In silico dissection of transcriptomes with a tumor immunology focus

Urzúa Traslaviña, Carlos

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General introduction and Outline of the Thesis

1 BACKGROUND

CANCER THERAPY WITH IMMUNE CHECKPOINT BLOCKERS

Cancer cells share a set of characteristics called the hallmarks of cancer [1]. One cancer hallmark describes the ability of cancer cells to block or evade an anti-tumor immune reaction by interacting with immune cells present in the local tumor microenvironment and nearby lymph nodes. Programmed cell death 1 (PD-1) and cytotoxic T-lymphocyte associated protein 4 (CTLA-4) are membrane proteins called immune checkpoints that inhibit the activation of CD8+ T cells. Immune checkpoint inhibitors (ICIs) are monoclonal antibodies that block the inhibition of CD8+ T cells by cancer cells by disrupting the membrane interactions of immune checkpoints such as PD-1 and CTLA-4 [2]. Without the inhibition of immune checkpoints, a durable anticancer immune response can sometimes be triggered against the primary tumor and metastases.

ICI therapy has revolutionized cancer treatment by improving patient outcomes across numerous cancer types [2, 3]. Regretfully, many patients with cancer still do not benefit from ICI therapy because they do not develop a durable response upon treatment. Additionally, ICI therapy sometimes results immune-related adverse events that damage non-cancer tissues [4]. Interestingly, many immune-related adverse events induced by ICI therapy resemble inflammatory conditions (e.g., inflammatory bowel disease or, diabetes). Current research efforts are therefore focused to uncover the requirements of an anticancer immune response, explain why some patients do not respond to ICIs, and understand the mechanism behind the immune-related adverse events.

Research questions in the field of anti-tumor immune responses can be divided into three categories: 1) Which biological processes do cancer cells use to inhibit or evade an anti-tumor response. 2) Which biological processes trigger an anti-tumor immune response. 3) Which non-tumor patient characteristics influence the balance of inhibiting and triggering anti-tumor response. In this thesis, we focus on the first question: Which biological processes do cancer cells use to inhibit or evade an anti-tumor response.

Tumor factors associated with a durable anti-tumor immune response upon ICI treatment are: PD-L1 membrane expression, microsatellite instability (MSI), tumor mutational burden (TMB), tumor-infiltrating lymphocytes, and an intact antigen presentation machinery [5–8]. However, there are cases where patients fail to respond despite possessing all known conditions for ICI response. In these cases, cancer cells may have other unknown immune inhibiting processes still engaged [9, 10]. For example, copy number alteration burden has been negatively associated with response to ICIs in gastrointestinal tumors [11].

IN-SILICO STUDY OF THE TUMOR MICROENVIRONMENT

The complex interactions between cancer cells and immune cells make in-silico analysis an ideal tool to prioritize genes likely to participate in immune evasion. Currently, there is a wealth of publicly available transcriptome wide mRNA profiles obtained from patient

tumor samples using microarray and RNA-seq technology. Previously developed methods, such as CIBERSORT, can be applied to tissue mRNA profiles to estimate the type and amount of immune cells originally present in the biopsy[12]. Additionally, dimensionality reduction methods such as principal component analysis (PCA) can robustly segregate latent mRNA transcriptional patterns present in multiple tumor samples [13]. Independent component analysis could improve PCA-based segregation of transcriptional patterns by extracting transcriptional signals that are as statistically independent from each other as possible [14]. For example, this independence condition could help segregate different biological processes into different mRNA transcriptional signals thus increasing the interpretability of the latent transcriptional patterns. Analyses that study latent mRNA transcriptional patterns and estimated immune cell fractions in tumor mRNA profiles could help prioritize the genes most likely to participate in immune evasion.

PATIENT PERSPECTIVE OF NOVEL ANTICANCER THERAPIES

More and more anticancer therapies are approved every year for use in the clinic which results in situations where more than one option is available for the same clinical situation [15]. Patients, doctors, and stakeholders have several priorities when evaluating a treatment, including the expected survival improvement, quality of life, adverse events, and cost of the treatment. Tools like the ESMO Magnitude of Clinical Benefit (ESMO-MCBS) facilitate the standardization and communication of the expected clinical benefit of anticancer therapies [16, 17]. To achieve this, the ESMO-MCBS distils into a score the survival, quality of life, adverse effects, and other outcomes that represent the clinical benefit of an anticancer treatment. Currently, this procedure results in a single number or letter score that does not communicate the characteristics of the treatment that resulted in such a score. Visualizations are practical tools to effectively communicate complex information to a non-technical audience [18–20]. Therefore, an opportunity arises to design a visualization that encodes the aspects that compose the clinical benefit of an anticancer treatment as evaluated by the ESMO-MCBS. This visualization could further help the decision-making process of patients, doctors and stakeholders and facilitate an informed conversation about the expected benefit of anticancer treatments.

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THESIS OUTLINE

The main aim of this thesis is to use transcriptomic data to gain more insight into the biological processes of cancer cells and their relationship with the immune tumor microenvironment. A sub-aim of the thesis is to develop visualizations that distil clinical trial evidence to help make more informed treatment decisions.

Transcritome-wide mRNA expression profiles of tumor tissue encode the transcriptional changes of tumor cells. However, the mRNA expression of genes in these profiles represents the accumulated mRNA expression contribution of cancer and non-cancer cells present in the tumor biopsy. Additionally, all mRNA profiles are confounded by exprimental artifacts originating from the mRNA expression measurment platform. Consensus independent component analysis (c-ICA) of mRNA expression profiles can segregate mRNA profiles into a mixture-model of independent transcriptional components. Each component thus captures different transcriptional footprints originating from genetic, non-genetic and experimental sources. The desired subset of components can then be used for further study.

In **Chapter 2**, we aimed to identify transcriptional components with c-ICA that are associated with metabolic processes in patient and cell line samples. Transcriptional components associated with metabolic processes were selected using gene set enrichment analysis of manually curated metabolic gene sets. This enabled the exploration of associations between metabolic transcriptional components and survival, drug sensitivity, and immune cell infiltration.

In **Chapter 3**, we aimed to improve gene function predictions for coding and non-coding genes. Using PCA and c-ICA preprocessed mRNA profiles we generated gene function predictions using a guilt-by-association strategy. We generated predictions for 16 different gene set collections to showcase which gene sets are better suited for gene function prediction. We compared predictions obtained using microarray and RNA-seq profiles as input to evaluate the robustness of the methods. We explored case studies of genes prioritized in CRISPR-based screens to validate the gene function predictions. This enabled us to compare the two methods and reveal the advantages and disadvantages of using each preprocessing method to predict gene functions.

In **Chapter 4**, we aimed to identify transcriptional components associated with the effect of copy number alterations (CNAs) in patient and cell line samples. To obtain transcriptional components, we applied c-ICA to patient and celline-derived mRNA profiles. Those transcriptional components with high weights disproportionally present on contiguous genomic segments were considered to capture the effect of CNAs. Reconstruction of the mRNA profiles using only CNA transcriptional components resulted in transcriptional CNA profiles of all tumor mRNA profiles. Analyses of the CNA transcriptional profiles revealed the landscape of CNA transcriptional effects and degree of gene transcriptional adaptation in tumors.

In **Chapter 5**, we aimed to identify transcriptional components associated with immune processes in patient samples. A sub aim was to determine if the immune transcriptional components active in patients that respond to immune checkpoint blockers are similarly active in patients with an immune disease or during the response to an immune influencing treatment. To obtain transcriptional components, we applied c-ICA to a

patient-derived microarray mRNA profile dataset and three studies of RNA-seq profiles of tumor tissues from patients with melanoma. Transcriptional components associated with immune processes were selected using gene set enrichment analysis of manually curated gene sets related to with immune processes. Studies of immune-related diseases or immune influencing treatments were manually curated from the Gene Expression Omnibus. The immune-associated transcriptional components were used to compare the activity of immune processes in samples with immune-related diseases (e.g., autoimmune diseases), samples being treated with immune influencing treatments (e.g., brodalumab, infliximab, and diphenylcyclopropenone), and melanoma samples treated with immune checkpoint blockers.

In **Chapter 6** We aimed to develop a visual tool for the European Society for Medical Oncology Magnitude of Clinical Benefit Scale (ESMO-MCBS). We generated a prototype, and more than 120 example visualizations were generated for field testing of the understandability of the visual tool. The collected feedback from the field testing was then used to amend the final design of the tool for publication.