TGFβ signaling in cancer progression

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Chapter 1

General introduction

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Cancer is a large diverse group of genetic diseases that can be triggered virtually everywhere in our body. Cancer cells differ from normal cells in that they are frequently shaped abnormally, grow uncontrollably, pass through their usual boundaries to invade, survive in blood circulation and metastasize to other organs or tissues (1). Among all cancers, breast cancer is the most prevalent cancer in females worldwide, impacting approximately 2 million women each year and leading to a huge number of cancer-related deaths (1). Distant metastasis is the cause of almost 90% of breast cancer-related deaths (2). Once metastases have been triggered, current therapies frequently fail to provide durable treatments (3). Cancer patients may present themselves in the clinic when their cancer has already spread to other tissues or organs. Therefore, a better understanding of the underlying mechanisms of the key initial steps in the metastasis process is needed to find new biomarkers for early diagnosis, make existing standard chemo/radiotherapy more effective and less toxic, develop (new and combinatorial) targeted therapies that provide long-lasting effects and increase the number of cancer patients who respond to immune therapies.

The cytokine transforming growth factor-β (TGFβ) is frequently produced at high levels by breast tumors and correlates with poor prognosis (4). TGFβ is a strong driver of epithelial-mesenchymal transition (EMT), which plays an important role in mediating cancer cell migration, invasion and metastasis (Figure 1) (5). Cancer cells with a mesenchymal phenotype are also more prone to become chemotherapy resistant than cancer cells with an epithelial phenotype (6). Combinatorial targeting or subsequent interference with TGFβ signaling after radiotherapy/chemotherapy has been shown to make cancers more responsive or regain responsiveness to therapy (7). TGFβ not only acts directly on cancer cells in the late stages of tumorigenesis but also manipulates the microenvironment to create a favorable niche for rapid tumor growth and metastasis by stimulating angiogenesis, activating cancer-associated fibroblasts and suppressing the immune system (8).

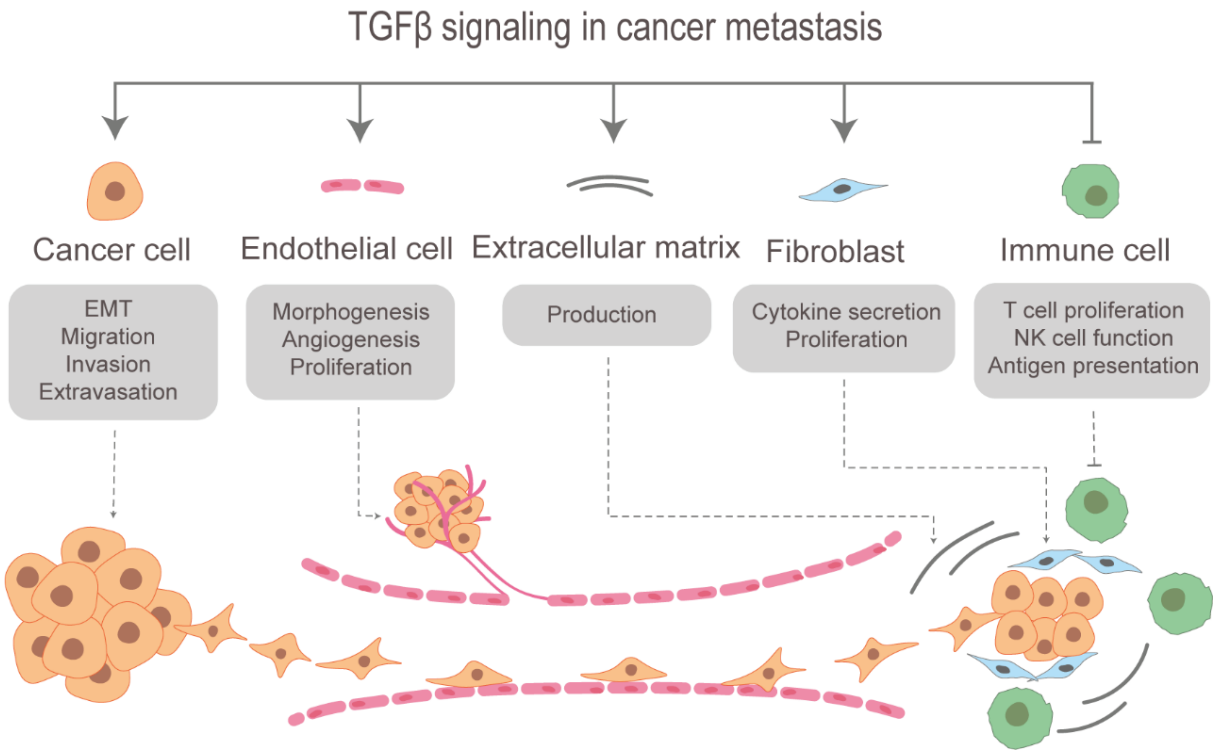

Figure 1. The role of TGFβ signaling in cancer metastatic progression. TGFβ promotes epithelial-mesenchymal transition (EMT), migration, invasion and metastasis of late-stage cancer cells. The TGFβ signaling networks between cancer cells and the microenvironment (fibroblasts, immune and endothelial cells) contribute to cancer metastasis by blocking the immune system, stimulating angiogenesis and promoting cytokine secretion and extracellular matrix production.

Breast cancer is a highly heterogeneous disease that can be classified into different subtypes based on histological and molecular characteristics (Figure 2). Based on the cellular origin from which the tumor evolved, cancer can be classified as (i) carcinoma, when derived from epithelial cells, or (ii) sarcoma, when derived from stromal parts. Based on gene expression profiling, breast cancer can be classified into five major molecular subtypes: luminal A and luminal B (expressing the estrogen receptor (ER)), human epidermal growth factor receptor 2 (HER2) and basal-like (9). Different subtypes show different clinical features; for example, basal-like breast cancers are more aggressive than luminal-like breast cancers (10), and ER-negative breast cancers are more aggressive than ER-positive breast cancers (11). However, among all breast cancer cases, triple-negative breast cancer (TNBC) is the most aggressive subtype, accounting for 12-17% of total breast cancers. TNBC lacks amplifications of ER, progesterone receptor (PR), and HER2 (12). As TNBC does not respond to anti-hormonal therapies and has a low response to chemotherapy/radiotherapy, TNBC remains the most challenging subtype to treat (13). Therefore, there is an unmet need for clinically meaningful molecular targets and effective pharmacological inhibitors to improve the therapy of TNBC patients.

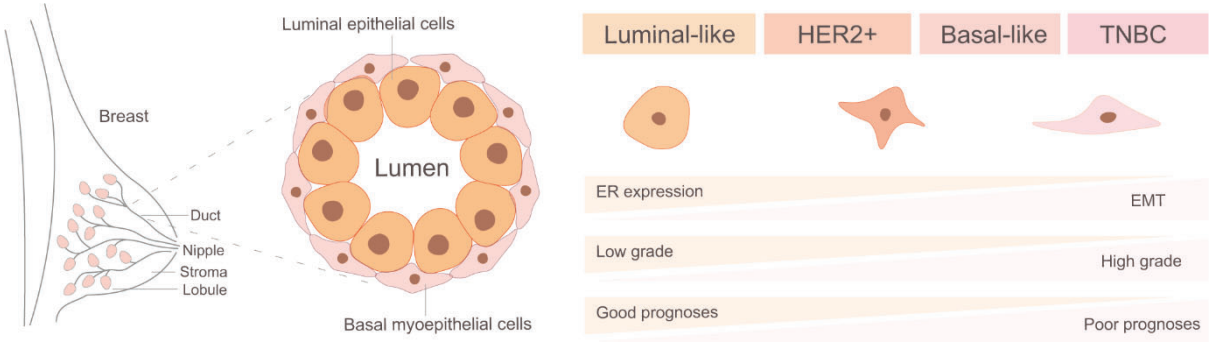

Figure 2. Histological and molecular characteristics of breast cancer. The histological subtype described in the left panel is ductal carcinoma, the most frequent subtype of breast cancer. The molecular characteristics of different breast cancer subtypes described on the right are important indicators for clinical prediction and therapy.

Ubiquitination is emerging as an important posttranslational modification for regulating protein stability, localization and functions in cancer cells (14). Ubiquitination is regulated by E1, E2 and E3 enzymes and reversed by deubiquitinases (DUBs). In humans, there are around 100 DUB family members, and some of them have been discovered to play pivotal roles during cancer progression (15). A catalytic cysteine in the catalytic domain is present in most DUBs, which renders them attractive targets for small-molecule drug development (16). The first clinical drug to target the ubiquitin system for cancer therapy was *bortezomib* (*Velcade*), which is a proteasome inhibitor that has been successfully applied in the treatment of multiple myeloma and mantel cell lymphoma (17). Currently, first-generation DUB inhibitors are undergoing clinical trials (16). The discovery of DUB activity-based probes

(ABPs) provides important tools to obtain fundamental new insights into DUB function and for drug discovery and development (16,18).

Melanoma is the most aggressive skin cancer in the world, with more than 60 thousand deaths in 2018 (19). Somatic mutation of BRAF (V600E) is often found in metastatic melanoma with poor prognosis (20). The clinical application of BRAF inhibitors such as *vemurafenib* (*Zelboraf*) and *dabrafenib* (*Tafinlar*) has significantly increased the median survival of metastatic melanoma patients by approximately 6 months (21-22). However, clinical trial data have demonstrated that 40% of patients develop drug resistance, for which the underlying mechanism remains unclear (21-22). Recent studies found elevated TGFβ signaling in drug-resistant melanoma with BRAF mutations, but the potential for targeting TGFβ signaling in the treatment of drug-resistant melanoma was not investigated.

In this thesis, I start with a general introduction in **Chapter 1** to introduce the general role of TGFβ signaling during cancer progression. In **Chapter 2**, I provide a mechanistic overview of all the DUBs that have been shown to impact the TGFβ signaling pathway in cancer and discuss the therapeutic value of DUB inhibitors for cancer treatment. In **Chapter 3**, we provide detailed working protocols for studying the metastasis of breast cancer cells in zebrafish xenograft models. In **Chapter 4**, I provide details on DUB activity profiling experiments, in which we found UCHL1 as a potential tumor-promoting protein that facilitates TGFβ-induced TNBC metastasis. In particular, we focus on UCHL1 as a new therapeutic target and demonstrate its promise in the stratification of breast cancer subtypes. In **Chapter 5**, we describe our development of an activity-based probe for monitoring UCHL1 activity in live cells and zebrafish embryos. In **Chapter 6**, we investigate the feasibility of targeting TGFβ signaling in BRAF inhibitor-resistant melanoma. In **Chapter 7**, I summarize all the studies in the thesis and provide some future projects related to our results.

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