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Uncovering the particularities of the dynamical interaction between cancer-related Epithelial-Mesenchymal Transition and the Mammalian Cell Cycle: a feedback-based Boolean networks interconnection approach

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Abstract

Epithelial-to-Mesenchymal Transition (EMT) plays a key role in epithelial-cancer. The state trajectory of its underlying Gene Regulatory Network (GRN) includes three fixed-point attractors characterizing epithelial, senescent, and mesenchymal, cell phenotypes, which implies specific cell-to-cell and cell-to-tissue interactions. The interplay between the GRN driving EMT and the one regulating the Mammalian Cell Cycle (MCC) influences cancer-related cell growing and proliferation. We expose the characteristics of the network arising from the interconnection of the gene regulatory networks associated to EMT and MCC. Our purpose is twofold: first, to elucidate the dynamical properties of cancer-related gene regulatory networks. Subsequently, to propose a computational methodology to address the interconnection of networks related to cancer. Our approach is based on feedback-based interconnection of networks described in discrete Boolean terms.

1 Introduction

It has been shown the pivotal role that Epithelial-to-Mesenchymal Transition (EMT) plays in human epithelial-cancer [4]. Moreover, from a systems-based analysis we know that the EMT

state trajectory of the underlying core transcriptional Gene Regulatory Network (GRN) includes three fixed-point attractors. These stable stable configurations characterize the epithelial, the senescent, and the mesenchymal, cell phenotypes. As far as the Mammalian Cell Cycle (MMC) is concerned, this consists of a succession of molecular events leading to the reproduction of the genome of the cell (Synthesis or S phase) and its division into two daughter cells (Mitosis, or M phase), is regulated by a highly structured gene regulatory network, as reported in [5, 2]. The constituting phases of MMC are then specified by the corresponding attractors landscape of the underlying regulatory network. Of particular interest is the systems level understanding of the behavior of human cells during the various stages of cancer genesis and cancer progression. Our main goal here is to contribute to this understanding. For this, we explore in this paper the characteristics of the cell dynamics that results from the interaction of the regulatory mechanisms driving EMT and MMC. Taking epithelial carcinogenesis into account, the feedback-based interaction between the GRN driving EMT and the one regulating MCC rules cancer-related cell growing and proliferation. In what follows we discuss the dynamic features of the regulatory network that arises from the interconnection of the gene regulatory networks associated with EMT and MCC. Our approach is based on the study of the feedback interconnection of associated core regulatory networks described in terms of discrete Boolean networks.

2 Methods

In the study of biological systems, Boolean models plays and important role because of most available data on regulatory interactions [2, 1, 6]. Using this approach we can model some specific biological systems but as we know biological systems do not work in isolation, they interact with other systems, so it is important to find a modeling technique that allows the interconnection among regulatory Boolean networks to form larger networks. On the other hand, some work has been done trying to establish a methodology to perform the interconnection of Boolean networks with shared nodes [3]. The result is a more complicated to analyze network. However, there is a *division method* of Boolean networks that can divide a given Boolean network under the scheme of asynchronous update in two modules that allows computing the dynamics of the network through the cross products of the attractors isolated from each node [8]. This method can be used so that instead of splitting a network we can join two existing subnetworks each representing a particular biological process and form then a larger network and then get the system dynamics by the method of division of Boolean networks. In what follows we recall some concepts concerning Boolean description of systems dynamics.

2.1 Dynamics of Boolean Models

2.1.1 Boolean Networks and Modules

In a Boolean model the variables take only the values 0 or 1. The state space of a Boolean Model is finite and is represented by a vector with the set of Boolean variables $\{x_1,\ x_2, \ldots, x_n\}$ [9, 7]. In a Gene Regulatory Network (GRN), x_n is the state of expression of the n^{th} gene and n is the total number of genes in the network. Each gene changes its state of expression according to the dynamic equation $x_n(t+\tau) = fn(x_{n_1}(t),\ x_{n_2}(t), \ldots, x_{n_k}(t))$, where $\{x_{n_1}(t),\ x_{n_2}(t), \ldots, x_{n_k}(t)\}$ are the regulators of the gene x_n and f_n is a Boolean function constructed by the combinatorial action of the regulators of x_n. τ is a measure of the relaxation time for a gene to change its state of expression undergo a change in the expression of its regulators. It is common to take

$\tau = 1$. A Boolean module is an extension from a Boolean Network, and its finite state space is represented by a vector with the set of Boolean variables $\{{}^A x_1, {}^A x_2, \ldots, {}^A x_n\}$, A indicates that it is module-A state space. A Boolean module has an input space represented by a vector with the set of Boolean variables $\{{}^A u_1, {}^A u_2, \ldots, {}^A u_p\}$ and also has an output space $\{{}^A h_1, {}^A h_2, \ldots, {}^A h_q\}$. In this embodiment and in the case of a GRN expression status of each gene is represented by the following dynamic equation $x_n(t+\tau) = fn({}^A x_1, {}^A x_2, \ldots, {}^A x_n, {}^A u_1, {}^A u_2, \ldots, {}^A u_p)$, where $\{{}^A x_1, {}^A x_2, \ldots, {}^A x_n, {}^A u_1, {}^A u_2, \ldots, {}^A u_p\}$ are the regulators of the gene x_n and f_n is a Boolean function constructed by the combinatorial action of the regulators of ${}^A x_n$.

2.2 Interconnection of Boolean Networks

One way to make the interconnection of two autonomous Boolean networks is through the nodes that are common to both networks. An example is given of such interconnections in [3]. Common to both networks nodes serve as a means of linking networks, but we know each node has defined a Boolean function, so at the time of interconnection between two networks we have two different Boolean functions *per node*. This ask then to implement a procedure to shape a function specifically resulting from the interconnection. This decision should be made based on knowledge, importance and effect of the Boolean function regulators. Two simple proposals by the interconnection are:

- The conjunction $\vee$.- We consider two autonomous Boolean networks A and B, both have a node in common ${}^A x_i$ and ${}^B x_i$ and in the case of a GRN representing the state of the same gene common to both networks. Therefore for the network A we have the ${}^A f_i$ Boolean function and for the network B we have the ${}^B f_i$. The resulting interconnection function is defined as ${}^{AB} x_i = {}^A f_i \vee {}^B f_i$.
- The disjunction $\wedge$.- In the same case considering two Boolean networks A and B for the disjunction Boolean function for interconnection is ${}^{AB} x_i = {}^A f_i \wedge {}^B f_i$.

This is a simple way to make the interconnection of autonomous Boolean networks. Nevertheless, if more is discussed in detail you can even propose a new Boolean function. The same strategy should be repeated with all the elements common to both networks.

2.3 Asymptotic dynamics by interconnections of Boolean modules

Under synchronous update strategy, an algorithm is proposed to achieve asymptotic dynamic attractors through two isolated Boolean modules. Let an attractor (steady state) of a Boolean module represented by ${}^A_\alpha \bar{X}^i_\beta$ where $\alpha \in U$ and $\beta \in H$. Let W the set of attractors crossed pairs between two modules A and B Boolean $({}^A_U \bar{X}^i_H \times {}^B_U \bar{X}^j_H)$ [8]. We can perform a asymptotic transition graph representing the dynamic interconnection between Boolean modules under the scheme of synchronous update using the following algorithm. Let the input space of Boolean module A $\{\gamma, \gamma_1, \beta, \beta_1\}$ and the input space of the Boolean module B $\{\sigma, \sigma_1, \alpha, \alpha_1\}$. The set of crossed pairs between attractors represent asymptotic graph nodes and transitions between cross pairs are defined as: ${}^A_\gamma \bar{X}^{i_1}_\alpha \times {}^B_\sigma \bar{X}^{j_1}_\beta \longrightarrow {}^A_\gamma \bar{X}^{i_2}_\alpha \times {}^B_{\sigma_1} \bar{X}^{j_2}_{\beta_1}$, if $\gamma = \beta$, $\alpha \neq \sigma$ and $\alpha = \sigma_1$; ${}^A_\gamma \bar{X}^{i_1}_\alpha \times {}^B_\sigma \bar{X}^{j_1}_\beta \longrightarrow {}^A_{\gamma_1} \bar{X}^{i_2}_{\alpha_1} \times {}^B_\sigma \bar{X}^{j_2}_\beta$, if $\gamma \neq \beta$, $\alpha = \sigma$ and $\beta = \gamma_1$; ${}^A_\gamma \bar{X}^{i_1}_\alpha \times {}^B_\sigma \bar{X}^{j_1}_\beta \longrightarrow {}^A_\gamma \bar{X}^{i_2}_\alpha \times {}^B_\sigma \bar{X}^{j_2}_\beta$. These equations define transitions between crossed product attractors. The last equation indicates that the cross product passes himself what it means that it is an interconnection attractor. If the transitions form a loop then this is a cyclic attractor. Graphically we show the methodology

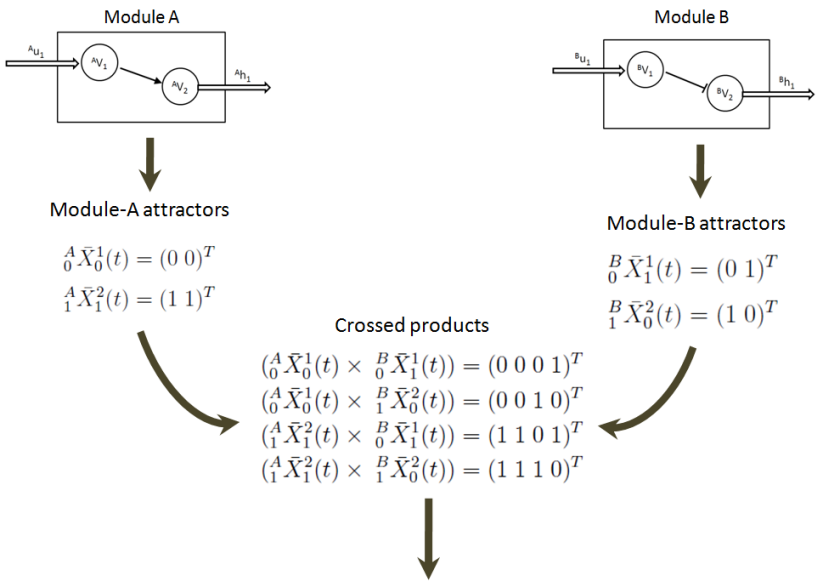

Figure 1: **Asymptotic dynamics by Boolean modules part 1.** This figure illustrates with an example the attractors landscape that results from the interconnection of two modules.

to be followed by an example in Figures 1 and 2. Let us now briefly describe the regulatory networks driving MMC and EMT.

2.4 The MMC and EMT regulatory networks

2.4.1 The Mammalian Cell Cycle

For the mammalian cell cycle, we use the Boolean model proposed in [2, 5] . This is a Boolean model of a GRN, this network has ten nodes representing major genes that interact and regulate the behavior of the cell (for the details concerning the specific biomolecular agents see the referenced sources). Under the scheme of synchronous update, this network has a fixed attractor $\bar{X}^1(t) = (0\,0\,1\,0\,1\,0\,0\,0\,1\,0)^T$ and a cyclic attractor:

$$\hat{X}^2(t) = \begin{bmatrix} 0 & 1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 1 & 1 & 1 \\ 0 & 0 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 1 & 1 & 0 & 0 & 0 & 1 \\ 1 & 0 & 0 & 0 & 0 & 1 & 1 \\ 0 & 0 & 0 & 0 & 1 & 1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 1 & 1 & 1 & 1 & 1 & 1 \end{bmatrix}$$

corresponding to the quiescent state and the cell division cycle.

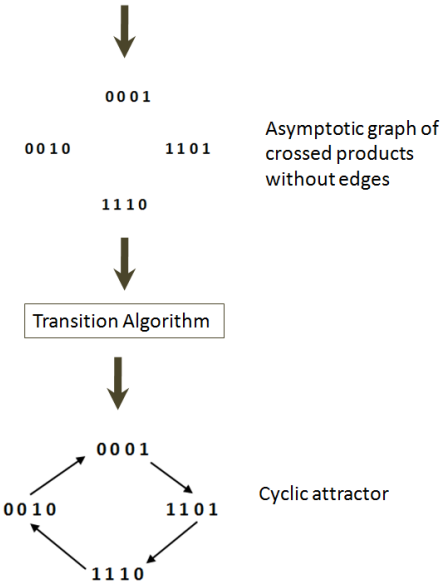

Figure 2: **Asymptotic dynamics by Boolean modules part 2.** This figure illustrates with an example the attractors landscape that results from the interconnection of two modules.

2.4.2 The Epithelial-Mesenchymal transition

This GRN represented by a Boolean network constitutes a robust developmental module for epithelial carcinogenesis that may partly underlie the cell types that are formed in carcinomas [4]. This network is considered a core regulatory network and consists of only 9 nodes. In this network the corresponding attractors landscape include three fixed attractors, that are achieved depending on the initial state of the system. Attractors represent the normal epithelial cell phenotype $\bar{X}^1(t)$, the senescent cell phenotype $\bar{X}^2(t)$, and the cancer (mesenchymal) attractor, *i.e.* $\bar{X}^1(t) = (0\,1\,0\,0\,1\,0\,1\,0\,1)^T$, $\bar{X}^2(t) = (0\,1\,1\,0\,0\,1\,0\,0\,1)^T$, and $\bar{X}^3(t) = (1\,1\,0\,1\,1\,0\,0\,1\,0)^T$.

2.5 Interconnection between MCC and EMT Boolean networks

The interconnection methodology between the two Boolean networks begins with the identification of nodes that are common to both networks, *i.e.* CycD = Cyclin, Rb and E2F. Subsequently Boolean functions of each node are fused and thereby interconnection is achieved. As a next step it is decided to form Boolean modules using this interconnection. We can make different divisions but we are interested in those representing functional modules from the biological standpoint. Therefore we select the structure shown in Figure 3. We can proceed now to explore the dynamics of this combined network.

3 Computational experiments

The division into modules will allow us to implement the strategy for the attractors calculation using isolated modules under the scheme of synchronous update. For the mammalian cell

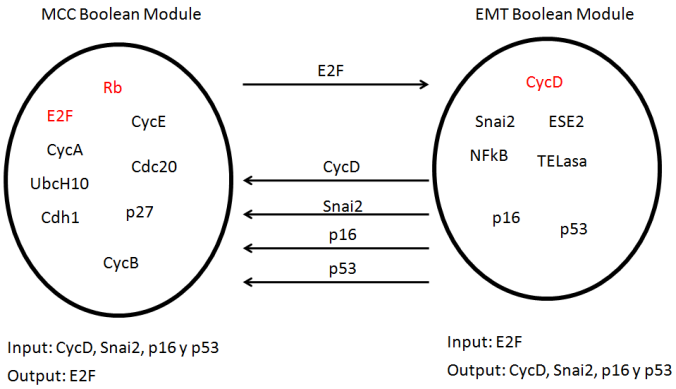

Figure 3: **Choice of the interconnection for the EMT-MMC network.** This figure schematically represents the structure selected for the interconnection of the Boolean modules that correspond to the Epithelial-to-Mesenchymal transition and the cell cycle.

cycle, and considering all possible combinations of inputs to the module, the following set of fifteen fixed attractors and a cyclic attractor of seven states is given: ${}^{A}_{0000}\hat{X}^{1}_{0}(t) = (0\,0\,1\,0\,1\,0\,0\,0\,1)^{T}$, ${}^{A}_{0010}\hat{X}^{2}_{0}(t) = (0\,0\,1\,0\,1\,0\,0\,0\,1)^{T}, \cdots, {}^{A}_{1111}\hat{X}^{15}_{0}(t) = (0\,0\,1\,0\,0\,0\,0\,0\,1)^{T}$, and:

$$ {}^{A}\hat{X}^{1}(t) = \begin{bmatrix} 0 & 1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 1 & 1 & 1 \\ 0 & 0 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 1 & 1 & 0 & 0 & 0 & 1 \\ 1 & 0 & 0 & 0 & 0 & 1 & 1 \\ 0 & 0 & 0 & 0 & 1 & 1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}. $$

In the EMT Boolean network, the module structure is give rise to seven fixed attractors when all possible inputs to the module are applied:

$$ {}^{B}_{0}\hat{X}^{1}_{0001}(t) = (0\,1\,0\,0\,1\,1)^{T},\ {}^{B}_{1}\hat{X}^{2}_{0001}(t) = (0\,1\,0\,0\,1\,1)^{T},\ \ldots,\ {}^{B}_{1}\hat{X}^{7}_{0110}(t) = (0\,1\,1\,1\,0\,0)^{T}. $$

Attractors calculated by module allow us to determine the interconnection attractors. In Figure 4 the network formed by the two interconnected modules Boolean is shown.

As a result of the interaction between all the cross products two simple attractors are obtained ${}^{AB}\bar{X}^{1}(t) = (0\,1\,1\,0\,0\,1\,0\,0\,1\,0\,0\,0\,0\,0\,0\,1)^{T}$ and ${}^{AB}\bar{X}^{2}(t) = (1\,1\,0\,1\,1\,0\,0\,0\,1\,0\,1\,0\,0\,0\,1\,0)^{T}$.

Furthermore a cyclic attractor is also obtained:

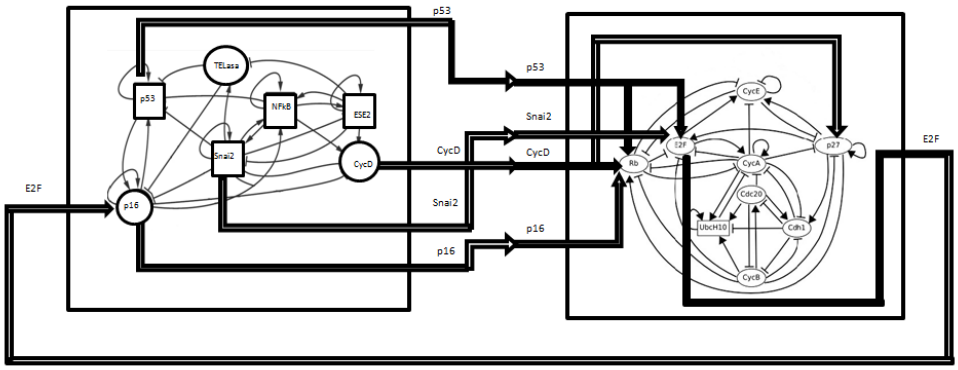

Figure 4: **EMT-MMC common nodes.** This figure represents the combined EMT-MMC gene regulatory Boolean network.

$$
{}^{AB}\hat{X}(t) = \begin{bmatrix}
0 & 0 & 0 & 0 & 0 & 0 & 0 \\
1 & 1 & 1 & 1 & 1 & 1 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 \\
1 & 1 & 1 & 1 & 1 & 1 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 1 & 0 & 0 & 0 & 0 \\
0 & 1 & 1 & 1 & 1 & 0 & 0 \\
0 & 0 & 0 & 1 & 1 & 1 & 1 \\
0 & 0 & 1 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 \\
1 & 1 & 1 & 0 & 0 & 0 & 1 \\
1 & 0 & 0 & 0 & 0 & 1 & 1 \\
0 & 0 & 0 & 0 & 1 & 1 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 \\
1 & 1 & 1 & 1 & 1 & 1 & 1
\end{bmatrix} \tag{15}
$$

We proceed now to discuss the biological meaning of the landscape resulting from the regulatory networks for EMT and MMC.

4 Results

In order to explore the asymptotic dynamics of the network resulting from the interconnection of the regulatory networks for EMT and MMC, we developed a computer-based tool based on the R language platform. The results are summarized in Figure 5 and Figure 6. In these figures the attractors are represented through a rectangular column, where each one of the gene nodes are specified; the corresponding activation state for each gene is represented in green if active, and in red if inactive. In the top of each column is indicated the percentage of the total states that define the corresponding basin of attraction. In each figure we represent a global specific

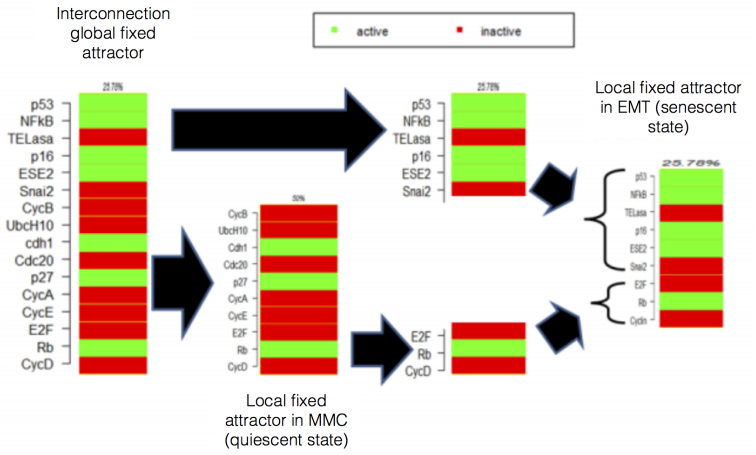

Figure 5: **Result 1.** First global fixed attractor resulting from the interconnection of the regulatory networks for EMT and MMC.

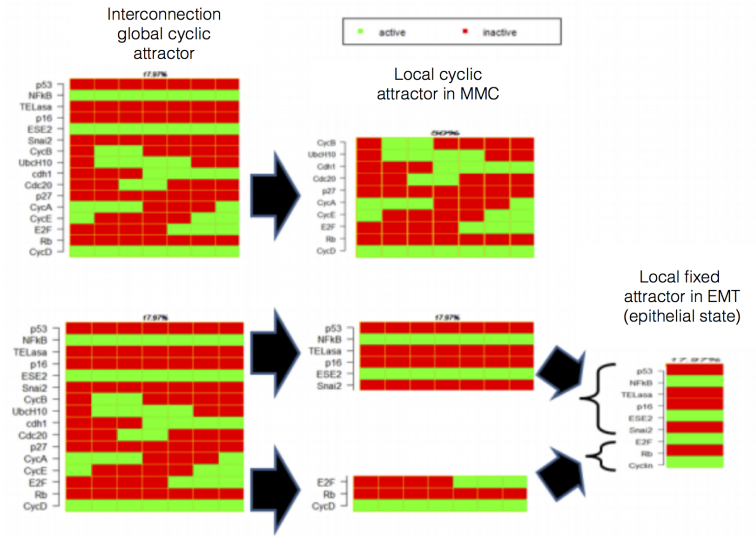

Figure 6: **Result 2.** Global cyclic attractor resulting from the interconnection of the regulatory networks for EMT and MMC.

attractor, and we describe how the trace of the attractors corresponding to the MMC and EMT regulatory are manifested in it. We have then the following results:

Result 1: As shown in Figure 5, in the global fixed attractor ${}^{AB}\bar{X}^1(t)$, it appears the trace of both the local fixed attractor corresponding to the quiescent state of the Mammalian Cell Cycle, and the local fixed attractor corresponding to the senescent state of the Epithelial-to-Mesenchymal Transition.

Result 2: As shown in Figure 6, in the global fixed attractor ${}^{AB}\bar{X}^3(t)$, it appears the trace of both the local fixed attractor corresponding to the local cyclic state of the Mammalian Cell Cycle, and the local fixed attractor corresponding to the epithelial state of the Epithelial-to-Mesenchymal Transition.

5 Discussion

Note that Result 1 and Result 2 correspond well with what is expected when the regulatory network of the Epithelial-to-Mesenchymal Transition and the regulatory network of the Mammalian Cell Cycle interact. Which is to say, carcinogenesis implies that the cancer-related senescent state constraint the cells to display a quiescent phenotype; in the epithelial cancer-related state, the cells display uncontrolled proliferation. As far as the global fixed attractor ${}^{AB}\bar{X}^2(t)$ is concerned, it appears the trace of both the local fixed attractor corresponding to an unknown local state of the Mammalian Cell Cycle, and the local fixed attractor corresponding to the mesenchymal state of the Epithelial-to-Mesenchymal Transition. It is necessary to analyze this result carefully, to know if it has a specific biological significance or if it is not simply an artifact of the modeling process.

6 Conclusions

We implemented in this paper a computer-based methodology to proceed to the feedback-based interconnection of the regulatory networks underlying cancer-related Epithelial-Mesenchymal Transition and the Mammalian Cell Cycle. We based our procedure on deterministic discrete-time Boolean descriptions. The results obtained validates our approach. We must point out that actual gene regulatory networks are stochastic in nature. However, the deterministic approach followed here allows us to uncover main asymptotic and structural characteristics of the cell dynamics that concerned us here. As far as the robustness properties associated to the network attractors are concerned, the probabilistic epigenetic landscape formalism offers a tool-of-choice to explore the interplay between stochastic fluctuations and attractor-to-attractor transitions. We are quite conscious that in order to understand cancer-related cell-to-cell and cell-to-tissue dynamics, we require both a multi-scale continuous time-description of the underlying biological processes, as well as the inclusion of spatial related issues (the influences of endogenous and exogenous mechanical forces should also be taken into account).

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