudin and Schlumberger, 2007).

It is known that thyroid carcinomas present quite complex patterns of evolution (Espinal-Enríquez et al., 2015). The entangled dynamics of these tumors is shaped by a non-trivial interplay of genomic and epigenomic changes including not only mutations and gene expression changes but also effects of copy number variations, gene translocations, methylation deregulation and altered signaling pathways. A detailed analysis of all the afore-mentioned features seems unattainable, however a complex systems approach based on integrating most of this information into a simplified dynamic model may allow us to have a global but coarse-grained view of the phenomenology behind thyroid cancer evolution.

Histologically, thyroid carcinomas are classified into follicular adenoma (FA), follicular thyroid cancer (FTC), papillary thyroid cancer (PTC) and anaplastic thyroid cancer (ATC). FTC and PTC are differentiated tumors with low risk of recurrence and good prognosis. On the other hand, ATC is more aggressive, usually diagnosed at an advanced stage, therefore frequently leading to a fatal prognosis (Baudin and Schlumberger, 2007). A transition from FTC to PTC and PTC to ATC has been reported depending on the differential expression of molecules related to degradation of Extracellular matrix (Espinal-Enríquez et al., 2015).

A relevant feature present in the most aggressive thyroid carcinoma and also a well known hallmark of cancer (Hanahan and Weinberg, 2000, 2011) is the invasiveness and migra-
tion of tumor cells via degradation of extracellular matrix (ECM) (Gu et al. 2005; Li et al. 2006). The components of ECM, collagen, elastin and gelatin, are degraded by the matrix metalloproteinases (MMPs). The activity of MMPs are negatively controled by the tissue inhibitors of matrix metalloproteinases (TIMPs). So far, four TIMPs have been described and characterized: TIMP-1, TIMP-2, TIMP-3 and TIMP-4. The interplay between MMPs and TIMPS in normal conditions maintains the balance of extracellular matrix. However, in metastatic tumors, overexpression of MMPs falls into an exacerbated destruction of ECM and the consequent invasion of adjacent tissues (Jacomasso et al. 2014). MMPs are also negatively regulated by other molecules, such as A2M, TSP2, TFPi2 and RECK (Krady et al. 2008; Oh et al. 2001). MMPs have positive regulation by TGFβ (Kim et al. 2004), plasmin (Ramos-DeSimone et al. 1999) or furin (Remacle et al. 2006). At the same time, other molecules participating in the matrix metalloproteinases pathway are a1CT, a1PI, a2AP, which regulate the levels of plasmin; ADAM17, LRPI, and ELANE (Figure 1).

The dynamics of this pathway is still unknown. Understanding the temporal behavior of all those elements in this pathway becomes crucial to have a better image of the ECM maintenance in cancer. To achieve this, to construct a model way becomes crucial to have a better image of the ECM (Gu et al. 2005; Ii et al. 2006). The components of our findings and the usefulness of such dynamical approaches between the repeated configurations is the transient time and the number of it-

Steady state conditions

For Boolean networks (finite number of nodes which take a finite number of values), all initial conditions lead to a periodic behavior where the network configuration is replicated after a certain number of steps. This pattern of repeated network configurations is called an attractor. For the same network, several attractors may coexist. The total amount of initial conditions which reach a particular attractor is called the basin of attraction. The time required to reach this condition is known as the transient time and the number of iterations between the repeated configurations is the period of the attractor. These values reflect global properties of the network. In this case the steady states of the network allow to us to identify the temporal behavior of particular phenotypes, by observing the interplay between metalloproteinases and their regulators. Furthermore, the Boolean approach that we have implemented is also capable to determine the steady state conditions after elimination of one node (in silico knock outs). This has been done to observe the global properties of the perturbed network simulating the action of a directed drug, the ultimate goal of this model.
Figure 1: **The regulatory network for thyroid cancer invasiveness.** A) This biochemical pathway takes place in the plasma membrane as well as in the extracellular space. Here, the main molecules involved in the pathway are depicted. B) The graph consistent with the minimal network representing the invasive phenotype response in thyroid cancer.
Figure 2: Network evolution pattern. Columns represent the states in terms of molecule activity: Green = active, Black = inactive or absent. Rows represent discrete time steps. Time runs from above to below. As it can be observed, after a small set of iterations, the network reaches a steady state condition (black and green squares do not change anymore). This is the attractor for that particular initial condition (first row).

Results

A Boolean dynamic network model for thyroid network invasiveness

Gene regulatory networks have been used previously to characterize the complexity associated with thyroid cancer phenotypes, such as malignancy associated with mechanisms of cell death resistance (Hernández-Lemus and Mejía 2012). It has also been discussed the important role that extra-cellular matrix (ECM) maintenance and repair processes play in the development of invasive tumors which are the more aggressive forms of thyroid carcinomas (Espinal-Enríquez et al. 2015). Among such processes it was established that the regulation of ECM remodeling by the family of Matrix metalloproteinases (MMPs) and their inhibitors is of foremost importance. For this network, calculated over all initial conditions ($2^{24}$), the dynamics converges to 10 different attractors (Figure 3). Two out of the ten attractors are period three, meanwhile the other eight are punctual attractors. For clarity, figure 3 contains only 4 attractors, two of period 1 (3A and 3B), and two of period 3 (3C and 3D).

Figure 3: Dynamic landscape for different attractors.
This representation shows the basin of attraction (fan-like structures). By observing from the outside of the figure to the center, each point of the fan-like structure represent one configuration of the network, meanwhile the lines correspond to time steps of the network dynamics. Two dots are connected if one of them is a successor of the other under the dynamics. In this sense, the fan-like structures are a set of network configurations which converge to one dynamical network state. Eventually, all configurations converge to an attractor. For panels A and B, the attractor is a single-point attractor, whereas Panels C and D present three-state attractors. The colors of links are different for graphical purposes only.

Elimination of nodes remarks the relevance of TIMP regulation

As we mentioned before, the interplay between MMPs and their inhibitors TIMPs, is the most relevant feature to determine whether the ECM is compromised or not. To quantify this parameter, we construct an invasiveness score ($IS$) which consists on the ratio between MMPs and TIMPs state values once an attractor has been reached. We eliminate one node of the network for performing the dynamics and observe whether the $IS$ grows or decreases. The $IS$ after elimination of each node is presented in table 1. As it can be observed, the $IS$ changes depending on which element was eliminated. Elimination of the node corresponding to TIMP-1 presents the largest $IS$, meanwhile the elimination of TNFA present the less aggressive situation. This last result is not intuitive, however, the network dynamics shows this is the most important node to have less invasiveness. This could be relevant in the context of directed therapies targeting the nodes whose elimination cause a decreasing in the invasiveness.
Table 1: **Invassiveness score IS after elimination of one node in the network dynamics.** The value of the Wild type network IS (with all nodes present) is bold. An IS above the WT represent dynamics which are more invasive after elimination of that node, meanwhile lower IS means the deletion of the node decreases the invasiveness and concomitant destruction of the ECM. It is worth to mention that the less aggressive dynamics is observed by eliminating the tumor necrosis factor α, (TNFA).

<table>
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<tr>
<th>knock-out node</th>
<th>IS</th>
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<tr>
<td>TIMP-1</td>
<td>2.2</td>
</tr>
<tr>
<td>TIMP-4</td>
<td>2.2</td>
</tr>
<tr>
<td>a2m</td>
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<tr>
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<tr>
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<tr>
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<tr>
<td>ELANE</td>
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<tr>
<td>ADAM17</td>
<td>0.833333333</td>
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<td>MMP2</td>
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**Dynamical regime of the network exhibits criticality**

**Dynamical Regime** Discrete networks can operate in three different dynamical regimes: ordered, critical and chaotic. These regimes are characterized by how perturbations are propagated across the network and also by how these perturbations modifies or not the network dynamical state. In the ordered regime, the network is not sensitive to perturbations. In the chaotic regime, a small perturbation often generates a perturbation avalanche that grows in time. However, in the critical regime, small perturbations neither increase nor decrease in time. A more profound description of the dynamical regime of discrete networks can be found in Espinal et al. [2011].

To determine the dynamical regime in which this network is operating, it can computed the Derrida map $M(x)$. This mapping relates the size of the perturbation avalanche at two consecutive time steps: $x(t+1) = M(x(t))$. It is known that $S = \frac{dM(x)}{dx} \bigg|_{x=0}$, (the slope of this map at $x = 0$), determines the dynamical regime: ordered if $S < 1$, chaotic if $S > 1$ and critical if $S = 1$. [Espinal et al. 2011; Balleza et al. 2008; Derrida and Weisbuch 1986; Aldana and Cluzel 2003].

Quite remarkably, the Derrida map of the MMP network shows with high accuracy that this network operates in the critical regime. This is evident from Fig. 4, where it is shown that the slope at the origin is close to 1. Systems operating close to a critical point have remarkable properties that would be very difficult to understand in the absence of criticality. In particular, a property of regulatory networks operating close to criticality relevant to the present study, is the interplay between robustness and adaptability observed in this network. Maintenance of the homeostasis of ECM is a major issue in the cell, since the stability of the tissue structure is highly dependent of the status of the ECM. It is necessary to have the mechanisms to degrade ECM but, at the same time, with a strong and sophisticated mechanism for a correct negative feedback of them. The property of criticality remarks the fact that this network can evolve with those characteristics (robustness under perturbations and evolvability to change under certain conditions) with a high accuracy. Therefore, the critical dynamics revealed in figure 5 is indicative of an optimized mechanism of degradation and maintenance of extracellular matrix via the interplay of MMPs and TIMPs as well as their regulators.

**Perturbation of the network remarks loss of criticality**

As we mentioned in the previous section, in a Derrida plot, pairs of initial states are sampled at defined initial distances, $H(0)$, from the entire state space, and their mean Hamming distance, $H(t)$, after a fixed time, $t$, is plotted against the initial distance $H(0)$. For this case, $t = 1$. The curve above/below the line (slope), $H(1) = H(0)$, reflects instability/stability, respectively [Kauffman 1969].
To investigate the significance of each node in the network, we calculated the perturbation measure for the individual nodes, by modifying the Derrida plot. Perturbation calculations were performed between the normal network and each of the 24 mutated networks. A mutated network for a specific node contains forced (0) value for that specific node in the input and output states, therefore it contains $2^{n-1}$ dynamical states. For perturbation calculation of each individual node, we perform a modified Derrida plot by measuring the drop in the Derrida map. This modified Derrida plot highlights the effect of a directed drug whose target is a particular node in the network. The perturbation calculations were normalized with respect to the slope, $H(t) = H(0)$. In figure 5 it is shown the modified Derrida map according to Gupta et al. [2007] and Espinal et al. [2011] in which after elimination of one node, the Derrida map is plotted and shows the dynamical regime of the network under a perturbation. The most important nodes to achieve the critical regime are those shown in the figure. Loss of them cause a chaotic behavior. As it can be observed, the nodes which maintain the dynamical regime are mainly the TIMPs and MMPs. Elimination of them in the pathway cause a disregulation which is also observed under cancer phenotypes.

**Discussion**

Thyroid cancer is an important disease that involves several processes related to remodeling of extracellular matrix, which becomes in invasiveness and migration of tumor cells. The main mechanisms which govern the interplay between matrix metalloproteinases and TIMPs is not fully understood yet. In this work we constructed a Boolean network model of the MMPs pathway, we observed the steady state conditions for the wild type network as well as networks without one node, to simulate the action of a specific drug or a mutation in one of those elements. We also developed an invasiveness score $IS$, which reflects the ratio between action of TIMPs and MMPs once the steady state conditions have been reached. We observe the property of criticality in the WT network as well as the loss of this property after elimination of some particular nodes, mainly TIMPs and MMPs.

**Figure 4:** Critical dynamics for the MMPs signaling network. Plot of the Derrida map $M(x)$ which relates the size of the perturbation cascade at two consecutive time steps. The convergence of this mapping to a stationary value under successive iterations, determines the dynamical regime in which the network operates. This figure relates an initial separation $x(t)$ against separation $x(t + \delta(t))$, $0 = \delta$, averaged over all states which are initially separated by $x(t)$. The slope of the curve near the origin is practically 1 in a sizeable neighborhood of the origin, an indicative that this network operates in the critical regime.

Loss of criticality as a global property of the network may be indicative for a severe damage of the network which impedes the recovery from a perturbation or, on the other hand, loss of flexibility under certain conditions. Those nodes that cause the chaoticity are thus relevant in the context of global maintenance of the dynamical features of the network.

**Figure 5:** Derrida modified plots. Analogously to the Derrida map shown in Figure 5, this modified map relates the size of perturbations at two consecutive time steps. Curves above/below zero (black horizontal line) reflect instability/stability, respectively. Curves above zero represent those networks whose the knock-out produces a chaotic regime. Curves close to zero remain in the critical regime after the knock-out of a node. Those curves which do not change their dynamical regime after node elimination, indicate that the deleted node is not relevant to maintain the dynamics. That is the reason for which it can be eliminated. The most relevant nodes to preserve the dynamics of the network are shown in the upper right part of the figure.

Elimination of nodes in this work was implemented systematically in order to find crucial elements for the progres-
sion of the disease. This implementation could be applied elsewhere in larger networks to find critical nodes which are capable of determine the behavior of the whole network.

An important result is that the smallest IS was obtained after eliminating the Tumor necrosis factor α, TNFA. This result can be a promising therapy against the migration process that occurs during the most aggressive thyroid carcinomas. Therapies regarding blockage of TNFA has been developed for other pathologies, such as Reumathoid arthritis [Brenner et al., 2015; Keffer et al. (1999)]. An opportunity to anti-TNF therapy could be opened with this study. Other approaches to find crucial nodes in a boolean network have been developed [Kim et al., 2013]. An interesting open question is whether the results observed here could be also obtained with other methodologies. That is matter of further research.

To our knowledge, this is the first time that a discrete theoretical model is implemented to understand the matrix metalloproteinases pathway, and furthermore, the particular dynamics of thyroid carcinoma progression. The finding of the TNFA as a crucial element for progression of this carcinoma could only be achieved with an approach such as the presented here.

It is worth to mention that the criticality exhibited by the WT network is consistent with the fact that most biological networks operate in this regime (Balleza et al., 2008; Shmulevich et al., 2005). Moreover, the loss of the property after node elimination could be explained as a loss of the equilibrium between those elements which degrade the ECM and those which maintain the basal levels of MMPs in order to preserve the homeostasis of Extracellular matrix.

This kind of approaches give to us a more accurate insight of how the temporal behavior of any biochemical network can be observed. It is worth to mention that despite the majority of reaction rates among the pathway elements are not known, the boolean modeling only needs the qualitative nature of the relationships. This is one of the greatest advantages of this coarse-grained approach. Experimental procedures must be performed to corroborate the results observed here. Notwithstanding, the boolean network developed in this work could suggest directed experiments in order to understand the complex nature of the invasiveness on thyroid cancer.

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References


