Remote control: the cancer cell-intrinsic mechanisms that dictate systemic inflammation and anti-tumor immunity

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Appendices

English summary

Tumors are not just a collection of cancer cells, but comprise a large variety of cell types, which include immune cells. Cells in this tumor microenvironment are in constant crosstalk with each other and this cellular interplay has profound effects on the behavior of cancer cells within the tumor. As such, tumors can be considered complex ecosystems. Furthermore, tumors have a systemic component as well: they can signal long distances using soluble molecules, hijack non-neoplastic cells (such as immune cells) throughout the body for their own benefit, and cancer cells themselves can spread throughout the body in a process called metastasis. The phenotype of immune cells in tumors and in systemic environments is a key determinant of cancer progression and response to therapy.

The immune response to cancer is dualistic. Similar to its role in pathogenic infections, the immune system exerts cytotoxic functions in tumors. When properly activated, cell types such as T cells and NK cells are able to potently kill tumor cells in in vitro cell culture, murine model systems and in cancer patients. However, tumors often induce chronic inflammatory conditions, which are dominated by cells that promote cancer cell proliferation and migration, blood vessel development and suppression of cytotoxic immune responses; processes that benefit cancer development and progression. These mechanisms are mainly facilitated by cell types such as neutrophils and macrophages. In some cancer types, such as breast cancer, these immune cell types dominate the non-cancerous cellular compartment of the tumor, often resulting in an impaired anti-tumor immune response. Therefore, understanding the mechanisms that dictate the composition and activation state of the immune system in primary and metastatic breast cancer is important to improve treatment.

This thesis aims to understand what governs the tumor-immune ecosystem. We argue that cancer-intrinsic genetic aberrations, mutations that drive tumorigenesis, can have a dominant role in determining the tumor immune contexture, as well as systemic inflammatory activation. Understanding the intricate connection between the genetics of breast cancer and anti-tumor immune responses will help develop personalized immune intervention strategies for cancer, tailored to the genetic makeup of a patient's tumor. Moreover, we examine in detail the role of cancer-induced systemic inflammation, which is dominated by neutrophils, in the progression and spread of breast cancer. While tumors can be highly heterogeneous in nature, we show that neutrophils themselves also have a tremendous phenotypic diversity. Mapping this heterogeneity in neutrophil phenotypes may help to utilize these cells in cancer immunotherapy.

Chapter 2 examines how the study of cancer evolved from a genetic point-of-view, to the insight that the immune system plays a key role in tumor development and progression, to the synthesis of these two notions; that tumor genetics can shape the tumor immune environment. How this relationship between the tumor genetic makeup and immune activation can be exploited therapeutically is revealed in **chapters 3** and **4**. In **chapter 3**, we have uncovered a novel role for tumor suppressor gene p53 in controlling systemic inflammation. By examining a panel of genetically engineered mouse models for breast cancer, we show that when tumors have a genetic deletion of p53, a signaling pathway called WNT is activated in cancer cells, which alters their crosstalk with intratumoral macrophages. This crosstalk sets about a cascade of immunological signaling events that drives the expansion and activation of pro-metastatic neutrophils. These neutrophils promote metastasis by creating an immunosuppressed environment in the lungs of tumor-bearing mice, thus enabling

metastatic colonization. Paracrine WNT signaling can however be therapeutically inhibited, and treating mice with p53-null tumors reduced systemic neutrophilia and metastasis.

In **chapter 4**, we demonstrate that specific mutations in p53 induce distinct immune phenotypes in mouse and human mammary tumors. Moreover, tumors with p53 mutations that induce a T cell-enriched phenotype respond to immunotherapy, while tumors with p53 mutations that induce a T cell-depleted phenotype do not. The molecular mechanism underlying these distinct responses to immunotherapy relies on the activation of autophagy. The work described in these two chapters is a testament to the use of tumor genetics in improving immune-based therapeutics for breast cancer.

Studying the relationship between cancer cells and their microenvironment can be performed in a variety of models, including genetically engineered mice, but also partly rely on reductionistic models. In **chapter 5**, we detail a method for studying the crosstalk between macrophages and breast cancer cells in vitro, as was utilized in **chapters 3** and **4**.

Chapter 3 demonstrates a metastasis-promoting role for systemic neutrophilia. While the tumor immune landscape can be heterogeneous in composition, neutrophils themselves also show highly heterogeneous phenotypes in the context of cancer. **Chapter 6** discusses the diverse roles that neutrophils can have in tumorigenesis, cancer development and metastasis, as well as the potential clinical utility of these cells. **Chapter 7** discusses work regarding a potential neutrophil-targeting strategy: metabolic reprogramming of these cells. **Chapter 8** describes the mapping of proteomic changes that occur in neutrophils of different organs and different maturation states in a mouse models for metastatic breast cancer, revealing marked differences in protein expression depending on the anatomical location in which these cells reside, as well as their maturation state.

Finally, **chapter 9** discusses the findings described in this thesis. It examines how neutrophil heterogeneity may potentially be exploited therapeutically. It gives a future outlook on the study of the connection between cancer genotypes and immune phenotypes, and its clinical applicability. Together, this work sheds light on some of the mechanisms shaping the tumor immune landscape and systemic inflammation.