Simulation of multiple microenvironments shows a putative role of RPTPs on the control of Epithelial-to-Mesenchymal Transition.

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Abstract

Epithelial-to-Mesenchymal transition (EMT) together with Mesenchymal-to-Epithelial transition (MET) are two celular transformations thought to participate in the process of cancer migration and metastasis acquisition. Multiple signals from the microenvironment have been reported to drive EMT. However, microenvironment signals that control EMT are still unknown. Here, we identified a hypothetical control mechanism of EMT by cell contact dependent activation of RPTPs. This mechanism was supported by simulation of relevant physiological scenarios, where six key EMT promoting microenvironment signals were taken into account. These simulations showed that RPTPs have the potential to prevent EMT and also to promote MET in several physiological scenarios, except when combined with hypoxia scenario. In these cases, FAT4 activation by cell contacts functions as an alternative control mechanism of EMT except under chronic inflammation, providing a theoretical explanation for the observed correlation between hypoxia and metastasis under chronic inflammation.

Keywords: Simulation; 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 loses the cell-cell adhesion to its neighbours and gains 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]. Mesenchymal-like phenotype is considered to be the main form of cancer single cell migration, whereas Epithelial-Mesenchymal hybrid phenotypes (Hybrid) that arise from incomplete EMT are the key driver collective migration of cancer cells [5]. Some cancer cells also show Amoeboid-like behaviour, a single cell mode of migration that often originates through a transdifferentiation process from Mesenchymal-like cells and acts as pathfinders [6].

Signals from the tumour microenvironment such as ECM stiffness, inflammatory signals, hypoxia, growth factors and Delta-Notch have already been proven to promote EMT in cancer cells *in vitro* [7–10]. However, microenvironment signals that prevent EMT and promote MET are not yet reported [11,12]. 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 [12–14]. 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 [15–18]. 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 [19]. This model was developed accounting for the published regulatory network models for EMT and further extended to include cell-cell contact dependent RPTP and FAT4 in the microenvironment [15,16]. Moreover, the model was extensively analysed and validated by comparing its results against phenotypic and activity observations from published experiments [19]. Here, we propose a natural control mechanism of EMT by cell contact signals on the cellular microenvironment based on the simulation of multiple physiological scenarios using a logical network model.

Methods

Modelling framework

The mathematical framework used in this work was the logical formalism, initially proposed by René Thomas [20]. This approach consists in defining an interaction map that reflects the regulatory network, which contains 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 only focused on the stable states (also called point attractors), 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 [19]. 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 (Figure 1). The model also considers 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. The model inputs include 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 with Boolean variables defining basal (value 0) or high (value 1) degrees of activity. The model was further adapted to associate cell-cell adhesion strength and focal adhesion dynamics nodes through interactions to define phenotypes as model readouts (Figure 1) according to their typical cell adhesion properties [19,21]. Logical functions of this model were developed to abstract the biological mechanisms involved in the activation of each model component (e.g. translation and phosphorylation). The model is publicly available on https://github.com/rjpais/CALMproj (see file Camodel_withPhen.zginml).

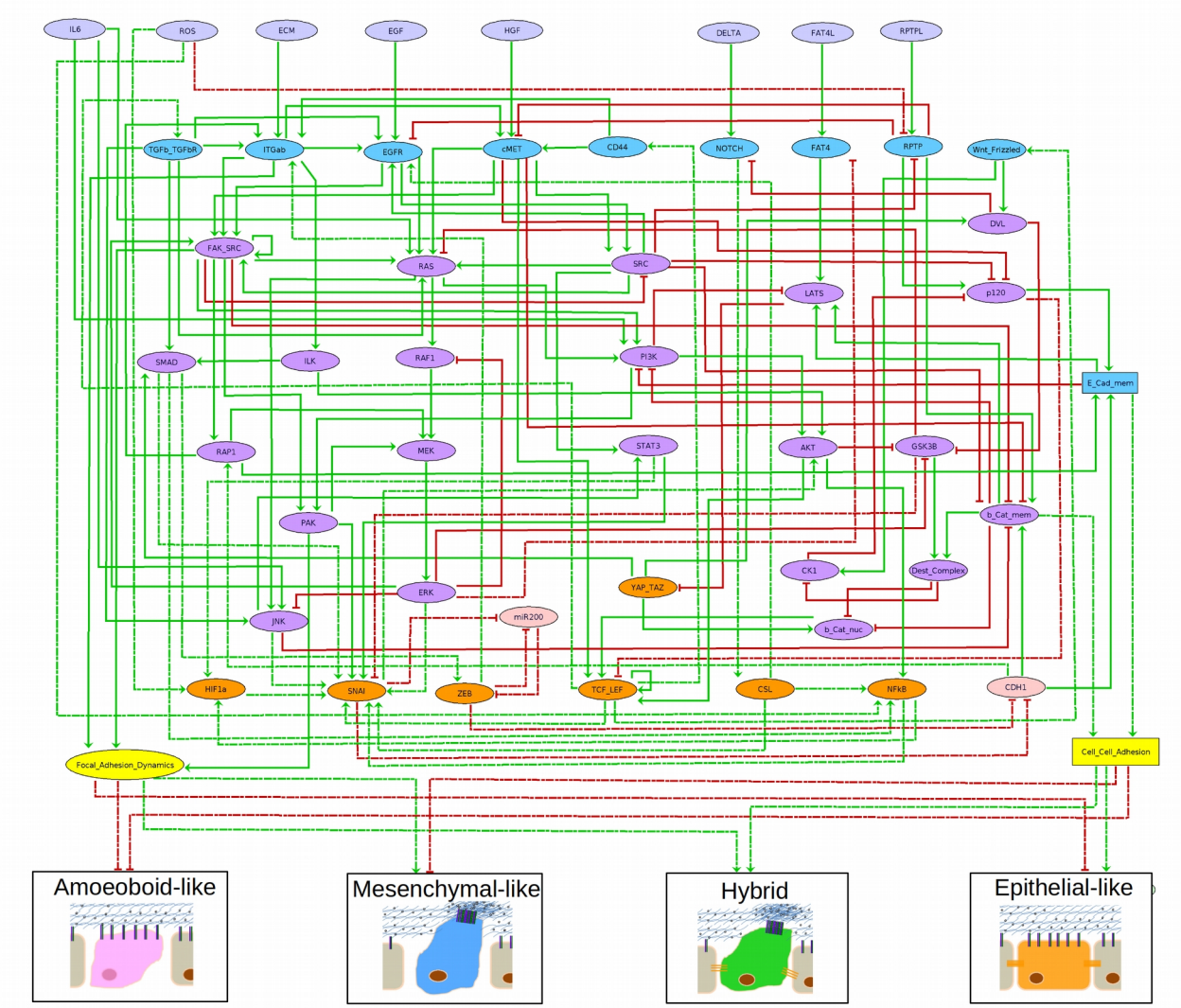


Figure 1. Network model and phenotypes. Microenvironment signals are depicted in grey, membrane protein/receptors in blue, signalling components in purple, transcription factors in orange, genes and RNA in pink, cell adhesion properties in yellow; elliptic nodes denote Boolean variables and rectangular nodes denote multi-valued variables; red arcs are inhibitions and green arcs activations. Fast interactions are denoted by plain arcs and slow interactions by dashed arcs.

Model simulation

Simulations were carried using GINsim, a free software tool for modelling regulatory networks [22]. The reachable stable states and their associated phenotypes were obtained in GINsim by generating the state transition graph with the method described in [22,23]. Model inputs were set in each simulation to mimic the cell

microenvironment associated with a particular physiological scenario, starting from a defined stable state associated with the typical Epithelial-like or Mesenchymal-like phenotype [19]. All simulations were run under asynchronous updating policy, according to rules of priority that accounted for timescale constraints using the method described in [23,24]. 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

Simulation of relevant physiological conditions that epithelial tissues may be exposed allowed us to explore the role of cell contact dependent activation of RPTP ligands (RPTPL), FAT4 ligands (FAT4L) and DELTA on EMT/MET. The outcomes from simulations resulted in predicted microenvironment conditions for the transitions between Epithelial-like and Mesenchymal-like phenotypes (Figure 2). No other phenotypes were obtained in these conditions. The results showed 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 indicates 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 can be a key driver of MET, explaining the observed MET in cancer cell lines due to signals from co-cultured normal Epithelial cells [25].

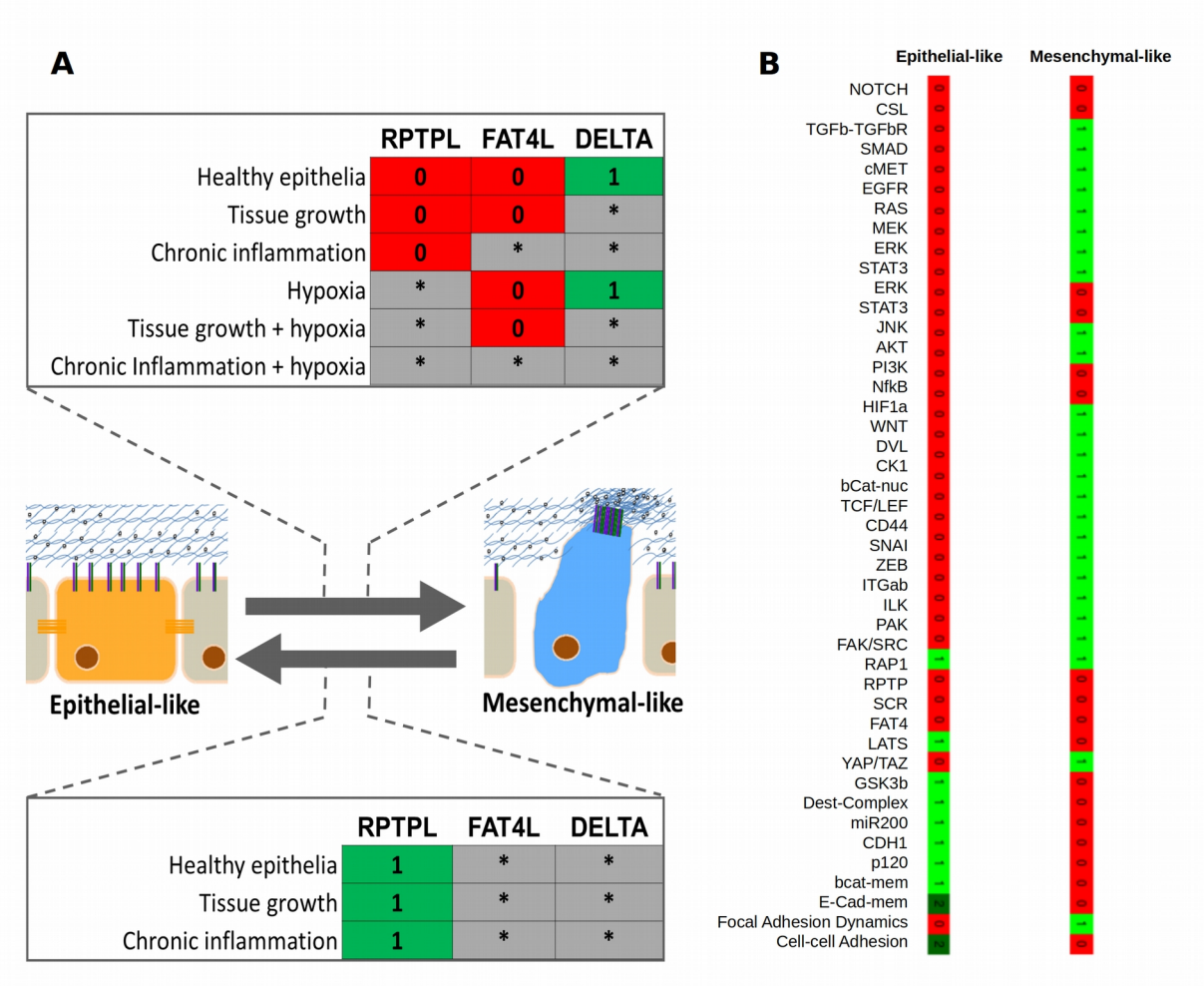


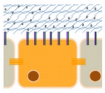
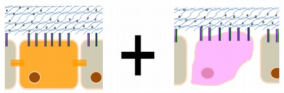
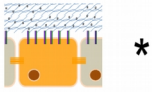
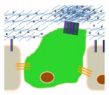
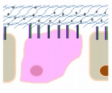
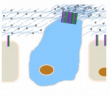
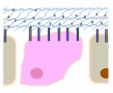
Figure 2. Simulation of the transitions between model phenotypes under physiological scenarios. Transitions between phenotypes are represented in panel **A** by dark grey arrows and the compatible conditions in linked tables. In tables, green(1) denotes high activity, red(0) denotes basal activity and grey(*) denotes all possible degrees. The conditions of the physiological scenarios in simulations are indicated in table S1 in supplementary material and the initial base stable states for Epithelial and Mesechymal like phenotypes denoted in panel **B**.

Further, simulations showed that the model input conditions that mimic hypoxia in combination with tissue growth, chronic inflammation or DELTA signal were capable of inhibiting the RPTP control over EMT and preventing MET. This is explained by the oxidative inhibition of RPTP by ROS generated under, which in turn was accounted in the model as a regulatory interaction [26,27]. On the other hand, hypoxia could not inhibit FAT4L capacity to prevent EMT, which suggests 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 multiple model perturbations that knockout the RPTP regulatory effects. For this purpose, we simulated EMT under a combination of EMT driving signals and analysed the resulting phenotypes (Table 1). The results demonstrated that removing the regulatory interactions with EGFR and cMET would result in the stability of Mesenchymal-like or Hybrid phenotypes, indicating that these molecular interactions are required for the control of EMT. The results also showed that the knockout of the regulatory interaction between RPTP and p120 would result in the stability of the amoeboid-like phenotype. In addition, we have analyzed the activity of the nodes involved downstream of RPTP activation on the resulted model stable states and reconstructed a proposed mechanism of action for the control of EMT (Figure 3). The changes in activity of nodes 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 necessary 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, the 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. Together, these results suggest that RPTPs need to target both catenins, MET and EGFR to prevent EMT and the stability of phenotypes with cell migration capacity.

Table 1. Effect of Knocking out RPTP interactions on model predicted EMT control by RPTPs. All effects were obtained through simulation, starting from the basal Epithelial-like stable state (see Figure 2 B) with an input configuration that describes diverse EMT signals under RPTP activation conditions (IL6=ECM=EGF=HGF=DELTA=RPTP=1 and FAT4L=ROS=0). Multiple reachable phenotypes are indicated with + and * indicates an intermediate degree in cell adhesion.

KO Target interaction	Model Perturbation	Phenotypes resulting from simulation
none	none	
RPTP → p120	p120[RPTP@0]	
RPTP → β-catenin	b-Cat_mem[RPTP@0]	 *
RPTP ⊣ EGFR	EGFR[RPTP@0]	
RPTP ⊣ MET	MET[RPTP@0]	
RPTP ⊣ EGFR RPTP ⊣ MET	EGFR[RPTP@0] MET[RPTP@0]	
RPTP → β-catenin RPTP ⊣ MET	b-Cat_mem[RPTP@0] MET[RPTP@0]	
RPTP → β-catenin RPTP → p120	b-Cat_mem[RPTP@0] p120[RPTP@0]	
RPTP ⊣ MET RPTP → β-catenin RPTP → p120	MET[RPTP@0] b-Cat_mem[RPTP@0] p120[RPTP@0]	
RPTP ⊣ EGFR RPTP → β-catenin RPTP → p120	EGFR[RPTP@0] b-Cat_mem[RPTP@0] p120[RPTP@0]	

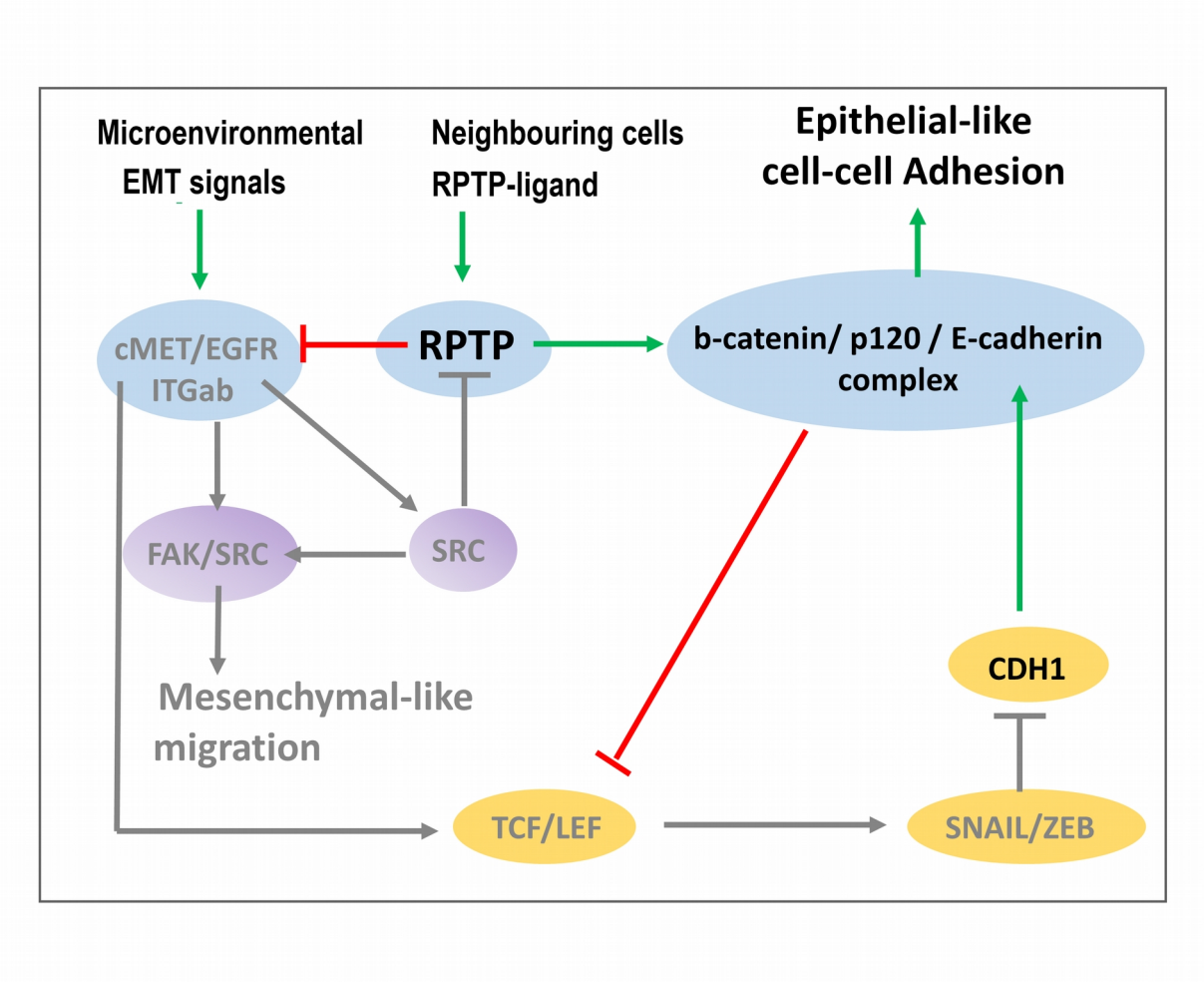


Figure 3. Proposed mechanism of EMT control by RPTP. Red arrows denote active inhibitions and green arrows denote active activations during simulations. Grey arrows indicate inactivity of processes during simulations. Active molecular components and phenotypic traits are indicated in black and inactive indicated in grey.

Discussion

In this work, we showed the potential capacity of RPTPs for 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 [11,12,28]. In addition, it also provides a mechanistic explanation for the observed high cell contact induced MET in cancer cell lines [25,29]. In theory, high cell contact dependent RPTP signal

is achieved in a scenario where a cell has enough neighbour cells expressing RPTP ligands, which would be the case of healthy epithelia [13]. 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 conveys the idea that EMT is tightly controlled based on the demand for Mesenchymal cells, explaining the transient behaviour in wound healing [29]. 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 [19]).

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) [14]. In general, RPTPs are highly expressed in Epithelial cells of most tissues, placing them as plausible candidates for a generic control mechanism of cancer migration and metastasis [13]. However, only a restricted set of RPTPs have been proven to be controlled by cell contact interactions through homophilic or another type of ligand interactions [13]. In cancer, two cell contact dependent RPTPs, the RPTP-k and DEP-1, are often mutated or down-regulated [13,30]. 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 [13,31–33]. 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 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 [26]. 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 epithelium, where cell contacts are high. This would exclude most analysed physiological scenarios based on the identified putative effect of FAT4, which may also prevent EMT but not trigger MET. 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 through 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 [8].

In conclusion, the model analysis in several physiological scenarios illustrated the role of cell contact dependent activation of RPTP a hypothetical mechanism for the microenvironment control of EMT and metastasis. 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 inhibiting the RPTP induced EMT control mechanism. This predicted mechanism is still not proven experimentally but yet pose substantial theoretical support placing it as a good hypothesis to be tested. Moreover, this control mechanism of EMT provides candidate targets towards the 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 is the founder and data scientist of BioenhancerSystems.

References

1. Savagner P. Epithelial-mesenchymal transitions: from cell plasticity to concept elasticity. Curr Top Dev Biol. 2015;112: 273–300. doi:10.1016/bs.ctdb.2014.11.021

2. Kalluri R, Weinberg R a. The basics of epithelial-mesenchymal transition. J Clin Invest. 2009;119: 1420–1428. doi:10.1172/JCI39104.1420

3. Steinestel K, Eder S, Schrader AJ, Steinestel J. Clinical significance of epithelial-mesenchymal transition. Clin Transl Med. 2014;3: 1–13.

4. Micalizzi DS, Farabaugh SM, Ford HL. Epithelial-mesenchymal transition in cancer: parallels between normal development and tumor progression. J Mammary Gland Biol Neoplasia. 2010;15: 117–34. doi:10.1007/s10911-010-9178-9

5. Clark AG, Vignjevic DM. Modes of cancer cell invasion and the role of the microenvironment. Curr Opin Cell Biol. 2015;36: 13–22. doi:10.1016/j.ceb.2015.06.004

6. Panková K, Rösel D, Novotný M, Brábek J. The molecular mechanisms of transition between mesenchymal and amoeboid invasiveness in tumor cells. Cell Mol Life Sci. Springer; 2010;67: 63–71. doi:10.1007/s00018-009-0132-1

7. Landskron G, De la Fuente M, Thuwajit P, Thuwajit C, Hermoso MA. Chronic inflammation and cytokines in the tumor microenvironment. J Immunol Res. 2014;2014: 149185. doi:10.1155/2014/149185

8. Gilkes DM, Semenza GL, Wirtz D. Hypoxia and the extracellular matrix: drivers of tumour metastasis. Nat Rev Cancer. 2014;14: 430–439. doi:10.1038/nrc3726

9. Jolly MK, Boareto M, Huang B, Jia D, Lu M, Onuchic JN, et al. Implications of the hybrid epithelial/mesenchymal phenotype in metastasis. 2015;5: 1–19. doi:10.3389/fonc.2015.00155

10. Denef C. Contact-dependent Signaling. Cell Commun Insights. 2014; 1–11. doi:10.4137/CCi.s12484.TYPE

11. Gao D, Vahdat LT, Wong S, Chang JC, Mittal V. Microenvironmental regulation of epithelial-mesenchymal transitions in cancer. Cancer Res. NIH Public Access; 2012;72: 4883–9. doi:10.1158/0008-5472.CAN-12-1223

12. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol. Nature Publishing Group; 2014;15: 178–96. doi:10.1038/nrm3758

13. Xu Y, Fisher GJ. Receptor type protein tyrosine phosphatases (RPTPs) - roles in signal transduction and human disease. J Cell Commun Signal. Springer; 2012;6: 125–38. doi:10.1007/s12079-012-0171-5

14. Hertog J den, Östman A, Böhmer F-D. Protein tyrosine phosphatases: regulatory mechanisms. FEBS J. Blackwell Publishing Ltd; 2008;275: 831–847. doi:10.1111/j.1742-4658.2008.06247.x

15. Steinway SN, Zanudo JGT, Ding W, Rountree CB, Feith DJ, Loughran TP, et al. Network modeling of TGFβ signaling in hepatocellular carcinoma epithelial-to-mesenchymal transition reveals joint Sonic hedgehog and Wnt pathway activation. Cancer Res. 2014;74: 5963–5977. doi:10.1158/0008-5472.CAN-14-0225

16. Cohen DPA, Martignetti L, Robine S, Barillot E, Zinovyev A, Calzone L. Mathematical Modelling of Molecular Pathways Enabling Tumour Cell Invasion and Migration. PLoS Comput Biol. 2015;11. doi:10.1371/journal.pcbi.1004571

17. Flobak Å, Baudot A, Remy E, Thommesen L, Thieffry D, Kuiper M, et al. Discovery of Drug Synergies in Gastric Cancer Cells Predicted by Logical Modeling. PLoS Comput Biol. 2015;11. doi:10.1371/journal.pcbi.1004426

18. Remy E, Rebouissou S, Chaouiya C, Zinovyev A, Radvanyi F, Calzone L. A modeling approach to explain mutually exclusive and co-occurring genetic alterations in bladder tumorigenesis. Cancer Res. 2015;75: 4042–4052. doi:10.1158/0008-5472.CAN-15-0602

19. Pais RJ. How cells initiate Epithelial-to-Mesenchymal Transition? A computational modelling of cellular and supra-cellular networks to unravel the control of EMT. [Internet]. Universidade Nova de Lisboa. 2018. Available: https://run.unl.pt/handle/10362/61583

20. Thomas R. Regulatory networks seen as asynchronous automata: A logical description. J Theor Biol. Academic Press; 1991;153: 1–23. doi:10.1016/S0022-5193(05)80350-9

21. Kawauchi T. Cell adhesion and its endocytic regulation in cell migration during neural development and cancer metastasis. Int J Mol Sci. Multidisciplinary Digital Publishing Institute (MDPI); 2012;13: 4564–90. doi:10.3390/ijms13044564

22. C. Chaouiya, A. Naldi DT. Logical Modelling of Gene Regulatory Networks with GINsim. Methods in Molecular Biology,. 1st ed. Springer; 2012. pp. 463–479. Available: http://dx.doi.org/10.1007/978-1-61779-361-5_23

23. Bérenguier D, Chaouiya C, Monteiro PT, Naldi a, Remy E, Thieffry D, et al. Dynamical modeling and analysis of large cellular regulatory networks. Chaos. 2013;23: 025114. doi:10.1063/1.4809783

24. Faure A, Naldi A, Chaouiya C, Thieffry D. Dynamical analysis of a generic Boolean model for the control of the mammalian cell cycle. Bioinformatics. Oxford University Press; 2006;22: e124–e131. doi:10.1093/bioinformatics/btl210

25. Ding S, Zhang W, Xu Z, Xing C, Xie H, Guo H, et al. Induction of an EMT-like transformation and MET in vitro. J Transl Med. BioMed Central; 2013;11: 164. doi:10.1186/1479-5876-11-164

26. Xu Y, Shao Y, Voorhees JJ, Fisher GJ. Oxidative Inhibition of Receptor-type Protein-tyrosine Phosphatase κ by Ultraviolet Irradiation Activates Epidermal Growth Factor Receptor in Human Keratinocytes. J Biol Chem. 2006;281: 27389–27397. doi:10.1074/jbc.M602355200

27. Godfrey R, Arora D, Bauer R, Stopp S, Müller JP, Heinrich T, et al. Cell transformation by FLT3 ITD in acute myeloid leukemia involves oxidative inactivation of the tumor suppressor protein-tyrosine phosphatase DEP-1/ PTPRJ. Blood. 2012;119: 4499–511. doi:10.1182/blood-2011-02-336446

28. Jolly MK, Tripathi SC, Somarelli JA, Hanash SM, Levine H. Epithelial/mesenchymal plasticity: how have quantitative mathematical models helped improve our understanding? Mol Oncol. Wiley-Blackwell; 2017;11: 739–754. doi:10.1002/1878-0261.12084

29. Leopold PL, Vincent J, Wang H. A comparison of epithelial-to-mesenchymal transition and re-epithelialization. Semin Cancer Biol. 2012;22: 471–483. doi:10.1016/j.semcancer.2012.07.003

30. Du Y, Grandis JR. Receptor-type protein tyrosine phosphatases in cancer. Chin J Cancer. BioMed Central; 2015;34: 61–9. doi:10.5732/cjc.014.10146

31. Holsinger LJ, Ward K, Duffield B, Zachwieja J, Jallal B. The transmembrane receptor protein tyrosine phosphatase DEP1 interacts with p120(ctn). Oncogene. Nature Publishing Group; 2002;21: 7067–76. doi:10.1038/sj.onc.1205858

32. Palka HL, Park M, Tonks NK. Hepatocyte Growth Factor Receptor Tyrosine Kinase Met Is a Substrate of the Receptor Protein-tyrosine Phosphatase DEP-1. J Biol Chem. 2003;278: 5728–5735. doi:10.1074/jbc.M210656200

33. Xu Y, Tan L-J, Grachtchouk V, Voorhees JJ, Fisher GJ. Receptor-type Protein-tyrosine Phosphatase-κ Regulates Epidermal Growth Factor Receptor Function. J Biol Chem. 2005;280: 42694–42700. doi:10.1074/jbc.M507722200