Network modelling of Epithelial-to-Mesenchymal transition reveals a putative role of RPTPs for the control of metastasis.

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Abstract

Epithelial-to-Mesenchymal transition (EMT) together with Mesenchymal-to-Epithelial transition together (MET) are two natural processes thought to participate in the process of cancer migration and metastasis acquisition. Multiple signals from microenvironment have been reported to drive EMT. However, the key microenvironment signals that prevent EMT and promote MET are still unknown. Here, we report the analysis of a network model accessing the putative effects of combinations of 8 microenvironment signals in relevant physiological scenarios. The model analysis demonstrated that cell contact dependent RPTP have the potential to control EMT and promote MET. The analysis also showed that this mechanism of control is sensitive to hypoxia. Moreover, the model also provided a mechanistic explanation for the observed correlation between hypoxia and metastasis under chronic inflammation.

Keywords: Logical modelling; Cancer; EMT; Tumour microenvironment; Metastasis control.

Introduction

Epithelial-to-Mesenchymal transition (EMT) is a complex and reversible trans-differentiation process observed in embryogenesis and wound healing, where a cell lose the cell-cell adhesion to its neighbours and gain migration capacity [1–3]. In cancer, EMT together with Mesenchymal-to-Epithelial transition (MET) is believed to participate in the metastasis process of carcinomas through the transitions between Epithelial-like and Mesenchymal-like cancer cells [1,4]. Signals from the tumour microenvironment such as ECM stiffness, inflammatory signals, hypoxia, growth factors and Delta-Notch have been already proven to promote EMT in cancer cells *in vitro* [5–8]. However, signals that prevent EMT and promote MET are not yet reported [9,10]. The Receptor Protein Tyrosine Phosphatases (RPTP) and FAT4 are two receptors dependent of cell contacts potentially capable of inhibiting the growth factor signalling (RPTP) and the Wnt signalling (FAT4), two pathways involved in EMT [10–12]. Logical modelling of regulatory networks in cancer has been proven to be a successful tool for exploring multiple hypotheses, describing observed behaviours and identifying novel biomarkers in cancer [13–16]. Previously, we developed a logical network model of the regulation of two critical cell adhesion properties involved in EMT, accounting for 8 key microenvironment signals, including cell-cell contact dependent RPTP and FAT4 [17]. This model was extensively validated by comparing its results against phenotypic and activity observations from published experiments [17]. Here, we report the model-based analysis focusing on the transitions between Epithelial-like and Mesenchymal-like phenotypes, and exploring the role of microenvironment signals that can control EMT and promote MET.

Methods

Logical formalism

The mathematical framework used in this work was the logical formalism, initially proposed by René Thomas [18]. This approach consists in defining an interaction map that reflects the regulatory network, which contain the regulators (nodes) connected through arcs representing the activations or inhibitions (interactions). In this framework, the nodes are the variables and are often binary (Boolean), describing two qualitative states of biological activity/concentration for the respective network components. In this approach, nodes can also be defined as multi-valued, where multiple discrete and finite degrees of activity/concentration can be associated with molecular components. Thus, it is assumed that activation degrees of biological entities can only be strong, intermediate (in the case of multi-valued) or basal. The behaviours of the model are defined by logical functions, which result in the evolution of the variables towards attractors, according to an update scheme. In this work, the model analysis was only focused on point attractors, called here by stable states, which are fixed points where functions are no longer updatable.

Network model

Modelling the transitions between Epithelial-like and Mesenchymal-like phenotypes were performed using a literature-based logical model for the regulation of two critical cell adhesion properties in EMT described in [17]. This model is composed by a total of 51 regulatory components (nodes) and 134 regulatory interactions, accounting for TGFβ, Integrin, Wnt, AKT, MAPK, HIF1, Notch, and Hippo signalling. We also considered the transcriptional regulation of E-cadherin and the post-transcriptional regulation of E-cadherin/β-catenin/p120 complex. For details on model components

and interactions see file CAmodeldoc.xhtml publicly available on https://github.com/rjpais/CALMproj. As model inputs, we included 3 inflammatory signals (IL6, ROS, and the ECM stiffness), 2 growth factors (EGF and HGF) and 3 cell-cell contact signals (DELTA, FAT4L, and RPTPL). These were associated to Boolean variables defining basal (value 0) or high (value 1) degrees of activity. Cell-cell adhesion strength and focal adhesion dynamics (cell adhesion properties) were defined as model readouts, where their combinations where associated to the Epithelial-like and Mesenchymal-like phenotypes [17,19]. Logical functions were developed to abstract the biological mechanisms involved in the activation of each model component (e.g. translation and phosphorylation).

Model setup and simulation

The logical model was implemented in GINsim, a free software tool for modelling regulatory networks [20]. The model is publicly available on https://github.com/rjpais/CALMproj (see file CAmodel.zginml). Reachable stable states and their associated phenotypes were obtained by simulation in GINsim using the method described in [20,21]. Model inputs were set in each simulation to mimic the cell microenvironment associated to a particular physiological scenario, starting from a defined stable state associated to the typical Epithelial-like or Mesenchymal-like phenotype [17]. All Simulations were run under asynchronous updating policy, according to rules of priority that accounted for timescale constraints using the method described in [21,22]. Knockout perturbations on regulatory interactions were also defined in GINsim by setting the values of the effects of regulators to 0 and analysed through simulation of particular physiological scenarios.

Results

Using the logical network model, we explored the role of cell contact depend activation of RPTP ligands (RPTPL), FAT4 ligands (FAT4L) and DELTA on EMT/MET under relevant physiological conditions that tumour cells may be exposed (Table 1). Simulation of these physiological scenarios resulted in predicted microenvironment conditions for the transitions between Epithelial-like and Mesenchymal-like phenotypes (Figure 1A). The results show that EMT was not compatible with high degree of RPTPL signal in the microenvironment under conditions that represent tissue growth, chronic inflammation and healthy epithelia with high activity of DELTA. Similar incompatibility was also obtained for FAT4L, except for the case of chronic inflammation. Interestingly, the model predicted that MET can only be triggered under high RPTP activity (RPTPL = 1). Simulations also showed that MET was achieved in microenvironment conditions compatible with tissue growth, chronic inflammation and healthy epithelia conditions under high DELTA signal. This indicate that RPTP activation triggers MET in the presence of EMT inducing signals such as EGF, HGF, ECM stiffness, and DELTA. These results suggest cell contact dependent RPTP is a key driver of MET, explaining the observed MET in cancer cell lines due to signals from co-cultured normal Epithelial cells [23].

Further, the analysis showed that the model input condition that mimic hypoxia in combination with tissue growth, chronic inflammation or DELTA signal where capable of inhibit the RPTP control over EMT and prevent MET. This is explained by the oxidative inhibition of RPTP by ROS generated under, which in turn was accounted in our model as a regulatory interaction [24,25]. On the other hand, hypoxia could not

inhibit FAT4L capacity to prevent EMT, which suggest that it could only have an effect under chronic inflammation conditions.

To understand the mechanism by which RPTP control EMT and promote MET, we analysed the impact of several model perturbations that knockout regulatory interactions downstream from RPTP. We analysed the reachability of phenotypes under chronic inflammation conditions and identified the interactions that are absolutely required for the RPTP predicted effect (Figure 1B). Only the combined knockout of 4 regulatory interactions resulted in reachable stable states compatible with Mesenchymal-like and not Epithelial-like phenotype (data not shown). This demonstrated that RPTP regulatory interactions with EGFR, MET, β-catenin, and p120 are required to ensure the control on EMT and promote MET. The perturbation analysis on multiple targets also showed that the inhibition of cMET together with activation of p120 and β-catenin is critical to ensure the typical high Epithelial cell-cell adhesion. Unexpectedly, cMET inhibition by RPTP was critical for preventing the inhibition of E-cadherin expression (CDH1) by SNAIL/ZEB via TCF/LEF activation of Wnt and TGFβ signalling. On the other hand, combined inhibition of cMET with EGFR by RPTP was found to be required to ensure an absolute inhibition of the high focal adhesion dynamics via inhibition of FAK/SRC complex. This suggests that RPTPs must target both MET with EGFR to prevent cell migration capacity.

Discussion

The model analysis allowed us to identify the RPTPs potential capacity of controlling EMT by preventing EMT and promoting MET. This already makes an important contribution to the cancer field with the identification of a novel mechanism that could control cancer migration through EMT and eventually prevent metastasis [9,10,26]. In

addition, it also provides a mechanistic explanation for the observed high cell contact induced MET in cancer cell lines [23,27]. In theory, high cell contact dependent RPTP signal is achieved in a scenario where a cell have enough neighbour cells expressing RPTP ligands, which would be the case of a healthy epithelia [11]. On the other hand, low RPTP signal can be achieved in scenarios where enough neighbour cells are destroyed by either apoptosis or tissue damage such as wounds. This is compatible with our model results and convey the idea that EMT is tightly controlled based on the demand for Mesenchymal cells, explaining the transient behaviour in wound healing [27]. Although EMT control by RPTPs is not yet proven, the model was based on well supported reports that demonstrate the individual regulatory interactions on Epithelial cells (see references in CAmodeldoc.xhtml). Moreover, it was also demonstrated that the network model was able to generate results consistent with a substantial number of observations reported in the literature (see model validation in [17]).

Potentially, RPTPs can be a good inhibitor of EMT since they typically have higher activity rates (about 1000-fold higher) in comparison with RTK dependent growth factors signalling involved in EMT (e.g. EGFR and cMET) [12]. In general, RPTPs are highly expressed in Epithelial cells of most tissues, placing them as plausible candidates for a generic control mechanism [11]. However, only a restricted set of RPTPs have been proven to be controlled by cell contact interactions through homophilic or other type of ligand interactions [11]. In cancer, two cell contact dependent RPTPs, the RPTP-k and DEP-1, are often mutated or down-regulated [11,28]. This together with the model analysis, supports the hypothesis that de-regulations on RPTPs are relevant for cancer invasion and metastasis. In addition, RPTP-κ is reported to target both EGFR and β-catenin, whereas DEP-1 targets p120 and MET [11,29–31]. Based on the model analysis, all above mentioned targets were

required for the EMT control by RPTPs. Thus, it is plausible to hypothesise that both RPTP-κ and DEP-1 may be collectively activated for an effective control of EMT.

The model analysis further showed that oxidative stress generated during hypoxia plays a key role in inhibiting the control of EMT by RPTPs. This is particularly evident in RPTP-k of keratinocytes under UV, suggesting that it could also be the case of other sources of oxidative stress [24]. This places the antioxidant usage as a candidate for cancer therapy to prevent excessive accumulation of Mesenchymal-like cancer cells in tumours. Importantly, our analysis provides the conditions by which these Mesenchymal-like cancer cells may accumulate in tumours. However, not all conditions are plausible in the case of tumours growing in an epithelia, where cell contacts are high. This would exclude most analysed physiological scenarios based on the identified effect of FAT4 on preventing EMT. Therefore, the tumour microenvironment under chronic inflammation conditions in combination with hypoxia is the most likely condition for promoting the accumulation of Mesenchymal-like cancer cells. According to model analysis, once these Mesenchymal-like cancer cells escape the primary tumour and migrate trough blood, they can colonize other organs through MET if they encounter a high cell contact and oxygen rich microenvironment. Thus, our results provide a mechanistic explanation for the correlation observed between metastasis and hypoxia under chronic inflammation conditions [6].

Conclusion

The model analysis in several physiological scenarios illustrated the role of cell contact dependent activation of RPTP and FAT4 as hypothetical mechanisms for the microenvironment control of EMT. Importantly, cell contact dependent RPTP activation played a central role in controlling EMT by either preventing or promoting MET. Hypoxia was identified using our modelling approach as a microenvironment

signal capable of inhibit the RPTP induced EMT control mechanism. These predicted mechanisms are still not proven experimentally but yet pose substantial theoretical support placing them as good hypothesis to be tested. Moreover, the analysis of the RPTP control mechanism of EMT provided candidate targets that may help in future design of new therapeutic strategies to prevent tumour cells to gain the capacity to invade the primary site.

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Conflicts of interest

The author declare no conflict of interest.

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Table 1. Model input variables used in simulation of particular physiological scenarios. For model inputs, the values 0 indicate basal and value 1 high degrees of activity.

	Description	Model Input
Healthy Epithelia	Scenario where the Epithelial cells of an epithelia surrounding a tissue are not massively dividing under tissue repair or wound healing. In these conditions, the degree of growth factors, cytokines, ROS and ECM stiffness are low, considered here as basal (value 0) [5,6,32].	IL6 = 0 ROS = 0 ECM=0 EGF=0 HGF=0
Tissue growth	Scenario with high secretion of growth factors by Fibroblasts and adjacent Epithelial cells [32–34]. Stimulates cell proliferation to balance cell death. Occurs during tissue repair, tissue size homeostasis and in a tumour proliferative state.	IL6=0 ROS=0 ECM=0 EGF=1 HGF=1
Chronic Inflammation	Scenario with a prolonged state of inflammation resulting in the accumulation of collagen I, IL6 cytokine and HGF secreted by recruited Fibroblasts and Macrophages to the inflammatory site [5,33]. Frequently observed during wound healing and in cancer.	IL6=1 ROS=0 ECM=1 EGF=0 HGF=1
Hypoxia	Scenario achieved under fast growth of tumours, where oxygen available in the tumour microenvironment is consumed. Low levels of oxygen trigger the generation of high levels of intracellular ROS [36]. Frequently observed in invasive tumours in combination of chronic inflammation conditions [5,6,35].	IL6=0 ROS=1 ECM=0 EGF=0 HGF=0

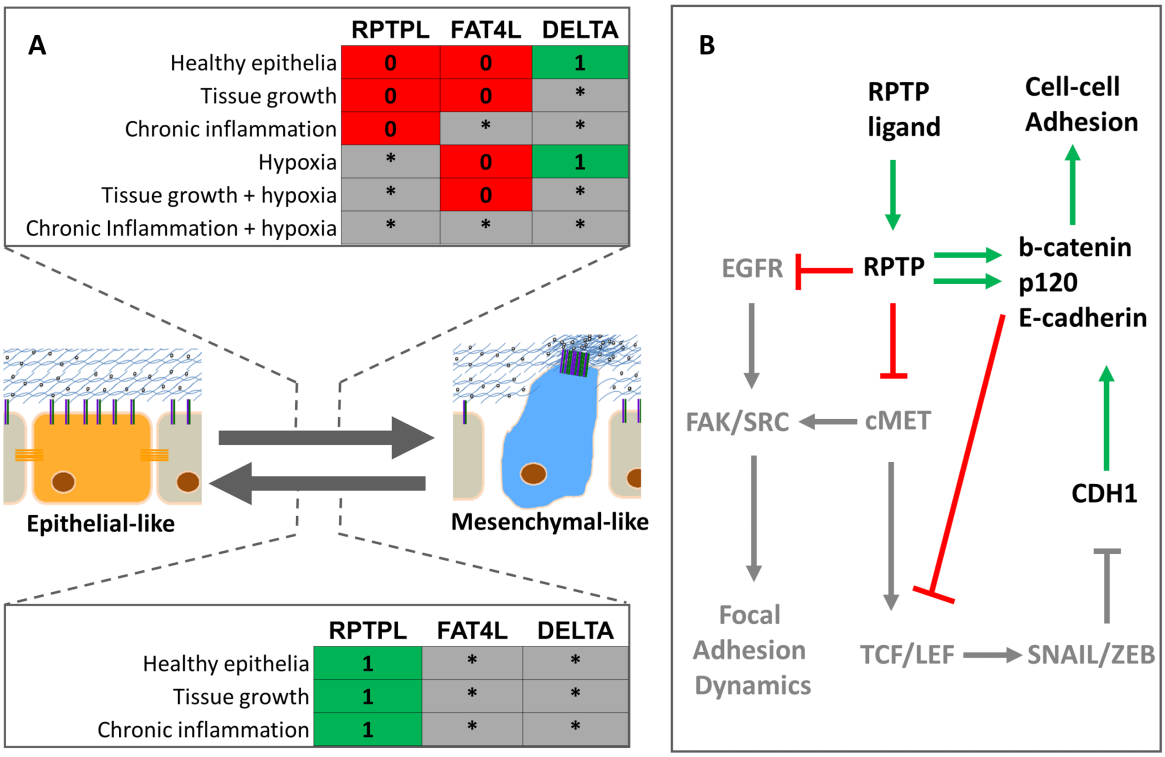

Figure 1. The predicted effect of RPTPs on the transitions between Epithelial-like and Mesenchymal-like phenotypes. The microenvironment conditions that trigger EMT and MET by simulation of physiological scenarios **(A)**.The mechanism of RPTP control over EMT**(B)**. In panel A, transitions between phenotypes are represented by dark grey arrows and the compatible conditions in linked tables. In tables, green(1) denotes high activity, red(0) denotes basal activity and grey(*) denotes all possible degrees. In panel B, red arrows denote active inhibitions and green arrows denote active activations during simulations. Grey arrows indicate inactivity of processes during simulation.