Computational modeling of cellular dynamics in tumor cell migration

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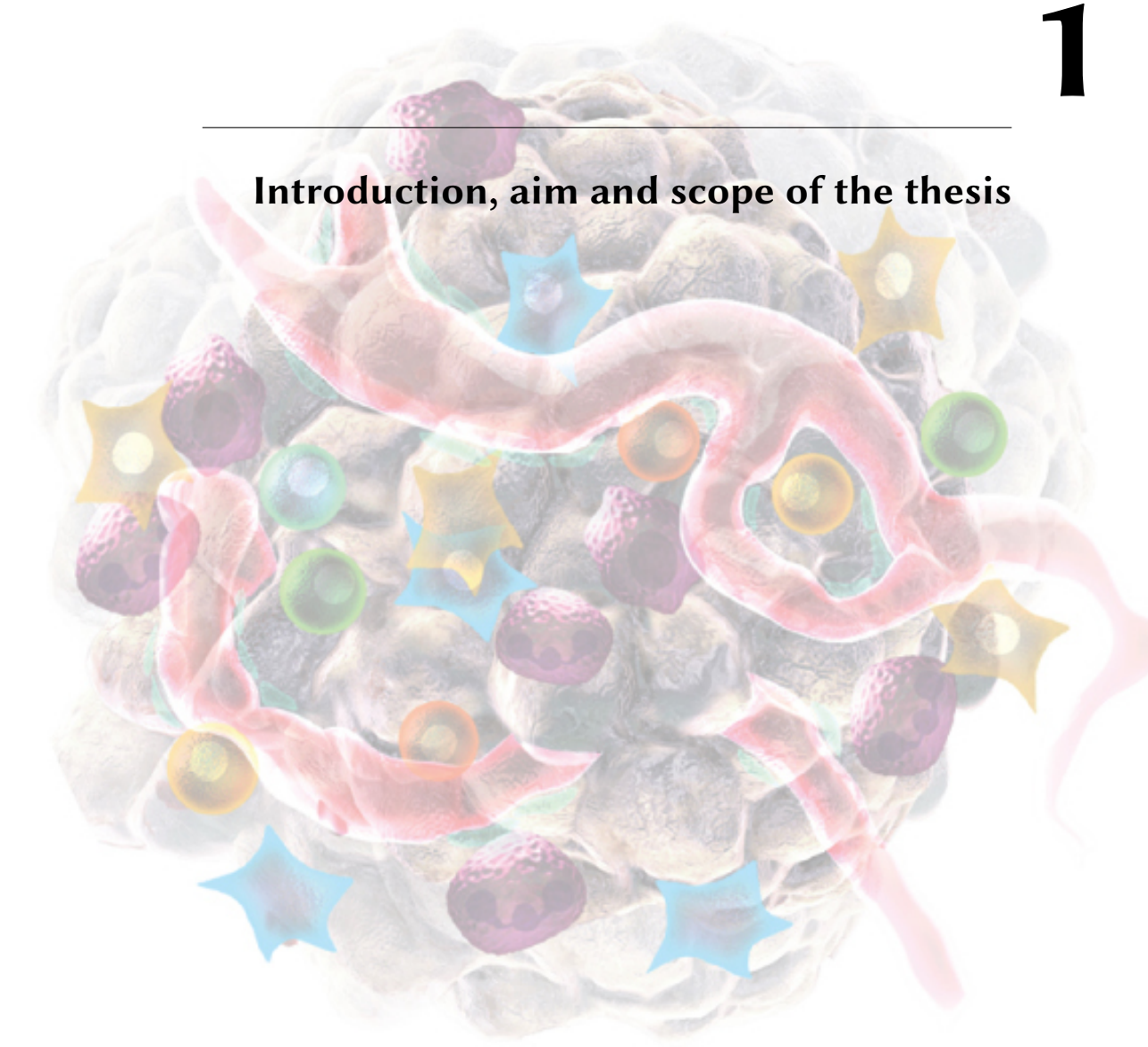
Introduction, aim and scope of the thesis



Background graphic: Tumor microenvironment. Adapted from Cancer Discov (2022) 12 (1): 31–46, D. Hanahan, Hallmarks of Cancer: New Dimensions, with permission from AACR.

1.1 Introduction

Cancer is the second-leading cause of death worldwide (IHME, 2020) and is expected to overtake cardiovascular disease as the leading cause of death in many countries this century (Bray et al., 2021). In 2020, an estimated 19.3 million new cancer cases were diagnosed worldwide, most commonly breast, lung, colorectal, prostate, stomach, and liver cancer (Fig. 1.1, Sung et al. 2021).

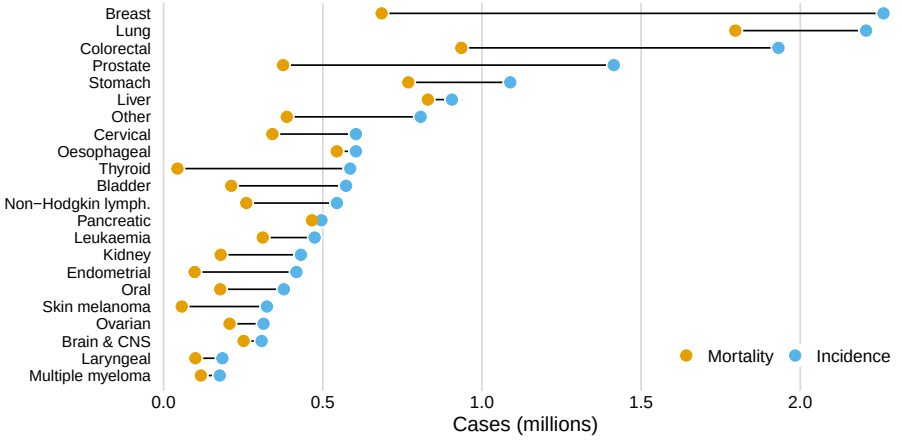


Figure 1.1: Global cancer incidence and mortality in 2020. Based on GLOBOCAN 2020 data (Sung et al., 2021; Ferlay et al., 2020).

Although cancer survival continues to improve, likely due to earlier diagnosis and improved treatment (ICBP SURVMARK-2, Arnold et al., 2019; Santucci et al., 2020), we are still a long way from fully understanding this immensely complex disease. In the "Hallmarks of Cancer" review series (Hanahan and Weinberg, 2000; Hanahan and Weinberg, 2011), of which the third installment was recently published (Hanahan, 2022), Hanahan and Weinberg aim to distill the vast complexity of cancer biology into cancer "hallmarks", functional capabilities that are crucial for human cells to form malignant tumors, and "enabling characteristics", conditions under which cells can acquire these hallmark capabilities (Fig. 1.2). The Hallmarks of Cancer framework has been embraced with much enthusiasm by the community of cancer researchers (Weinberg, 2014), with the previous installments amassing an incredible ~40 and ~60 thousand citations at the time of writing this thesis. However, a point of criticism is that most of these hallmarks are shared by both benign and malignant tumors alike (Lazebnik, 2010; Meirson et al., 2020). Since metastasis is the primary cause of death for the vast majority of cancer patients, it has been argued that "activating invasion & metastasis" should be the defining hallmark for malignant tumors (Fares et al., 2020; Meirson et al., 2020).

In the metastatic cascade of solid tumors, five steps can be distinguished (Fig. 1.3): (1) invasion of cells from the primary tumor in the surrounding tissue, (2) intravasa-

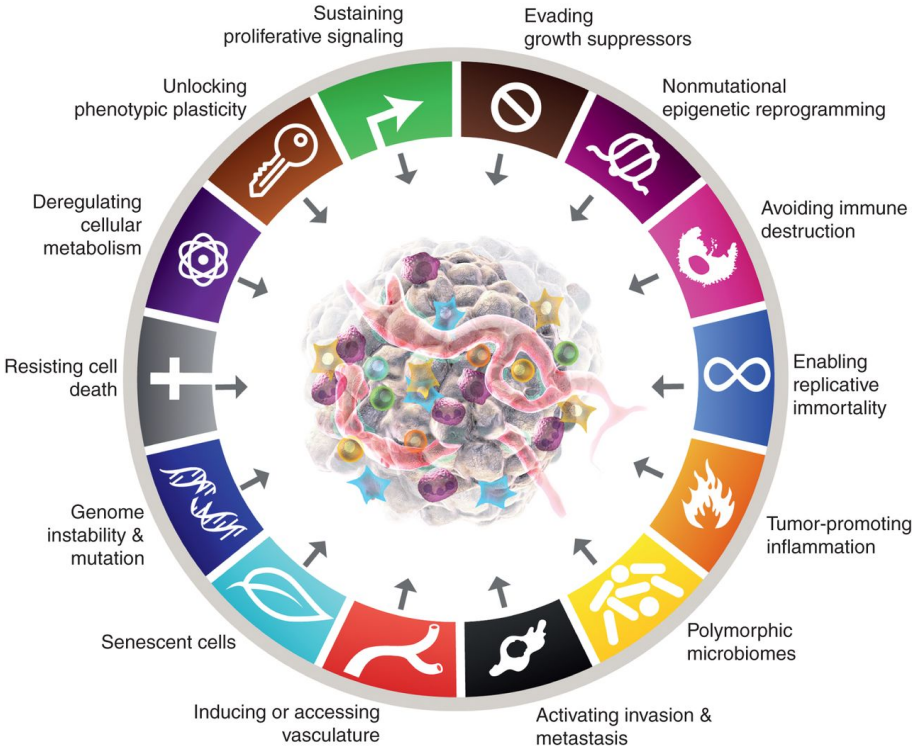


Figure 1.2: Cancer hallmarks and enabling characteristics. Reproduced from Cancer Discov (2022) 12 (1): 31–46, D. Hanahan, Hallmarks of Cancer: New Dimensions, with permission from AACR.

tion of tumor cells into the bloodstream (or lymphatic system), (3) survival and arrest in the circulation, (4) extravasation at a secondary site, and finally (5) colonization (Steeg, 2006). Targeting metastasis is challenging, as several steps (invasion, intravasation, and circulation) usually happen before diagnosis, and therapies successful in the overt metastatic (macrometastatic) setting are not always successful in the adjuvant (micrometastatic) setting (Mina and Sledge, 2011). Instead, current therapeutic approaches often target other cancer hallmarks and reduce tumor cell viability (i.e., are cytotoxic), which, if successful, results in a partial or complete response (i.e., shrinkage or disappearance of all lesions) (Meirson et al., 2020). Anti-metastatic therapies do not necessarily produce such a response (i.e., they are cytostatic). Therefore, despite their metastasis-preventive activity, many are likely to have failed in traditional clinical trials only focused on lesion shrinkage (Steeg, 2016). Importantly, cytotoxic treatments can paradoxically promote metastasis by inducing pro-metastatic states in "post-near-death" cells (Conod et al., 2022), further stressing the need to shift the invasion and metastasis hallmark to the center of anti-cancer research (Meirson et al., 2020).

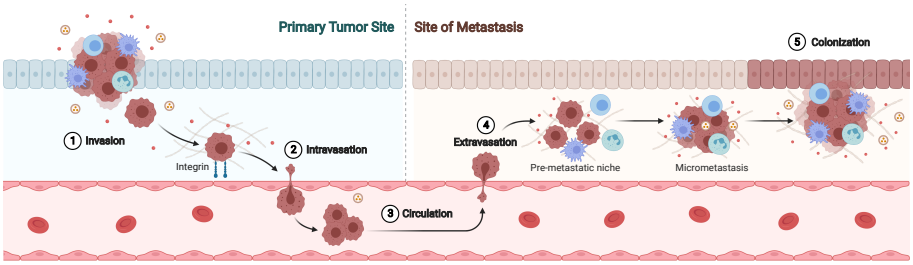


Figure 1.3: Metastatic cascade. For a solid tumor to metastasize, cells need to invade the surrounding tissue (1), intravasate (2), survive the circulation (3), extravasate (4) and colonize (5) at a distant site. Created in BioRender.com.

Epithelial-Mesenchymal Plasticity

Epithelial-mesenchymal transition (EMT) is a cellular process during which epithelial cells convert into motile mesenchymal cells, and plays an important role in development and wound healing (Kalluri and Weinberg, 2009). During this process, cells lose epithelial properties such as cell-cell adhesion and gain mesenchymal features such as front-back polarity and increased cell-matrix interactions (Fig. 1.4; Nieto et al. 2016). Rather than all or none, EMT is now recognized as a plastic process, allowing cells to interconvert between intermediate E/M phenotypes along the EMT spectrum (Panchy et al., 2019; Simeonov et al., 2021), which is best described by the term epithelial-mesenchymal plasticity (EMP) (Yang et al., 2020). There is a vast body of evidence that EMP plays a major role in the metastatic progression of carcinomas (i.e., cancers of epithelial origin) (Williams et al., 2019; Brabletz et al., 2021). In such cancers, EMT allows cells to migrate and invade (Yang et al., 2020), eventually followed by the reverse mesenchymal-epithelial transition (MET), which allows metastatic colonization and outgrowth (Williams et al., 2019; Bakir et al., 2020). Such plasticity is an example of a more general phenotypic plasticity, recently recognized as an additional (emerging) cancer hallmark (Fig. 1.2; Hanahan 2022). Moreover, EMP is linked to several other hallmarks of cancer, such as "deregulating cellular metabolism" and "avoiding immune destruction" (Fig. 1.2, reviewed in more detail in **Chapter 2**). For example, EMT is linked with immunoevasion via immune checkpoint protein programmed death-ligand 1 (PD-L1). In tumors, PD-L1 is primarily upregulated through transcription factor (TF) interferon regulatory factor 1 (IRF1) in response to interferon gamma (IFNγ) signaling (Chen et al., 2012). Apart from the regulation by TFs, PD-L1 expression can also be influenced by various mutations and is also regulated by microRNAs (miRNAs) (Bruns and Beltman, 2022). With respect to the latter, the miR-200 family plays the most notable role, because its members repress master EMT regulators (Bracken et al. 2016; and studied in **Chapter 3**).

It is also becoming evident that hybrid EMT phenotypes, displaying mixed epithelial and mesenchymal features, are particularly associated with cancer progression and metastasis (Derynck and Weinberg, 2019; Simeonov et al., 2021; Lüönd et al.,

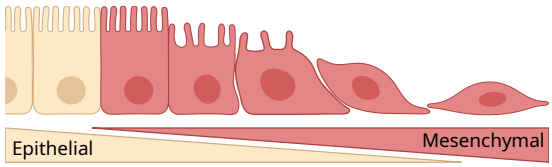


Figure 1.4: Epithelial-Mesenchymal Transition. During EMT, cells lose epithelial properties, such as cell-cell adhesion, and acquire mesenchymal properties, such as a solitary, highly migratory phenotype. Created in BioRender.com.

2021). However, due to the context-specific involvement of hundreds of TFs and miRNAs, understanding of EMP regulation remains limited (Cook and Vanderhyden, 2020; Bracken and Goodall, 2022).

Cell migration

A key characteristic of EMT is the acquisition of a migratory phenotype, which plays an essential role in several steps in the metastatic cascade (Stuelten et al., 2018). Apart from the long-range translocation of cells from the primary tumor to potential metastatic sites, cell migration is also important for the short-range dispersal of cells within the tumor, thus allowing accelerated tumor growth (Waclaw et al., 2015; Gallaher et al., 2019). Cancer cells display a wide variety of migratory behaviors, which can be classified into two main groups: individual (mesenchymal and amoeboid) and collective migration, and cells can interconvert between these migration modes (Fig. 1.5; Friedl and Wolf 2010). So far, most biophysical studies of cell migration have focused on either single-cell migration or collective migration of confluent tissues, and migration of cell clusters has received limited attention (Debets et al., 2021). A partial EMT (pEMT) can result in such circulating tumor cell (CTC) clusters, because cells can acquire mesenchymal traits without (completely) losing epithelial traits, such as cell-cell adhesion (Brabletz et al., 2018). These CTC clusters are of particular interest as they can have a 20- to 100-fold increased metastatic potential compared to single CTCs (Aceto et al., 2014; Schuster et al., 2021). Moreover, the expression of genes associated with migration is negatively associated with breast cancer survival (Nair et al., 2019), further illustrating that a detailed understanding of (collective) cancer cell migration is essential to obtain insight into cancer progression and metastasis (Stuelten et al., 2018).

The role of computational models

Due to immense experimental efforts, there has been considerable progress in our understanding of EMP and cell migration. In recent decades, also computational and mathematical modeling have made a significant contribution to this understanding, as we and others have reviewed (e.g., for EMP see **Chapter 2** (Burger et al., 2017) and

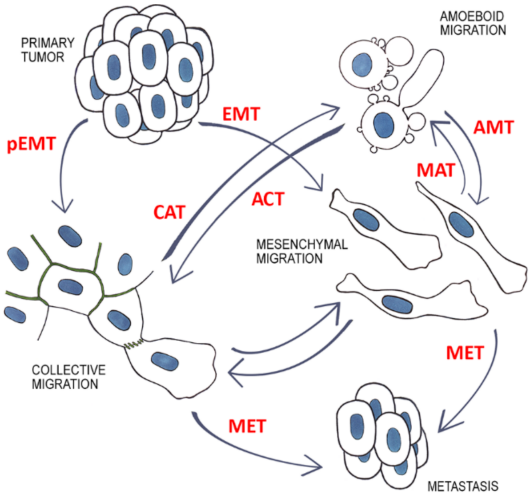


Figure 1.5: Cell migration modes, their transitions, and their hypothesized role in the metastatic cascade. (p)EMT = (partial) Epithelial-Mesenchymal Transition, ACT = Amoeboid-Collective Transition, and AMT = Amoeboid-Mesenchymal Transition; and their reverse MET, CAT, and MAT transitions. Adapted from Rubtsova et al. (2021).

Jolly et al. (2017); for cell migration see Van Liedekerke et al. (2015), Te Boekhorst et al. (2016), and Buttenschön and Edelstein-Keshet (2020)).

Cancer research benefits from computational modeling in several ways: First, computational modeling can be used to verify hypotheses that arise from data interpretation by constructing a minimal model that incorporates mechanistic descriptions of the hypothetically involved processes. Second, computational models can be used as hypothesis-generating machines, providing hypotheses and predictions that can be verified experimentally, guiding experimental research into promising directions. This, in turn, should lead to improved computational models, synergistically accelerating research. Third, it is envisioned that these models will play a crucial role in personalized medicine, for example, by using patient data to create "digital twins" that can be used to adjust treatment and monitor response using real-time data (Hernandez-Boussard et al., 2021). Last but not least, computational modeling is an animal-free, relatively environment-friendly, and (with no shortage of pre-existing data) cost-effective approach to generate new knowledge.

Depending on the application, different model formalisms can be employed. To model gene regulatory networks (GRNs), quantitative ordinary differential equation (ODE) models are typically applied to model small networks, and qualitative logic-based models, such as Boolean models, are typically applied to model large networks containing many regulators (see also Calzone et al., 2018). To model multiple cells in a spatial context, one can use agent-based models (ABMs) (Gorochowski, 2016), either off- or on-lattice, where the cellular Potts model (CPM) is an example of the

latter. Furthermore, various hybrid and multiscale approaches have been developed that combine the above-mentioned approaches. This allows the study of interactions between different mechanisms at the same scale, or how dynamics at a finer scale lead to emergent behavior at a coarser scale. This approach is often used in developmental biology, where the CPM has been combined with ODEs and partial differential equations (PDEs) to model intracellular (e.g. protein expression) and extracellular (e.g. chemical signal) dynamics (Boas et al., 2018).

In this thesis, we develop ODE models of EMP regulation and a CPM model of tumor cell migration. With respect to modeling of EMP regulation, we are particularly interested in how cells can undergo a stable partial EMT, as the hybrid E/M phenotype is especially associated with cancer progression and metastasis. Results from earlier modeling work have shown that multistability (i.e., the existence of multiple stable states in a regulatory network) can explain the existence of a stable partial EMT phenotype, as we discuss in more detail in **Chapter 2**.

We implement biologically-inspired mechanisms in both our ODE and CPM modeling approaches to discover which combination is minimally sufficient to explain experimentally-observed cancer biology. If significant inconsistencies between observations and model output exist, this points to alternative mechanisms not yet considered, leading to new hypotheses and directions for future (experimental) work.

1.2 Thesis outline

In the first part of this thesis (**Chapters 2 to 4**), we study the GRNs underlying EMP, and the mechanisms behind its relationship with immunoevasion. In the second part of this thesis (**Chapter 5**), we study cell migration of triple-negative breast cancer (TNBC) cells. The thesis concludes with a discussion of the findings and limitations of our work and potential future research directions (**Chapter 6**).

In **Chapter 2**, we review different computational approaches to model EMP and its relationship to other tumor characteristics. We first focus on the "core" regulatory network of EMT, which consists of the miR34-SNAI1 and miR200-ZEB1 axes, driven by EMT-inducing signals such as transforming growth factor beta (TGFβ), for which competing models exist (Jia et al., 2017a). Subsequently we review extensions to this core network with other EMT-TFs and phenotypic stability factors (PSFs) that can stabilize cells in a particular (e.g., hybrid) phenotype (Yaswen, 2014). Moreover, we review systems-level approaches that include a large number of regulators. Finally, we discuss EMP in relation to other tumor characteristics such as stemness, metabolism, and immune evasion.

In **Chapter 3**, we study the interaction between EMP and immune resistance by developing an ODE model of the crosstalk between TGFβ-induced EMT and IFNγ-induced PD-L1 expression. We show that the multistability of zinc finger E-box-binding homeobox 1 (ZEB1) in our EMP model is mirrored in the PD-L1 expression levels, which are further amplified by IFNγ stimulation. In addition, we show that

IFNγ stimulation allows cells to undergo EMT for lower amounts of inducing TGFβ, accelerates EMT, and decelerates MET.

In **Chapter 4**, we develop an ODE model of an alternative GRN of EMT that was recently proposed by Fazilaty et al. (2019). Specifically, we consider the interplay between the EMT transcription factors snail family transcriptional repressor 1 (SNAIL1) and paired related homeobox 1 (PRRX1) and their role in the regulation of downstream targets. We first show that the addition of a SMAD–GLI relay to this network is required to describe the "two-wave" expression of SNAIL1 observed in Madin-Darby canine kidney (MDCK) NBL-2 cells. Next, we extend our model to include interactions between the EMT-TFs and epithelial and mesenchymal markers. Consistent with published data, we show that these TFs have a differential role in inhibiting epithelial and activating mesenchymal features. Here, SNAIL1 primarily inhibits epithelial-related genes, whereas PRRX1 primarily promotes mesenchymal-related genes.

In **Chapter 5**, we study the migratory behavior of TNBC cell lines HCC38 and Hs578T at various cell densities. Analysis of 2D random cell migration experiments reveal a counter-intuitive increase in speed and persistence with increasing density in HCC38 cells. Moreover, HCC38 cells exhibit strong cluster formation and high pseudopod activity, especially at low densities. We develop a CPM with explicit pseudopod dynamics to obtain a mechanistic understanding of the observed behavior. We show that pseudopods exerting a pulling force on the cell and interaction via increased adhesion at pseudopod tips can explain the experimentally observed increase in speed and persistence with increasing density in HCC38 cells.

Finally, in **Chapter 6**, we summarize all findings discussed in this thesis, discuss their limitations and implications, and provide directions for future research.