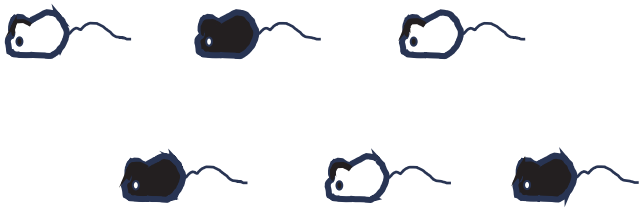
Dissecting the immune microenvironment of breast cancer

Ciampricotti, M.

Chapter 6



Addenda

English Summary

The heterogeneous nature of cancer, inter- and intra-tumor heterogeneity, is not only a consequence of aberrant mutations but also of the composition and activation states of the inflammatory tumor microenvironment (TME). The TME does not only consists of cancer cells but it also contains fibroblasts, endothelial cells, and immune cells. These cells secrete inflammatory mediators, such as elements of the extracellular matrix, metabolites, cytokines, and chemokines, that play a vital role in the cancer cells' ability to grow and metastasize. The immune system has shown to be an important player in tumorigenesis, being able to attack and kill cancer cells but also to promote tumorigenesis. To date, it remains largely unclear why certain tumors elicit anti-tumor immune responses whereas other tumors elicit pro-tumor immune responses or are not regulated by the immune system at all. For the development of therapeutic strategies that target the tumor-associated stroma, it is important to understand how cancer-promoting and cancer-inhibiting immune responses are regulated.

Chemotherapy is the mainstay treatment for most cancer types. Chemotherapeutic drugs do not only kill tumor cells but also influence the number and the phenotype of immune cells. Understanding how different chemotherapeutic agents impact the immune system could facilitate the rational design of combination therapies and thereby increase chemo-responsiveness and/or prevent chemo-resistance.

Macrophages are frequently the most abundant immune cell type present in cancer and represent key orchestrators of various tumor-promoting processes. Therefore, macrophages have become interesting putative targets for therapeutic intervention. Immunomodulatory agents that target macrophage function via CSF-1/CSF-1R signaling have recently been developed and are currently being tested in clinical trials. To maximize the clinical success of therapies targeting macrophage function, we need to understand what the exact mechanisms are by which these agents lead to therapeutic benefit and identify the optimal partner among existing conventional anti-cancer therapies to maximize their efficacy.

The scope of the work in this thesis (outlined in **Chapter 1**) is to advance the development of anti-cancer therapies by understanding the impact of the immune system on breast tumorigenesis and chemotherapy response. This involves the assessment of the adaptive immune response in tumorigenesis

of HER2+ breast cancer, the assessment of the involvement of the adaptive immune system in chemotherapy response, and the evaluation to optimize macrophage-targeted based therapies in breast cancer genetically engineered mouse models (GEMMs). In **Chapter 1**, I also review the current understanding of the paradoxical roles of adaptive immune cells and macrophages in tumorigenesis and chemotherapy response. Furthermore, I discuss how to further understand the inherent complexity of the immune system in cancer for the identification of novel prognostic and predictive biomarkers, and for the design of novel immunomodulatory treatment strategies to fight cancer.

Adaptive immune response in HER2+ breast tumorigenesis

Different cancer types and subtypes have been described to be regulated differently by the adaptive immune system. In **Chapter 2**, we assess the impact of the adaptive immune system on HER2+ breast cancer during (pre-) malignant progression and pulmonary metastasis formation in MMTV-*NeuT* transgenic mice. By genetically eliminating the adaptive immune system from the transgenic MMTV-*NeuT* mouse model via intercrossing with *Rag2*$^{-/-}$ mice, lacking B and T lymphocytes, we reveal that, unlike other breast cancer subtypes, spontaneous HER2-driven mammary tumorigenesis and metastasis formation are neither suppressed nor promoted by the adaptive immune system.

Chemotherapy and adaptive immune responses in GEMMs

Tumor cell line transplantation models have shown that the adaptive immune system dictates the therapeutic efficacy of certain chemotherapeutics. Studies have elucidated major limitations of tumors produced by inoculation of cancer cells as they do not resemble de novo tumors with co-evolving tumor-host interactions and an immunosuppressive microenvironment. In **Chapter 3** of this thesis we generated T and B cell-deficient spontaneous mammary tumors of MMTV-*NeuT* and *K14cre; Cdh1*$^{F/F}$*; Trp53*$^{F/F}$ mice by intercrossing both mouse tumor models with *Rag*$^{-/-}$ mice. We evaluated the capacity of the adaptive immune system response to various conventional chemotherapeutic drugs. We describe that in both mammary tumor models, lack of T and B cells did not affect chemotherapy response. In **Chapter 3**, we then highlight that the role of the endogenous adaptive immune system

in chemotherapy response might not be as crucial as proposed previously when using tumor cell line transplantation models.

Targeting macrophages as anti-cancer therapy

Macrophages have been shown to counteract the anti-cancer effects of chemotherapeutic drugs. In **Chapter 4** of this thesis we show that targeting macrophages, by using CSF-1R inhibition in the non-immunogenic spontaneous *K14cre; Cdh1$^{F/F}$; Trp53$^{F/F}$* model for breast cancer, enhanced chemotherapy efficacy in a drug-dependent manner. We observed that anti-CSF-1R synergizes with platinum-containing drugs but not with docetaxel. In addition, we identified that CSF-1R inhibition stimulated intratumoral type I interferon signaling which is essential for the synergistic anti-cancer effect of cisplatin and the anti-CSF-1R combination. Finally, we show that also targeting immunosuppressive neutrophils in this setting was essential to unleash an effective anti-tumor immunity. In conclusion, in **Chapter 4** our findings underscore the potential of targeting macrophages and neutrophils to improve the therapeutic outcomes of chemotherapy in breast cancer, leveraging the activation of intratumoral type I interferon signaling and unleashing a robust anti-tumor immune response.

Concluding remarks and future perspectives

Chapter 5 contains the general discussion where I contextualize the findings of this thesis with the current literature and propose clinical implications based on our findings. This thesis is focused on the use of genetically modified mice and immunotherapeutic treatments to obtain an immunological relevant understanding of breast tumorigenesis and chemotherapy response for the development of anti-cancer therapies. As such the work presented may improve clinical applications of immunomodulatory therapies targeting macrophages.