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Tumor tissue architecture, immune cells and molecular characteristics in relation to immune escape in colon cancer: what rises must fall

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Chapter 7

English summary

中文摘要 (Chinese summary)

Nederlandse samenvatting

Curriculum vitae

PhD portfolio

Acknowledgements

Summary

Colon cancer is a frequent malignancy with heterogeneous clinical outcome that is not fully explained by conventional clinicopathologic staging. Tumor immune contexture and antigen presentation through HLA class I are key determinants of tumor progression and treatment response. At the same time, colon cancer represents a complex biological system in which germline HLA background, tumor intrinsic pathways and local immune microenvironment interact across organizational levels. A systems biology view emphasizes that these interactions give rise to emergent properties, such as effective immune control or immune escape, that cannot be understood from single markers in isolation. The overall aim of this thesis was to improve the understanding of tumor immune interactions in colon cancer by integrating spatial T cell patterns, HLA class I tissue expression and germline HLA class I diversity with molecular features and clinical outcome. The work builds on a multiscale atlas of colon cancer and adopts a systems biology perspective in which immune contexture, antigen presentation through HLA class Ia and HLA class Ib and locus specific characteristics of HLA-A, HLA-B and HLA-C are studied as interconnected components of one tumor immune system.

Chapter 2 introduces the systems biology framework for studying cancer and tumor immunity. It explains why reductionistic single-marker models are insufficient for complex phenotypes and proposes an integrated approach to connect multiscale interactions, from molecular variations and cellular networks to tissue-level immune contexture and patient outcomes. This framework positions colon cancer as a dynamic ecosystem in which tumor-intrinsic programs and the microenvironment jointly shape immune control and escape states. It sets the conceptual stage for the thesis, establishing how spatial architecture and antigen presentation biology must be evaluated collectively to understand tumor evolution.

Chapter 3 addressed the spatial organization of tumor-infiltrating T cell subsets in colon cancer. A multiplex immunofluorescence assay was used to identify CD3+CD8+ T cells, CD3+FoxP3+ regulatory T cells, and CD3+CD8-FoxP3- T cells. Proliferative Ki67+ and cytotoxic granzyme B+ T cells were mapped in relation to tumor epithelium, stroma, and invasive margin and linked to immune-related transcriptomic signatures. CD3+CD8-FoxP3- T cells formed the largest subset of infiltrating T cells, whereas CD3+CD8+ T cells and CD3+FoxP3+ T cells showed comparable densities. Ki67+ and granzyme B+ T cells were enriched within tumor epithelium,

indicating frequent direct contact with malignant cells. Tumors with high Immunologic Constant of Rejection (ICR) scores showed increased T cell densities and shorter distances between CD3+CD8+ T cells and tumor cells. Higher stromal densities of CD3+FoxP3+ T cells were associated with improved survival. Ultimately, these findings demonstrate that the spatial organization and functional activation of T cells, rather than abundance alone, are associated with immune activation and improved outcome in colon cancer.

Chapter 4 examined the relationship between HLA class I expression in tumor epithelium and the composition of the tumor immune infiltrate. In a large, well annotated retrospective cohort, immunohistochemistry for HLA class Ia heavy chains, $\beta 2$ microglobulin and the HLA class Ib molecules HLA-E, HLA-F and HLA-G was combined with quantitative assessment of CD45+ immune cells and selected lymphocyte subsets in tissue microarrays. Immune related gene signatures were used to characterize the transcriptional context. High HLA class Ia expression together with high CD45+ cell density defined tumors with immune active contexture, higher ICR scores and more favorable association with outcome. Tumors with loss or downregulation of HLA class Ia showed reduced immune infiltration and lower expression of rejection related signatures. HLA class Ib patterns were more heterogeneous and displayed context dependent associations with immune infiltrates and survival, suggesting immune regulatory roles within the microenvironment. These observations support a model in which antigen presentation through HLA class Ia and the presence of a robust immune infiltrate form a coupled system that is jointly related to patient outcome.

Chapter 5 focused on germline HLA class I diversity and locus specific HLA class I expression in colon cancer. Germline HLA-A, HLA-B and HLA-C alleles were used to calculate locus specific HLA evolutionary divergence, reflecting predicted breadth of the immunopeptidome. These germline metrics were integrated with molecular subtypes, microsatellite instability status, immune gene signatures and protein level expression of HLA-A, HLA-B and HLA-C heavy chains using epitope specific antibodies. Most tumors retained global HLA class I expression, but analyses with locus specific antibodies revealed distinct loss patterns. Selective loss of HLA-B or HLA-C was observed in a subset of tumors and coincided with specific immune cell compositions, including enrichment of neutrophils in some loss patterns. HLA evolutionary divergence associated with immune gene signatures within immune active tumors, with a

dominant contribution from HLA B rather than HLA-A or HLA-C. Hazard ratios for HLA evolutionary divergence metrics were modest, but the work provided a spatial and molecular map of HLA class I in colon cancer and suggested that locus specific HLA features interact with immune context is important.

Across **Chapters 3** to **Chapter 5**, spatial T cell organization, HLA class I tissue expression, and germline HLA class I diversity emerge as interconnected determinants that collectively shape the tumor immune microenvironment. The germline HLA background shapes the potential immunopeptidome, tumor-intrinsic pathways modulate HLA class I expression on epithelial cells, and local signals guide the spatial deployment and functional state of T cells in the tumor bed. Together, these processes give rise to distinct immune states. Tumors with preserved HLA class Ia expression, broad HLA-B divergence, dense and spatially engaged T cell infiltrates, and high ICR scores tended to have a more favorable association with outcome. Conversely, tumors with altered or reduced HLA class Ia expression, selective locus loss, sparse or distant T cell infiltrates, and immune-cold gene signatures were more often related to a poor outcome. This thesis therefore provides an integrated picture in which host HLA class I background, tumor-intrinsic antigen presentation, and local spatial immune contexture jointly shape the clinical course of colon cancer. Ultimately, this work demonstrates how integrated analysis of spatial immune organization, tumor HLA class I expression, and germline HLA diversity can be translated into tissue-based biomarkers of tumor immunity, providing a basis for improved patient stratification and future immunotherapeutic strategies.