Computational modeling of cellular dynamics in tumor cell migration

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Summary

Cancer is the second leading cause of death worldwide and has overtaken cardiovascular disease as the leading cause of death in many Western countries, including the Netherlands. Despite the significant progress made in recent decades in the early detection and treatment of cancer, we are still far from a complete understanding of, in particular, the process of metastasis, which is the main contributor to cancer mortality. Epithelial-mesenchymal plasticity (EMP) likely plays an important role in metastasis, with the forward epithelial-mesenchymal transition (EMT) allowing cancer cells to break free from the primary tumor and invade the surrounding tissue and vascular system (*invasion* and *intravasation*), and the subsequent backward mesenchymal-epithelial transition (MET) allowing the formation of a secondary tumor at a distant site (*colonization*). Even though tumors release surprisingly high numbers of so-called circulating tumor cells (CTCs) in the circulation, the vast majority of these CTCs are unable to survive and eventually metastasize, making metastasis less common than one might assume based on the number of CTCs. In recent years, it has become increasingly clear that MET is not an 'all-or-nothing' process but that cells can adopt a hybrid phenotype with both epithelial and mesenchymal characteristics and that precisely these hybrid cells can form clusters of CTCs that have an up to 100-fold metastatic potential compared to single CTCs. It is thus of great importance to get a better understanding of the mechanisms underlying EMP, and in this thesis, we do so through the development and analysis of mathematical and computational models.

First, in **Chapter 2**, we review and summarize various computational approaches applied in EMP research. EMP regulation is extremely complex and depends on hundreds of regulators, but ultimately these regulators converge on a network of so-called EMT transcription factors (EMT-TFs) that suppress epithelial properties and induce mesenchymal properties. Computational models of EMP can therefore be broadly classified into two groups: (1) quantitative models that include a small number of regulators, typically based on ordinary differential equations (ODEs), and (2) qualitative (often Boolean) models that incorporate a high number of regulators. For example, the first group includes ODE models of the so-called 'core' EMT network, consisting of EMT-TFs SNAIL1 and ZEB1. SNAIL1 and ZEB1 are part of the SNAIL, TWIST, and ZEB families of 'core' EMT-TFs, TFs that play a central role during EMT in different biological contexts. In the second group of models, this core regulatory network is typically extended to include more TFs and regulators, and the lack of detailed parameter knowledge then necessitates a more discrete approach. In our literature review, we describe how models from both groups have contributed to an improved understanding of EMP regulation and have also been used to study the relationship of EMP to other cancer hallmarks. In doing so, we noted that the relationship between EMP and immune invasion had received relatively little modeling attention.

Therefore, in **Chapter 3**, we develop an ODE model of the interaction between EMP and PD-L1-mediated immune invasion. PD-L1 on the tumor cell binds with

programmed death 1 (PD-1) on immune cells, and this 'immune checkpoint' blocks the immune system from attacking the tumor cell. PD-1 and PD-L1 checkpoint inhibitors have been successful in the treatment of various cancers but produce no, or only a transient response in a substantial number of patients. Therefore, a better understanding of the mechanisms that influence PD-L1 expression in tumor cells is necessary. We study the interaction between EMP and PD-L1 expression by coupling an existing 'core' model of EMT based on SNAIL1 and ZEB1 with a model of IFNγ-induced PD-L1 expression we develop. The EMT model we use is tristable, which means that there are three stable equilibria with low, medium, and high ZEB1 expression for an epithelial, hybrid, and mesenchymal phenotype, respectively. Analysis of the combined model shows that due to this coupling, the tristability in ZEB1 is mirrored in the expression of PD-L1, with low PD-L1 expression for an epithelial, medium expression for a hybrid, and high expression for a mesenchymal phenotype. In addition, our results also show that this coupling not only makes EMT more likely, but also accelerates it. We conclude that through these mechanisms, EMT may contribute to immune invasion and, additionally, that tumor cells are more likely to undergo EMT due to the presence of an immune response.

In **Chapter 4**, we study an alternative proposed network for EMP regulation based on complementary expression of SNAIL1 and PRRX1. Previous experiments have shown that these EMT-TFs are activated sequentially and have complementary roles in the EMT process: first SNAIL1, a strong transcriptional repressor, causes loss of epithelial properties, and subsequent activation of PRRX1, a transcriptional promoter, furthers EMT by the acquisition of mesenchymal properties. In this chapter, we first develop a generalized ODE model of this network, which we then fit to experimental data of EMT-TFs and EMT markers in the MDCK (NBL-2) cell line. Our analysis shows that this proposed model can only explain the experimental data with the addition of a SMAD–GLI relay, which is required to explain the dynamics consisting of two waves of SNAIL1. Furthermore, we show that PRRX1 is indeed a more potent mesenchymal promoter than SNAIL1; in particular, the expression of the mesenchymal marker ACTA2 is fully explained by PRRX1. Thus, our model provides insight into the role of PRRX1 during EMT, and can be used to further investigate the importance of this transcription factor (TF) during EMT.

A characteristic feature of EMT is the acquisition of a migratory phenotype, which is of great importance during different stages of the metastatic cascade. In **Chapter 5**, we study the migratory behavior of HCC38 and Hs578T, two rapidly migrating and invasive triple-negative breast cancer (TNBC) cell lines. Through analysis of cell migration experiments, we show that with increasing cell density, HCC38 cells migrate faster and more persistently and that at low density, these cells form clusters. Using simulations with the cellular Potts model (CPM) framework, we show that previously published CPM models cannot explain this observed HCC38 behavior. Because we observe high pseudopodial activity in the experiments, we implement realistic pseudopod-driven persistence, in which cells form dendrite-like

protrusions and move in the direction of these protrusions. With this model, we can qualitatively reproduce both the clustering and the observed speed and persistence change, although at high density there is no clustering in the experiments, whereas there is clustering in the simulations. This may indicate that there is less cell-cell adhesion at high densities. Given the high expression of Vimentin, a mesenchymal marker, cluster formation in HCC38 is surprising in itself because cluster formation is typically associated with a hybrid phenotype. It thus seems that HCC38 could indeed be a hybrid cell line, partly because of the high expression of EpCAM, which is associated with protrusion formation. This direct link between EMP and migratory behavior deserves further investigation.

Finally, in **Chapter 6**, we summarize our findings, place them in the context of recent research on EMP, and outline possible directions for follow-up research. Given the complexity of EMP, there is an urgent need for single-cell transcriptomics and proteomics data to develop and validate improved computational models of EMP regulation. However, fortunately, current models do already yield potential therapeutic targets to counteract cancer progression.

Overall, this thesis contributes to understanding the underlying mechanisms of EMP. In doing so, we show that mathematical and computational research has a valuable, and sometimes even indispensable, role in complementing experimental research on EMP.