Computational modeling of cellular dynamics in tumor cell migration

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Discussion

Background graphic: Reproducable R code using `targets` (Landau, 2021) and `renv` (Ushey, 2022) to generate the figures in Chapter 3. Available at `https://doi.org/10.5281/zenodo.6000437` (Burger, 2022).

6.1 Summarizing discussion

Epithelial-mesenchymal plasticity (EMP) and tumor cell migration play an important role in cancer progression, and an improved understanding of the mechanisms underlying these concepts is essential for developing new targeted approaches. In this thesis, we studied these mechanisms using mathematical and computational approaches, which we will discuss in more detail below.

Epithelial-mesenchymal plasticity

First, we summarized and reviewed previous computational approaches that have been used to decipher EMP regulation (**Chapter 2**). EMP regulation is incredibly complex, involving a network of interconnected pathways (De Craene and Berx, 2013) and hundreds of transcription factors (TFs) and microRNAs (miRNAs) (Bracken and Goodall, 2022) that become active in a context-specific manner (Cook and Vanderhyden, 2020). To induce epithelial-mesenchymal transition (EMT), the signals of these pathways eventually converge on a network of EMT-inducing TFs (EMT-TFs) that repress epithelial characteristics and induce mesenchymal characteristics.

Computational modeling of this complex regulation can be roughly categorized into two main categories: (1) quantitative (typically ordinary differential equation (ODE)) models including a limited number of regulators, and (2) more qualitative (e.g., Boolean) models that can include a large number of regulators. Examples of the first category are the Cascading Bistable Switches (CBS) (Zhang et al., 2014) and Ternary Chimera Switch (TCS) (Lu et al., 2013a) models. These models each describe a distinct topological/mechanistic interpretation of the EMT "core" regulatory network consisting of the miR34-SNAI1 and miR200-ZEB1 axes (Nieto et al., 2016), and were implemented using ODEs. Both models predicted tristability of EMT, but whereas in the CBS model, both axes function as bistable switches, in the TCS model, the tristability is due to the miR200-ZEB1 axis, and the miR34-SNAI1 axis functions as a noise-buffering integrator. At the time of writing **Chapter 2**, we concluded that it was hard to distinguish between these two core regulatory models based on the available experimental data, but since then, additional evidence has been acquired. Specifically, SNAIL1 has been shown to be a key signal integrator (Zhang et al., 2018), and the miR200-ZEB1 axis has been demonstrated to be a key hysteresis controller of EMT (Celià-Terrassa et al., 2018). Together, this suggests that the TCS model might be the preferred model, although it remains possible that this is context-specific.

An example of the second category of computational models is the Boolean model by Steinway et al. which includes 70 regulators (Steinway et al., 2014; Steinway et al., 2015). Interestingly, both the TCS model and the Steinway model allow the existence of hybrid EMT phenotypes: the TCS model by intermediate values of zinc finger E-box-binding homeobox 1 (ZEB1), and the Steinway et al. model, which by its Boolean nature cannot have intermediate values, by combinatorial expression of different regulators. This illustrates how these complementary approaches can provide insight

into different mechanisms through which hybrid phenotypes can emerge. Finally, we discussed computational efforts aimed at unraveling the association of EMP with other tumor characteristics. For instance, we highlighted that EMT-related therapy resistance and immune evasion had received only limited modeling attention.

Therefore, in **Chapter 3**, we studied the reported role of EMT in programmed death-ligand 1 (PD-L1)-mediated immune evasion (reviewed by Jiang and Zhan (2020), who emphasize the urgent need for additional mechanistic studies). One of the proposed mechanisms underlying crosstalk between EMT and immune evasion involves post-transcriptional regulation of PD-L1 by the miR200-ZEB1 axis, thus linking PD-L1 directly to a key part of the EMT core regulatory network. To study the expected interactions, we connected a simplified TCS model (Jolly et al., 2016) to a model for interferon gamma (IFNɣ)-induced PD-L1 expression, which we developed based on the JAK–STAT model by Quaiser et al. (2011). We connected these two models by a mutually inhibitory feedback loop between microRNA-200 (miR-200) and PD-L1, described using appropriate miRNA–mRNA dynamics (Lu et al., 2013b). The addition of this feedback loop led to mirrored tristability in PD-L1 expression levels, with low PD-L1 in the epithelial, intermediate PD-L1 in the hybrid, and high PD-L1 in the mesenchymal phenotype. Moreover, we showed that this crosstalk promotes EMT by reducing the amount of inducing signal required. Additionally, we studied the EMT and mesenchymal-epithelial transition (MET) temporal dynamics by running time course simulations where we suddenly increased and decreased the EMT-inducing signal. We found that the EMT–PD-L1 crosstalk accelerated the forward EMT process and, to a lesser extent, decelerated the reverse MET process, mainly by shortening the time spent around the hybrid E/M state. All effects discussed above were amplified by IFNɣ stimulation. Finally, we showed that these findings are consistent with a broad set of published experimental results.

A model by Sahoo et al. (2021b) also described the interaction between EMP and PD-L1. Instead of direct, mutual feedback between miR-200 and PD-L1 using miRNA–mRNA dynamics, they used an indirect feedback mechanism of PD-L1-mediated E-cadherin inhibition. Their model results matched qualitatively with our model in that hybrid E/M cells were expected to be PD-L1 positive and hence immune-invasive. However, there were substantial quantitative differences in the predicted PD-L1 expression levels for the different phenotypes: Sahoo et al. predicted an almost equally high PD-L1 expression for the hybrid and mesenchymal phenotypes, whereas we predicted that PD-L1 expression for the hybrid phenotype is closer to that of the epithelial phenotype. Given the potential context-specific role of the included crosstalk mechanisms, further research will be required to unravel this interplay, and our model forms a basis to include additional relevant factors. Overall, our analysis illustrates how crosstalk between EMP (modeled using the TCS core regulatory model) and IFNɣ-induced PD-L1 production can result in immune evasion and contribute to invasion and metastasis.

In **Chapter 4**, we investigated an alternative regulatory network of EMT proposed

by Fazilaty et al. (2019), in which snail family transcriptional repressor 1 (SNAIL1) and paired related homeobox 1 (PRRX1) were expressed in a complementary manner because of their mutual repression. PRRX1 is of particular interest because, despite being an EMT-TF, its expression is associated with good patient prognosis and lack of metastasis (Fazilaty et al., 2019; Chen et al., 2021). In vitro and in vivo experiments showed that upon transforming growth factor beta (TGFβ)-induction, SNAIL1 and PRRX1 were activated sequentially and had complementary roles: Early activation of SNAIL1, thought to be a strong epithelial repressor, led to the loss of epithelial features such as cell detachment, and subsequent activation of PRRX1, thought to be a strong mesenchymal promoter, led to the acquisition of mesenchymal features allowing cell migration and invasion, completing EMT.

Because we lacked knowledge of the involved kinetic parameters, we used network topology to generate ODE models automatically, in which we used non-linear shifted Hill functions to model inhibition and activation, similar to the RACIPE framework (Huang et al., 2018). We then fitted various models (network topologies) to quantitative PCR (qPCR) data of EMT-TFs and epithelial and mesenchymal markers in Madin-Darby canine kidney (MDCK) (NBL-2) cells, widely used to study EMP, to determine (a), whether the proposed network by Fazilaty et al. (2019) can account for the observed TF and marker dynamics, and (b), whether there is a differential role of SNAIL1 and PRRX1 in the regulation of downstream markers.

We first showed that the proposed regulatory network could not capture the early SNAIL1 peak in the observed two-wave SNAIL1 dynamics and that the addition of a SMAD–GLI relay was necessary to capture the SNAIL1 dynamics. In this SMAD–GLI relay, proposed by Zhang et al. (2018), SMAD initializes, and GLI maintains SNAIL1 expression, such that SNAIL1 functions as an integrator of TGFβ signaling, as suggested by the authors of the TCS model (Lu et al., 2013a). Depending on the duration of TGFβ signaling, this can result in a transient (early response) or sustained SNAIL1 expression in cells (Zhang et al., 2018). The updated regulatory network could also capture the observed EMT-TF dynamics of PRRX1. In contrast to Fazilaty et al.'s suggestion, our analysis predicted inhibition of SNAIL1 by PRRX1 but not of PRRX1 by SNAIL1. However, there are conflicting reports on the SNAIL1-PRRX1 interaction, as Ocaña et al. (2012) report no association between SNAIL1 and PRRX1.

Finally, we investigated the differential role of SNAIL1 and PRRX1 in inhibiting and activating epithelial and mesenchymal markers. In line with earlier reports, we found that PRRX1 is a stronger mesenchymal inducer than SNAIL1 and that SNAIL1 is a slightly stronger epithelial repressor than PRRX1, which we illustrated with virtual knockdown experiments. Of particular interest is the mesenchymal marker actin alpha 2 (ACTA2), which we predicted to be fully explainable by PRRX1. Overall, our work provides mechanistic insight into the role of PRRX1 during EMT and provides support for the SNAIL1-PRRX1 regulatory network that needs further investigation.

Cell migration

A hallmark EMT property is the acquisition of a migratory phenotype, and cell migration plays a crucial role in the metastatic cascade. In **Chapter 5**, we studied the migration of triple-negative breast cancer (TNBC) cell lines HCC38 and Hs578T, two highly migratory and invasive cell lines, using time-lapse microscopy and computational modeling. We were interested in whether the migratory behavior of these cell lines depended on their "social context," i.e., their cell density. To investigate this, we performed 2D cell migration assays using differential interference contrast (DIC) and fluorescence microscopy with cells plated at different densities. For HCC38 yet not for Hs578T, cell density clearly affected cell migration characteristics such as clustering, speed, and persistence. Specifically, HCC38 cells formed tight clusters at low densities, which loosened at high densities; this coincided with increased speed and persistence.

We could not reproduce this density-dependent behavior with cellular Potts model (CPM) simulations using various previously published persistence models. Since we observed high pseudopodial activity in the experimental videos, we implemented pseudopod-driven persistence dynamics by reusing a previously developed CPM extension used to simulate the migration of dendritic-shaped tissue-resident memory T cells (Ariotti et al., 2012). In this model, cells form dendrite-like protrusions that extend and retract, by which they move the cell in the direction of the protrusions. This model could not reproduce the observed density-dependent increase in speed and persistence, despite achieving the observed dynamic clustering. After extending this model with pseudopod-mediated pulling and increased adhesion of pseudopod tips, we could reproduce these features of the experimentally observed HCC38 migratory behavior. Thus, dynamic clustering and pseudopodial dynamics offer an attractive explanation for the observed speed and persistence increase with density. Recent modeling work by Debets et al. (2021) points in a similar direction: They showed that a density-dependent speed and persistence increase occurs for persistently migrating cell clusters of increasing size if the migration direction of cells is influenced explicitly by that of their direct neighbors. Our model is more mechanistic because the collective behavior emerges from the included single-cell pseudopod dynamics. In practice, the approach by Debets et al. may be more suited for large-scale simulations as it is computationally more efficient. Nevertheless, it is an open question how appropriate it is to use clusters of increasing size to rationalize the observed HCC38 density-dependent behavior as tight clusters were primarily observed for the slowly-migrating, low-density cells. In contrast, faster-migrating high-density cells did not display such clustering. An alternative hypothesis that requires experimental testing is that cell-cell adhesion at high densities might be weak, which we showed should also result in higher speed and persistence.

Given the importance of EMT for cell migration, an additional question is what the expected role of EMP is in the observed migratory behavior of HCC38 cells. We had initially considered HCC38 to be a mesenchymal cell line (mentioned as such by

Hollestelle et al., 2010; Kim et al., 2019a), in part because of its high vimentin (VIM) expression. Thus, it was surprising that HCC38 cells sometimes exhibited strong clustering, which could indicate an intermediate EMT phenotype (Bocci et al., 2019). Other evidence pointing to HCC38 having an intermediate phenotype includes its high EpCAM expression (Klijn et al., 2015; Koedoot et al., 2021), which has been reported to trigger dynamic protrusions (Litvinov et al., 1997; Guillemot et al., 2001). Even if HCC38 manifests itself as a hybrid E/M cell type at low cell densities, it could be that these cells become more mesenchymal at high densities. Such density-dependent EMT could be the result of TGFβ production, leading to amplified TGFβ and resulting in a full EMT. A similar phenomenon occurs in MDCK cells: These cells produce latent TGFβ, which becomes activated in subconfluent conditions (Moyano et al., 2010). Thus, the potential occurrence of density-dependent EMT in HCC38 requires further investigation.

Overall, we have shed light on the influence of cell density on the migratory behavior of HCC38 and Hs578T and illustrated how clustering and pseudopodial dynamics, potentially influenced by EMT-related factors, can alter the migratory behavior of these highly invasive TNBC cell lines.

6.2 Perspectives

Research on EMP has grown explosively over the last two decades, with over half of all EMP articles published in the last five years, and again half of those on studies of EMP in the context of cancer (Yang et al., 2020). Given the complexity of EMP regulation, it has been recognized that a combination of experimental and computational modeling is essential to gain a mechanistic understanding (Yang et al., 2020). This has been accompanied by a significant increase in computational studies of EMP, as evidenced by the number of reviews on computational EMP studies in the last five years (Table 6.1).

Table 6.1: Overview of computational EMP reviews since Burger et al. (2017).

Year	Article
2018	"Logical versus kinetic modeling of biological networks: applications in cancer research" — Calzone et al. (2018)
2019	"The basics of epithelial-mesenchymal transition (EMT): a study from a structure, dynamics, and functional perspective" — Das et al. (2019)
	"Investigating epithelial-to-mesenchymal transition with integrated computational and experimental approaches" — Xing and Tian (2019)
	"Quantifying cancer epithelial-mesenchymal plasticity and its association with stemness and immune response" — Jia et al. (2019a)
	"Landscape perspectives of tumor, EMT, and development" — Yu et al. (2019)

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Table 6.1 – continued from previous page

Year	Article
2020	"The physics of cellular decision making during epithelial-mesenchymal transition" — Tripathi et al. (2020)
	"Computational models to explore the complexity of the epithelial to mesenchymal transition in cancer" — Cortesi et al. (2020)
2021	"In silico logical modelling to uncover cooperative interactions in cancer" — Selvaggio et al. (2021)
	"Towards decoding the coupled decision-making of metabolism and epithelial-to-mesenchymal transition in cancer" — Jia et al. (2021)
	"Computational systems-biology approaches for modeling gene networks driving epithelial-mesenchymal transitions" — Katebi et al. (2021)
	"Measuring and modelling the epithelial-mesenchymal hybrid state in cancer: clinical implications" — Jolly et al. (2021)

As Tripathi et al. (2020) showed in their review, EMP is investigated at multiple scales (Fig. 6.1). Most work in this thesis has focused on the small, core networks driving EMT in isolation (**Chapter 4**), or in connection to immune evasion (**Chapter 3**), in a "bottom-up" manner, that is, relying on biological evidence for regulatory interactions to include (see Katebi et al., 2021, for a review on bottom-up and data-driven top-down approaches to EMP). We described these networks using ODE modeling, which is quite challenging because of the large number of kinetic parameters that need to be estimated from literature, calibrated directly using specific experimental data sets, or guessed in case there is no relevant data. An alternative is using the more qualitative Boolean models, in which it is straightforward to include a greater number of regulators (discussed in more detail in **Chapter 2**). Interestingly, attractors (steady

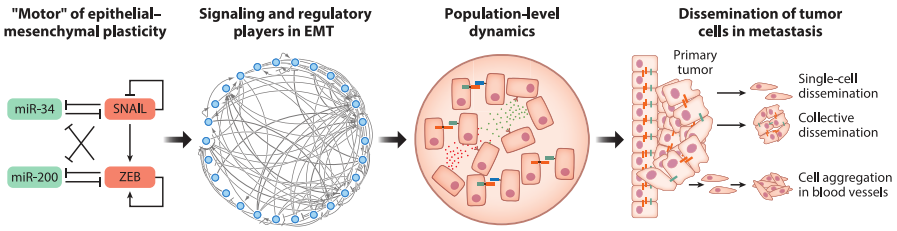

Figure 6.1: Different scales of the dynamics of EMP. A small, core circuit driving EMT and MET operates within a large network, including many EMT regulators. At the population level, EMT dynamics are affected by intercellular communication, mediated via multiple pathways. Finally, EMT-and MET-associated heterogeneity and plasticity determine the mechanism of cancer cell dissemination from the primary tumor. Reproduced from Tripathi et al. (2020), with permission.

states) in these Boolean models are conserved in Hill-type (ODE) models. However, ODE models can reach additional steady states not observed in Boolean models, and they characterize dynamic properties better than Boolean models (Saadatpour and Albert, 2016; Hari et al., 2020).

Under the assumption that network topology rather than specific values of kinetic parameters determines the functionality of a given network, various approaches have been developed to create "in-between" models that do not need detailed knowledge of kinetic parameters yet can provide more quantitative insight into network dynamics. One such approach is the use of "multi-state" Boolean models (Naldi et al., 2018), which have, for instance, been used to study the tristable dynamics in MCF10A (Silveira and Mombach, 2020) and the influence of microenvironmental signals (Pais, 2020) on EMP. Another approach is RACIPE (RAndom CIrcuit PErturbation), in which an ensemble of ODE models is generated using Hill kinetics with randomly chosen parameters (Huang et al., 2017; Huang et al., 2018). RACIPE can be used to predict likely biological functions of a regulatory network without the need for detailed kinetic parameters by identifying gene expression clusters. Besides such identification, this ensemble method can also be used to study environmental heterogeneity and cell-to-cell variability (Kohar and Lu, 2018). Since its inception, RACIPE has been used extensively in computational EMP research (Ramirez et al., 2020; Hari et al., 2020; Deshmukh et al., 2021; Sahoo et al., 2021a; Subbalakshmi et al., 2021), and we used a similar approach in **Chapter 4** to study an EMT core regulatory network involving SNAIL1 and PRRX1.

Another approach from dynamical systems theory that has been applied to EMP research is the landscape approach (Li and Balazsi, 2018; Kang et al., 2019; and reviewed in Jia et al., 2017b; Yu et al., 2019). Inspired by the classic Waddington landscape (Waddington, 2014), in this approach, cells are like balls "rolling" over a landscape consisting of different basins of attraction (different stable EMT states), and the barrier heights between the basins determine the difficulty in switching between these different states.

Nevertheless, despite these increased efforts and novel approaches, there are still many open questions, and we will discuss some of these below.

Deciphering the role of EMT-TFs

Numerous EMT factors control EMP, and they can roughly be divided into three groups: EMT-inducing signals, EMT-TFs, and EMT markers. The SNAIL, TWIST, and ZEB families are considered core EMT-TFs because they play conserved, central roles in EMT in various contexts, whereas other EMT-TFs (e.g., PRRX1) are only implicated in specific contexts (Yang et al., 2020). Therefore, it is no surprise that EMT regulatory networks studied in this thesis included SNAIL1 (**Chapter 4**) or SNAIL1 and Zeb1 (**Chapter 3**); however, it is surprising that we still do not know the exact role of SNAIL1 and ZEB1 in the EMT process. As we discussed in **Chapter 2**, the CBS model predicted a stepwise activation of the miR34–SNAIL1 and miR200–ZEB1 feedback

loops, where miR34–SNAIL1 transitions cells to a hybrid phenotype, and miR200–ZEB1 completes the transition to a mesenchymal phenotype, whereas, in the TCS model, the miR34–SNAIL1 loop functions as a noise integrator and the miR200–ZEB1 loop is responsible for both transitions.

Combined experimental and computational work by Celià-Terrassa et al. (2018) seemed to confirm predictions by the TCS model by presenting the miR200–ZEB1 axis as the key hysteresis controller of EMT. However, it is important to note that this conclusion was reached based on sensitivity analysis of a mathematical model that did not include the miR34–SNAIL1 feedback loop. Moreover, experimental testing was done by CRISPR/Cas9-mediated editing of the miR200–ZEB1 axis only (and not of the miR34–SNAIL1 axis), resulting in a loss of bistability (and thus hysteresis). This loss of hysteresis is not surprising, as the miR200–ZEB1 axis is a source of hysteresis in both the CBS and TCS models. Therefore, a similar edit of the miR34–SNAIL1 axis is required to completely rule out SNAIL1 as a source of hysteresis, especially because the TCS model predicts SNAIL1 tristability, and only loss of bistability was observed. More surprisingly, Celià-Terrassa et al. (2018) noted that changes in EMT markers and ZEB1 induction preceded SNAIL1 induction. This is surprising because SNAIL1 has been described as an "immediate-early response gene" for TGFβ (Cho et al., 2007), exhibiting a two-wave response after TGFβ induction (Zhang et al., 2018) with the first peak within one hour after TGFβ induction (**Chapter 4**). It is thus possible that this early peak was missed in the work by Celià-Terrassa et al. (2018).

Another possibility is that the "core" regulatory network of SNAIL and ZEB is even more context-specific than previously thought, and different cell types have different core regulatory networks, a possibility that we explored in **Chapter 4** by studying the role of PRRX1. Other EMT-TFs whose role in EMP remains to be elucidated are, for example, core EMT-TFs SLUG (SNAIL2) (Chen et al., 2021; Park et al., 2022) and TWIST1 (Kim et al., 2019b; Yang et al., 2020), and phenotypic stability factors (PSFs) such as the miR-200 family, GRHL2, OVOL2, NUMB, and NRF2 (Yaswen, 2014; Coban et al., 2021). Therefore, there is an urgent need for single-cell transcriptomics with sufficient time resolution (especially at early time points) to resolve conflicting reports on the role of different EMT-TFs (e.g., SNAIL1 and ZEB1). Given the post-transcriptional regulation by, for example, different miRNAs, this should be accompanied by measurements on the protein level and ideally also by quantification of cellular properties associated with EMT to couple molecular marker expression to cellular behavior (Yang et al., 2020). This can, for example, be achieved with imaging of reporter cell lines, which, as an additional benefit, also provides spatial information on expression and cellular behavior.

Multiscale modeling of EMP

The modeling work we presented in this thesis has mostly concerned EMP regulation as it is thought to occur inside a single cell with one or two constant inducing signals. However, some open questions regarding EMP require a multiscale modeling

approach, such as the influence of cellular heterogeneity and inter-cellular signaling on EMP, the interaction of tumor cells with the tumor microenvironment (TME), and how EMP shapes dissemination and cell migration dynamics (as Fig. 6.1 illustrates). Given the vast complexity of EMP regulation described in the previous section, it is understandable that most computational work has focused on unraveling EMP regulation itself without adding the additional complexity of multiscale approaches (see Table 6.1 for reviews). Fortunately, recent publications show that such multiscale questions are being investigated; for example, to elucidate the role of EMT on cell detachment at the boundary of the epithelial tissue (Murphy et al., 2021), the role of EMP on the size distribution of circulating tumor cell (CTC) clusters (Bocci et al., 2019), and the coupling of EMP with extracellular matrix (ECM) stiffness (Deng et al., 2021).

Another example where multiscale modeling would be helpful is in studying the interplay between EMP and the immune system because considerable heterogeneity in immune infiltration may be expected across a tumor. In **Chapter 3**, we presented a crosstalk model of IFNγ-induced PD-L1 production and TGFβ-induced EMT. This IFNγ is secreted by $CD8^+$ T cells and can have a long-range effect on bystander tumor cells (Hoekstra et al., 2020), yet this range is likely strongly affected by tumor dependent factors such as expression of galectins that affect IFNγ diffusion by cytokine trapping (Hoekstra et al., 2021). Thus, the effect of IFNγ may be relatively localized in some tumors, which could have consequences for the likelihood of EMT within the tumor as a whole. Moreover, TGFβ is produced by several cell types in the TME, including cancer cells themselves, and we currently have limited knowledge of the heterogeneity of TGFβ expression across tumor regions. Given its immunosuppressive properties (Batlle and Massagué, 2019) and the potential negative feedback to $CD8^+$ T cell effector function, this is also an important determinant to consider in future modeling approaches concerning the relation between EMP and anti-cancer immune responses. Therefore, it is essential to generate detailed data on cytokine signaling, for example, with signaling reporters (as used in Hoekstra et al., 2020), and spatial heterogeneity within the tumor with spatial omics approaches (reviewed in Lewis et al., 2021).

Therapeutic implications

How can the mechanistic insights into EMP presented here be translated into therapeutic opportunities? One might be tempted to target the EMT process itself, but at the time of diagnosis EMT typically has already taken place, and targeting EMT may promote MET in CTCs, thus promoting further colonization. Similarly, targeting MET may also promote EMT and result in an increased rather than a decreased dissemination. In these scenarios, we still fall victim to the incredible plasticity of EMT; thus, it has been proposed to target this plasticity itself (reviewed in Kvokačková et al., 2021). An illuminating argument in favor of this approach comes from the landscape approach: Rather than killing cancer cells (removing cells from landscape

basins), the main goal should be to alter the landscape topography to prevent adverse outcomes. Otherwise, due to perturbations (e.g., drug treatment), it is inevitable that cells eventually return to these basins of attraction (Li and Balazsi, 2018).

An example strategy could be the breaking of positive feedback loops in EMP regulation as these can be the cause of multistability (Hari et al., 2020). Celià-Terrassa et al. (2018) attempted this by editing the miR200-ZEB feedback loop to prevent hysteresis, indeed resulting in decreased metastasis in mice.

Another strategy to develop improved (combination) therapies could be to use mechanistic insight into the relationship between EMP and acquired resistance to various anti-cancer therapies, which are strongly correlated (Brabletz et al., 2021). Indeed, a therapy itself, or a therapy-induced immune response, can cause EMT, potentially to cope with the cellular stress induced by the treatment. Alternatively, such cellular responses to therapies might involve activity that aims to block an anti-cancer immune response, thereby indirectly affecting EMT. For example, antibodies for anti-PD-L1 may affect PD-L1 mRNA (CD274), resulting in increased binding with miR-200, which in turn increases ZEB1 expression and promotes EMT (**Chapter 3**). To circumvent EMT as by-product of therapeutics, therapies could be combined with knockdown of different EMT-TFs as this has been reported to resensitize cancers to different therapies (reviewed in Brabletz et al., 2021). Alternatively, mechanistic insight into the relationship between a treatment and EMP could be used to suggest improvements to existing therapeutic approaches. An example of this is our suggestion in **Chapter 3** to use therapeutic small interfering RNA (siRNA) instead of anti-PD-L1 antibodies because this may free up miR-200 for its anti-EMT effect on ZEB1.

6.3 Conclusion

In conclusion, in this thesis, we used computational modeling to contribute to the understanding of the mechanisms underlying EMP and tumor cell migration. We developed a model for the interaction between EMP and PD-L1-mediated immune evasion; an alternative core regulatory model for EMT consisting of SNAIL1 and PRRX1; and a spatial, pseudopod-driven migration model to explain density-dependent migration of TNBC cell lines. Jointly, our analysis showed that computational modeling can be used to test hypotheses based on experimental data, and generate testable hypotheses, making it a valuable addition to wet-lab experiments. Importantly, we identified mechanisms related to potential therapeutic targets, hopefully leading to improved targeted therapies and reduced cancer mortality.