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 1

General introduction and thesis outline

Colon cancer

Colon cancer is one of the most common malignancies worldwide and remains a major cause of cancer related mortality. Despite organized screening programs[1] and improvements in surgical techniques[2] and systemic therapy[3], a substantial proportion of patients still develop local recurrence or distant metastases after curative intent resection[4,5]. Current staging systems that are based on tumor invasion depth, nodal status and presence of metastasis capture important clinicopathologic information, but patients within the same stage often have very different clinical courses[6]. This heterogeneity illustrates that conventional staging does not fully reflect the underlying tumor biology. There remains a need for robust, clinically validated biomarkers that capture the dynamic crosstalk between malignant cells, the immune system, and other host determinants to refine risk stratification, inform treatment selection, and monitor response.

Tumor immune microenvironment in colon cancer

The immune system plays a central role in controlling tumor initiation, progression and dissemination. Tumor cells are recognized and eliminated by cytotoxic CD8+T cells, supported by CD4+helper T cells, B cells, natural killer cells and myeloid cell populations[7,8]. In colon cancer, dense infiltration by tumor infiltrating lymphocytes and formation of an active immune contexture have been associated with favorable outcome[9–11]. Quantitative measures of CD3+and CD8+T cells in the tumor center and invasive margin have shown strong association with survival in several cohorts and can outperform conventional TNM staging[12]. These observations support the concept that tumor immune interactions in the local microenvironment are a major determinant of disease course.

Not only the density but also the spatial organization and functional state of immune cells are important[13]. Effector T cells can localize within tumor epithelium, in the stromal compartment or at the invasive front, where they encounter malignant cells, cancer associated fibroblasts, endothelial cells and myeloid cells[7,14]. Spatially restricted niches such as tertiary lymphoid structures and immune excluded margins reflect distinct modes of immune surveillance or immune escape[15]. Chemokine gradients, adhesion molecules, stromal architecture and metabolic constraints shape T cell trafficking and retention[16,17].

Exhaustion markers, immune checkpoint molecules and regulatory T cells further modulate effector function[18,19]. A comprehensive understanding of this spatial organization in colon cancer remains incomplete and its relation to survival and therapy response requires further exploration.

Antigen presentation and HLA class I in colon cancer

For cytotoxic T cells to recognize and kill tumor cells, peptides derived from tumor associated antigens must be presented on human leukocyte antigen class I molecules at the cell surface[20–23]. HLA class I comprises the classical HLA class Ia loci and non-classical HLA class Ib molecules that have immune regulatory functions[24]. Alterations in HLA class I expression are frequent in many solid tumors and represent a key mechanism of immune escape. Loss or downregulation of HLA class I reduces visibility of tumor cells to CD8+T cells and can shift immune pressure towards natural killer cell recognition through missing self-sensing[25,26]. In colon cancer, altered HLA class I expression has been related to immune infiltration patterns and to patient outcome, but reported associations are not consistent[27–29].

HLA class I expression is influenced by oncogenic signaling, interferon pathways, antigen processing machinery and genomic instability[30–32]. In addition, HLA class I diversity at the germline level contributes to the breadth of the presented immunopeptidome and may influence susceptibility to cancer, immune editing and response to immunotherapy[33]. Yet the locus specific contribution of HLA-A, HLA-B and HLA-C alleles to antigen presentation in colon tumors is not fully defined. Protein level assessment of HLA class I in tissue often relies on antibodies that differ in epitope specificity, which makes comparison across studies challenging. Integrating allele level information with detailed tissue expression patterns and the surrounding immune context may help to disentangle these effects.

Tumor immune contexture, molecular subtypes and systems biology

Colon cancer is a heterogeneous disease at the genomic, transcriptomic and immune level. Consensus molecular subtypes and immune gene

signatures describe distinct patterns of immune activation, stromal remodeling and oncogenic signaling[34]. Tumors with microsatellite instability often have high mutational load, dense T cell infiltration and strong expression of immune checkpoints[11]. Tumors that are microsatellite stable show more variable immune landscapes that range from immune inflamed to immune excluded or immune desert states[35]. These patterns indicate that tumor immune contexture is tightly linked to underlying molecular programs.

Recent integrative studies that combine tumor genomics, spatial immune profiling and microbiome analysis in large colon cancer cohorts have shown that tumor cells, immune cells and microorganisms share ecological niches within the same tissue compartments[13,27]. Specific bacterial taxa associate with immune activation or immune suppression, with particular molecular subtypes and with distinct spatial patterns of immune infiltration[27]. These observations suggest that epithelial cells, stromal cells, infiltrating immune cells and microbial communities form a coupled system that jointly shapes disease behavior and outcome.

Such findings fit within a systems biology view of cancer. Biological systems can be regarded as networks in which molecules, cells, tissues and organs interact through dynamic feedback[36,37]. Processes at lower organizational levels contribute to emergent properties at higher levels, such as immune control or immune escape. At the same time, higher level events, including tissue damage, systemic inflammation and therapy, feedback to molecular and cellular processes. Systems biology aims to capture these multiscale interactions by integrating data from genomics, transcriptomics, proteomics, spatial imaging, microbiome profiling and clinical phenotypes into coherent models. In colon cancer, an integrated tumor, immune and microbiome atlas[27] has illustrated how multi-omics data can be combined to define immune ecosystems, microbial communities and molecular programs that together relate to prognosis and potential treatment response. This integrative framework underscores that tumor immune interactions in colon cancer cannot be understood by isolated markers alone, but require analysis of coordinated patterns across the whole system.

Rationale and knowledge gaps

Current evidence indicates that the immune contexture of colon cancer emerges from coordinated tumor intrinsic and host extrinsic processes,

including T cell infiltration, HLA class I mediated antigen presentation, stromal and vascular architecture, myeloid inflammation, and the intestinal microbiome, and these features are associated with clinical outcome[13,27,29]. However, important gaps remain when these processes are considered from a systems biology perspective. Most studies have focused on immune cell density in broad tumor regions, whereas the precise spatial distribution of distinct T cell subsets within tumor epithelium and stroma has been less systematically addressed. In addition, there are few studies that integrate protein level assessment of HLA class I expression with quantitative measurements of immune infiltration and with multi omics immune signatures in large, well annotated colon cancer cohorts.

Moreover, the locus specific diversity and tissue expression patterns of HLA class I molecules, and how these relate to immune microenvironmental states and molecular features, remain insufficiently defined[38]. How germline HLA class I diversity, tumor intrinsic HLA class I alterations and local spatial deployment of T cells together contribute to emergent properties such as immune control or immune escape is not well understood. A more integrated view on these aspects, embedded in a systems biology framework, may reveal patterns of tumor immune organization that better capture the dynamic balance between immune control and escape and that may guide the development of immunotherapeutic and systems based strategies.

Clinical motivation and translational perspective

Immune checkpoint blockade has become a standard option for microsatellite instability high or mismatch repair deficient metastatic colorectal cancer, yet most mismatch repair proficient colon cancers remain resistant[39–41]. This gap motivates biomarkers that capture antigen presentation competence and spatial immune engagement[42,43]. By integrating HLA class Ia and HLA class Ib tissue expression, CD45 density, spatial T cell architecture, and germline HLA class I diversity, this thesis aims to define immune states that can guide rational selection of immunotherapy and combination strategies.

Aim and outline of this thesis

The overall aim of this thesis is to improve the understanding of tumor immune interactions in colon cancer by integrating spatial T cell organization, HLA class I expression and molecular features with clinical outcome within a systems biology framework. **Chapter 2** provides the biological rationale for studying colon cancer as an integrated tumor ecosystem. It summarizes how tumor intrinsic programs, antigen presentation via HLA class I, and the spatial organization of immune cells jointly shape immune contexture and are associated with clinical outcome within a systems biology framework. This chapter defines the key concepts, measurements, and analytical strategy that support the spatial T cell analyses in **Chapter 3** and the HLA class I focused studies in **Chapters 4** and **5**. **Chapter 3** investigates the spatial dynamics of tumor infiltrating T cell subsets in colon cancer. In this chapter, detailed mapping of T cell localization within tumor epithelium, stroma and invasive margin is performed and related to immune gene signatures and survival, to define immune contexture patterns that are associated with patient outcome. **Chapter 4** examines how HLA class I expression in tumor epithelium interacts with the density and composition of tumor immune infiltrates. This chapter assesses HLA class I expression using complementary antibodies, quantifies CD45+ immune cells and selected lymphocyte subsets, and evaluates their association with molecular characteristics and survival in a large colon cancer cohort. **Chapter 5** focuses on locus specific HLA class I diversity and tissue expression in colon cancer. In this chapter, allele level information and epitope specific staining for HLA-A, HLA-B and HLA-C heavy chains are integrated with immune and genomic context, to delineate patterns of antigen presentation that are related to immune cell composition and clinical outcome. In the final chapter, the findings of these studies will be summarized and placed in the broader context of tumor immune biology and systems biology of colon cancer. The implications for biomarker development and for the design of immune based and systems oriented therapeutic strategies will be discussed, with the ultimate goal to contribute to more individualized care for patients with colon cancer.

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