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Short Communication

An In Silico Cell Signaling-Based Approach for Exploring the Activities Involved in Pre-Metastasis and Metastasis

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SUMMARY

In order to understand, identify and explore the activities involved in metastasis, as well as possible control points, in this work we model and simulate the sequential steps that a cell must follow from its transformation to metastasis, using the Cellulat bioinformatics tool, an in silico experimentation environment that complements and guides in vitro experimentation concerning intra and intercellular signaling networks.

KEYWORDS

Metastasis; Cancer cells; *In silico* experiments; Cellulat bioinformatics tool.

BODY

Metastasis is the clinical term for the process by which tumor cells leave a primary cancer tumor to transfer to different organs. The metastasis is considered to be the true killer in cancer patients, since usually when this process is triggered, it inevitably leads to death. To prevent the spreading of cancer, the most important is to avoid the metastasis of the primary tumor, as well as to find markers that allow early identification of the presence of a primary tumor [1]. Features that promote metastasis - i.e., sustained proliferation, replicative immortality, and evasion of growth suppression - allow cancer cells to grow uncontrollably in a tumor large enough to invade neighboring tissues. The ability of cells to resist cell death and prevent their destruction caused by the immune system response, allow them to survive on their way to metastasis.

The intrinsic complexity of biological systems and phenomena - such as protein-protein interaction, protein-ligand docking and protein folding, just to mention a few examples - has required the development of a wide range of computational tools dedicated to the modeling and simulation of them, so that these computational approaches - also known as computer simulation or *in silico* experimentation - can complement, corroborate and enrich both the advances in theoretical and experimental research in the study of such systems. Regarding cancer research, in the last years it has found valuable support in a wide range of modeling and simulation approaches, which cover a wide spectrum ranging from mathematical models - e.g., continuous models [2–4] and stochastic models [5–8] - to computational models - e.g., Monte Carlo method and cellular automata [9–11], Boolean networks [12], Petri nets [13], artificial neural networks [14–16] and expert systems [17]. These approaches have allowed the *in silico* experimentation in cancer at the cellular, system and patient level.

In order to understand, identify and explore the activities involved in metastasis, as well as possible control points, in this work we model and simulate the sequential steps that a cell must follow from its transformation to metastasis. We simulate and explore the complex interaction patterns of signaling pathways involved in pre-metastasis and metastasis using the Cellulat bioinformatics tool (http://bioinformatics.cua.uam.mx/node/10) [18, 19], a computational simulation tool developed by us and inspired by Biochemical Tuple Spaces for Self-Organizing Coordination model (BTSSOC) [20]. The main idea behind the Cellulat bioinformatics tool is to provide an *in silico* experimentation environment that complements and guides in vitro experimentation concerning intra and intercellular signaling networks.

Cellulat, as simulation tool, captures and mimics the behavior of complex networks of elements that interact with each other in different forms, i.e., linear, non-linear, positive feedback, negative feedback, among others. The interaction between two or more elements is expressed as a rule, law or reaction, whose condition and action are described as tuples of elements. The BTSSOC model, on which the bioinformatics platform was built, is strongly characterized by three key elements: 1) the notion of tuple space [21], 2) the concept of chemical reaction, which is characterized by a kinetic parameter (rate), while the elements involved are characterized by its availability or concentration, and 3) an action selection mechanism based on Gillespie's algorithm [22], a stochastic simulation algorithm typically used to mimic systems of chemical/biochemical reactions in an efficient and accurate way. On the other hand, two characteristic features of Cellulat - which makes it very suitable for the simulation of cellular signaling systems - are its multi-compartmental nature and multi-level representation. It is important to note

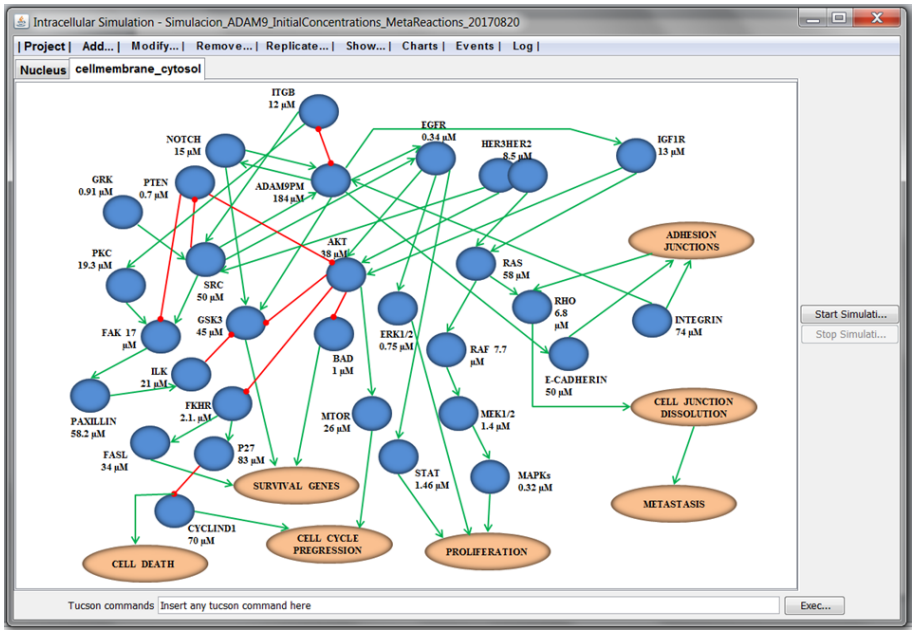

Figure 1: Simulation of the metastasis cellular signaling pathways. Cells, cell compartments, chemical reactions and reactants have been created as the initial components required by the simulation of activities involved in pre-metastasis and metastasis. Signaling elements - e.g., proteins and enzymes - are represented by solid blue spheres. Each signaling element is detailed by its name (acronym) and its initial concentration in micromoles. Red arrows indicate inhibition relationships and green arrows indicate activation relationships.

here that the majority of cell signaling simulation tools, suchas E-Cell [23], BetaWB [24, 25] and Cell Illustrator [26], provide abstractions to model only intracellular behavior. Thus, they are not suitable to model cells in their social context, along with all those biological mechanisms that involve two or more cells, that is essential in the scenario discussed in this work.

The methodology followed in this work is based on a continuous bidirectional feedback between the *in silico* approach and theoretical and experimental knowledge. That is, the proposed metastasis cellular signaling model and the results of its corresponding computational simulation- e.g., possible target elements or control points - should provide valuable support to guide in vitro experimentation; while the results of theoretical and experimental research should lead to both the improvement of the model - e.g., what other interactions should be added to the model?- and the design of the most appropriate *in silico* experiments - e.g., what virtual knockout experiments to carry out?

Figure 1 shows the initial state of the simulation once the simulation components - i.e., cells, cellular compartments, chemical reactions and reactants - have been created from the metastasis signaling pathway model, conceived and corroborated as initial phase of our methodological approach. The resulting signaling network is made up of 31 nodes representing signaling elements (i.e., proteins and enzymes), 7 nodes representing cell processes (such as cell death, metastasis and proliferation), and 58 arcs representing chemical reactions between the involved nodes (e.g., ITGB* + SRC -> SRC* and AKT* + GSK3* -> GSK3, where the symbol "*" means that the signaling element is active). The overall signaling network extends across 3 cell compartments (i.e., cell membrane, cytosol and nucleus) comprising key cellular signaling pathways involved in growth and metabolism leading to survival, proliferation, tumor progression and cell death, as well as integration with the formation of intercellular interactions (i.e., EGFR/MAPK, JAK/STAT, RAS/RAF/MEK/ERK and PI3K/AKT signaling pathways).

The metastasis cellular signaling model evolved significantly, from the first versions to later version, after multiple theoretical/experimental feedbacks which allowed to solve the following problems that emerged during the execution of the associated simulation: 1) the earliest models of metastasis cellular signaling did not include all the required chemical reactions, particularly negative feedback (or balancing feedback), 2) the initial concentration of some reactants did not match the required value, which prevented the expected solution to be reached , 3) the estimated reaction rate constant of some chemical reactions did not meet the required value, avoiding that such reactions were executed at the appropriate time by Gillespie's algorithm, 4) the relationship between the calculated rates of various chemical reactions was not properly adjusted, having as a result that some slower reactions were executed before the faster reactions.

The aim of this work was to identify - at an *in silico* level - a mechanism that prevents metastasis which is the true killer in cancer. Different experiments were made to prevent the cancerous cell from going to metastasis or its survival in the successive steps. For

this we gradually reduced each of the key signalling elements which participate in the pathways that precede metastasis - i.e., from the highest concentration of 1000 μM to the lowest concentration of 0.001 μM - considering the kinetic parameters and concentration values reported in literature. We saw how this simulation tool can be applied to the simulation of the activities involved in pre-metastasis and metastasis - particularly, signaling in tumor cells mediated by ADAM9 - and the identification of possible control points. By means of *in silico* experiments, using the Cellulat bioinformatics tool, we identified two possible key molecules to avoid metastasis, ADAM9 and ITGB.

The cellular signaling model and its associated simulation presented here provided invaluable support for the *in silico* experiments and proved to be very flexible, efficient and secure, both with regard to the schemes it provides for the representation of the cellular compartments, chemical reactions and reactants, as in relation to the discrete stochastic algorithm used for the selection and execution of chemical reactions with their own kinetic parameters. As part of our future work, 1) we will integrate elements of ”host” cells into the current cell signaling model, which favor the anchoring of metastatic cells, and we will also consider elements detected in exosomes, and 2) we will use other related simulation tools, such as MCell [27] and Virtual Cell [28] for comparison with Cellulat.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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