Immunosuppression in breast cancer: a closer look at regulatory T cells

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English Summary
Nederlandse samenvatting
Curriculum Vitae
Acknowledgements
List of publications
PhD portfolio

ENGLISH SUMMARY

The human immune system is equipped with powerful cellular mechanisms that provide protection against threats which may compromise tissue function and homeostasis. These mechanisms can be used to deal with external threats like pathogenic micro-organisms, but are also suitable as a counter to the internal threat of cancer. However, the manifestation of cancer in today's society as a leading global health challenge demonstrates this counter is not waterproof, and tumors are able to evade immune pressure. An important reason for this observation is that cancer cells are in essence not foreign cells, but have acquired characteristics, as a result of continuous accumulation of mutations, that distinguishes them from healthy cells. Thus, cancer cells balance on the edge of self- and non-self. This complicates detection by the immune system, which requires a threat to be clearly identifiable to be of foreign nature (i.e. immunogenic), to become fully activated. On top of being poorly recognisable, tumors actively employ other means to evade immune-mediated eradication. In general, immune effector mechanisms have to be carefully regulated to prevent self-inflicted tissue damage and auto-immune-related pathology which can result from excessive or misguided immune activation. However, mounting evidence shows that tumors can co-opt immunoregulatory mechanisms to limit immune responses against primary tumors and metastases. In cancer types like breast cancer, tumor-derived signals can guide the infiltration and anti-inflammatory polarisation of myeloid- and adaptive immune cells which, together with local stromal- and tumor cells, construct a dense network of immunosuppression. These immunosuppressive conditions are characterised by abundant expression of immune inhibitory receptors, anti-inflammatory cytokines and other immune-modulatory factors that can turn the tumor microenvironment (TME) into a hostile milieu for effector cells, thereby blunting anti-cancer mechanisms. Moreover, it is becoming increasingly clear that tumor-associated immunosuppression takes on systemic forms, and thereby helps cancer to spread beyond the primary tumor. By increasing our understanding of the immunoregulatory mechanisms that are hijacked in the context of breast cancer, existing immune-based therapeutic approaches can be improved to overcome immunosuppression, while new insights may also inspire novel avenues of immunotherapy.

An important cell type that is broadly involved in controlling immune activation is the regulatory FOXP3^{+}CD4^{+} T cell (T_{regs}). T_{regs} are equipped with a diverse arsenal of immunosuppressive mechanisms, and T_{reg} dysfunction stands at the basis of a wide range of auto-immune-related diseases. In cancer, T_{regs} are thought to be important allies of tumors, as the immunosuppressive function of T_{regs} can be directed against anti-tumor immunity. The work described in this thesis explores the intricate relationship of T_{regs} with primary and metastatic breast cancer, and T cell based immunotherapeutic approaches, using sophisticated mouse models that recapitulate the development and progression of poorly immunogenic breast tumors.

Chapter 2 reviews the exciting progress of the past years regarding our understanding of T_{regs} in (human) breast cancer, and discusses the future prospects of T_{reg}-targeting strategies. $FOXP3^+$ T_{regs} mainly develop in the thymus, but can also arise in the periphery through TGF- β mediated differentiation of conventional $CD4^+$ T cells. These peripheral T_{regs} (pT_{regs}) acquire FOXP3 expression and immunosuppressive activity, in a process called 'peripheral conversion'. However, if, and by which mechanisms , pT_{regs} accumulate in mammary tumors is largely unknown. Research in **Chapter 3** identifies that tumor-associated macrophages (TAMs), which are the most abundant immune cell type in spontaneously arising murine mammary tumors, play a key role in the accumulation of pT_{regs} in tumors. In part, this is mediated by TGF-β released by TAMs. We also show that TAMs induce PD-1 expression on intratumoral conventional $CD4^+$ T cells, which additionally "prepares" these cells for intratumoral conversion into pT_{regs}.

The local TME dictates the phenotype and function of intratumoral immune cells, but the systemic inflammation that accompanies tumor development and progression affects immune cell function far beyond the borders of the primary tumor. In **chapter 4**, we studied how mammary tumor development affects the phenotype, transcriptome and function of T_{regs} in tumors, blood and distant organs. During tumorigenesis, T_{regs} become systemically activated and acquire enhanced immunosuppressive potential. Interestingly, RNAseq analysis of T_{regs} in distant organs of tumor-bearing mice revealed that T_{regs} in different organs uniquely adapt to mammary tumorigenesis. Targeting these tumor-educated T_{regs} limits metastatic spread to lymph nodes but not to lungs, demonstrating that T_{regs} support metastasis formation in a tissue-specific manner. Mechanistically, tumor-educated T_{regs} that reside in the lymph node niche, but not those of the lungs, can limit the activation of anti-metastatic NK cells. In line, we show that T_{regs} increase, while NK cells decrease, in sentinel lymph nodes of breast cancer patients compared with healthy controls. This study identifies T_{regs} as key regulators of lymph node metastasis in breast cancer, and reveals that neo-adjuvant targeting of T_{regs} in breast cancer may have therapeutic benefit. **Chapter 5** further highlights the importance of immune regulation in tissue tropism of metastasis from the angle of neutrophils, by discussing how cancer-cell derived Cathepsin C drives neutrophil accumulation in lungs, thereby promoting metastasis.

Therapeutic strategies aimed at engaging T cell-mediated anti-cancer immunity, such as immune checkpoint blockade (ICB) are transforming the treatment landscape of cancer. Because T_{regs} can highly express immune checkpoint molecules, it is important to understand how ICB influences T_{reg} biology, which is the focus of **Chapter 6**. As spontaneous mammary KEP tumors are therapeutically unresponsive towards the combination of anti-PD1/anti-CTLA4, this model allows the study of immunotherapy resistance mechanisms. ICB-treated mice are characterized by expansion of immunosuppressive T_{regs} in blood and tumors, while effector T cells remain unchanged. Depletion of T_{regs} in the context of ICB remodels the tumor

immune landscape into a more pro-inflammatory state, and unleashes the accumulation of activated $CD8^+$ T cells and NK cells in blood. Moreover, T_{reg} depletion during ICB extends metastasis-related survival, showing T_{regs} form a hurdle for the response to ICB.

Besides phenotypic characterisation, functional assays are helpful to study how cancer, tissue-context, or therapeutics impact the immunosuppressive potential of T_{regs}. **Chapter 7** details an experimental protocol that has been used throughout the thesis, which enables quantitative and reproducible assessment of T_{reg} function isolated from tumors and other organs.

Finally, **chapter 8** discusses the insights from this thesis in a broader perspective, and provides my thoughts on how to further advance our fundamental understanding of T_{reg} biology in cancer. Ultimately, this may contribute to the development of therapeutic applications aimed at bypassing tumor-supportive immunoregulatory mechanisms in breast cancer.