Cell-cell Communication between CD8+ T cells and Dendritic cells in Melanoma Microenvironment

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I. Introduction

Skin cancer, specifically cutaneous melanoma, is the most prevalent form, and its occurrence has been notably escalating over recent years. It has been demonstrated that the immune system possesses the ability to combat tumors in various types of cancers, including melanoma and lung cancer [1].

Immune system cells consistently react to signals from their immediate surroundings and, in turn, influence the environment through temporary reactions, decisions in differentiation pathways, flexible adjustments in cellular states, and adaptable integration into their residing tissue. Genomic methodologies have paved the path for in-depth exploration of these coordinated responses [2].

Grasping the functionality of cell-to-cell communication necessitates insights into how ligand-receptor engagements impact the expression of target genes. Therefore, there's a demand for computational techniques that leverage the expression data from interacting cells to deduce the impacts of ligands from the sender cells on the gene expression of the receiver cells [3].

The complex interplay between Conventional Dendritic Cells (cDCs) and CD8+ T cells in the tumor milieu is crucial for driving effective immune responses against tumors. Focusing on melanoma, this report explores the intricate networks of cell communication that underpin this interaction. Using NicheNet to filter out the interactions between cDCs and CD8+ T-cells, not only helps to build immune response against Melanoma but also to find new methods in immunotherapy for cancer detection.

II. Literature Review

Recent research has highlighted the capacity of single-cell transcriptomics to explore the dynamics of communication between individual cells. In one of the papers, the genetic activity of around 6,000 individual cells from 18 patients with head and neck cancer, including cells from primary tumors and their spread to lymph nodes in five of these patients was analyzed. By combining detailed genetic data from individual cells with broader data from many tumors, the study helped improve classification of head and neck cancers based on their cancerous and supportive cell makeup. The study also identified a specific genetic program, partial epithelial-to-mesenchymal transition (p-EMT), as a reliable indicator for predicting the likelihood of the cancer spreading to lymph nodes, the severity of the cancer, and other harmful characteristics [4].

Another paper used use imaging-based deep learning to identify intratumoral cDC1-CD8+ T cell clustering as a unique feature of protective anti-cancer immunity. These clusters form selectively in stromal tumor regions and constitute niches in which cDC1 activate TCF1+ stem-like CD8+ T cells. A distinct population of immunostimulatory CCR7neg cDC1 that produce CXCL9 to promote cluster formation and cross-present tumor antigens within these niches, which is required for intratumoral CD8+ T cell differentiation and expansion and promotes cancer immune control was identified [5].

III. Methods

In this project, NicheNet, a bioinformatics computational approach was employed to study ligand-receptor interactions between CD8+ T cells and dendritic cells (DCs) based on single-cell RNA sequencing (scRNA-seq) data in tumor infiltration and immune signaling.

A. Data Preprocessing

The datasets utilized were scRNA-seq data for CD8+ T cell and conventional dendritic cells (cDC) [4]. The data was imported in R with the help of `readxl` package into which further analysis was done. Analysis included normalizing the data and cell metadata extraction. The expression data of CD8+ T cells in particular was transposed for convenient downstream analysis.

For both cells, Seurat objects (available in the `Seurat` package) were created and expression data and metadata information were retained. This then allowed access to the expression data for further analysis and ligand-receptor activity predictions.

B. Ligand-Receptor Network Construction

For ligand-receptor interactions, NicheNetR package was utilized to infer ligand activity based on single cell RNA sequencing gene expression datasets of different cell types. For this project, pre-built ligand-receptor maps for *Mus musculus* was employed from publicly available repositories. Ligand-target matrix and weighted networks data were also downloaded from these repositories and the ligand-target matrix data was read into R through custom functions to manage any issues that might occur during data loading.

The ligand-receptor network which is prebuilt gives information about the interactions between ligands and their corresponding receptors, and this is very crucial for identifying the respective communication pathways between CD8+ T cells and cDCs in tumour environment.

C. Cell Clustering and Differential Expression Analysis

CD8+ T cells were already clustered into 8 clusters and also were grouped into 3 conditions which are genetic constructs grouped together based on mutations in mouse models: CD4_Cre_Ptger2dd_Ptger4ff_BRAFV600E, GzmB_Cre_Ptger2dd_Ptger4ff_BRAFV600E, Ptger2dd_Ptger4ff_BRAFV600E. DCs were clustered into 5 clusters. Cells were assigned to cluster identities and these clustering results were saved into the metadata of each Seurat object.

The genes were identified in the receiver CD8+ T cells which were differentially expressed between every two pairs of genetic construct conditions and also the ones which were expressed between every two pairs of the 8 clusters. Only those genes were kept which were statistically significant. By combining the differentially expressed genes from condition based and cluster based analysis, the gene set of interest in receiver cells was obtained.

IV. Results

A. Ligand Activity Prediction

After identifying differentially expressed genes (DEGs), ligand activities were predicted using NicheNetR. The ligand activities are prioritized according to ranks and these ranks are selected by the statistical measure called Area Under the Precision Curve (AUPR) and top 30 ligands in the cDCs were selected.

In the figure above, each bin on the X- Axis represents range of AUPR values and Y- Axis represents number of ligands which have the AUPR value in that respective range. The dotted red vertical line is the cutoff line for the top 30 ligands.

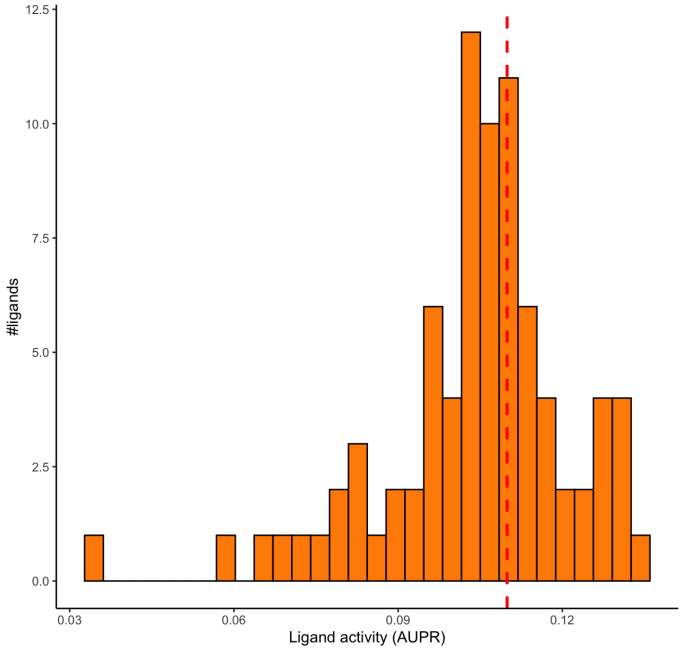

Fig. 1. Histogram of ligand activities (AUPR). The red dashed line indicates the cutoff for the top 30 ligands.

B. Ligand-Target and Ligand-Receptor Interaction Visualization

Now, after the top 30 ligands were identified, the target genes whose expression levels in the CD8+ T-cells were affected by these ligands are identified and ligand target pathways were known. Following heatmap shows this visualization of ligand target connections.

The `get_weighted_ligand_receptor_links` function gives the ligand receptor interactions between the two cell types. The input for this function are the best ligands (which have already been identified), receptor genes that are expressed in the gene set of interest, the pre-existing ligand-receptor network and the weighted network which weights these ligand-receptor connections. Fig. 3 shows this visualization of ligand receptor connections. It can be seen that the communication pathway between Tnf in the cDCs and Traf2 in the CD8+ T-cells is the strongest.

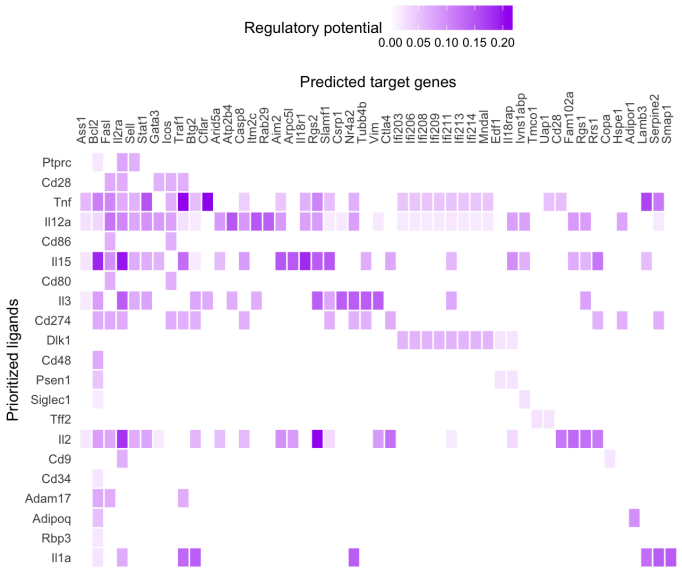

Fig. 2. Heatmap visualizing ligand target interactions.

C. Enrichment Analysis

Gene Ontology (GO) enrichment analysis was performed on the genes which were differentially expressed based on the conditions (genetic constructs) as well as the clusters of the CD8+ T-cells to help identify which biological functions are those genes responsible for. The below two figures show the same enrichment analysis.

Fig. 3. Heatmap visualizing ligand receptor interactions.

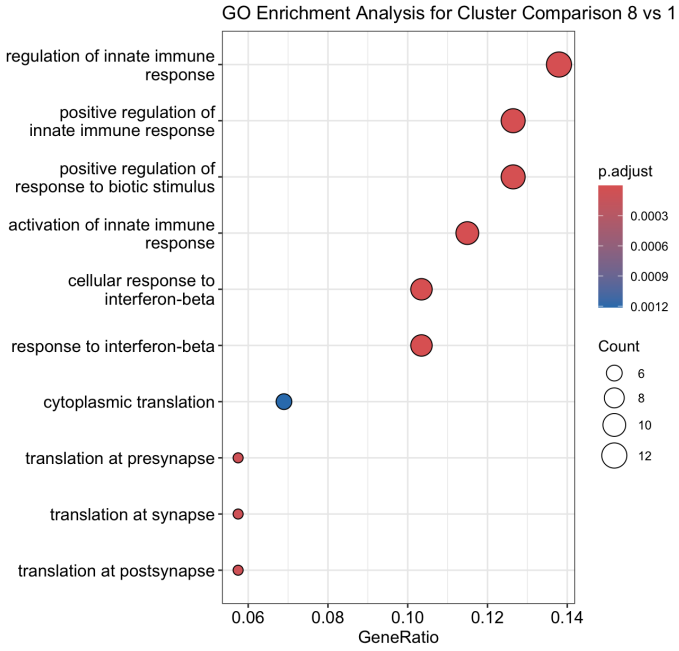

Fig. 4. Heatmap visualizing ligand receptor interactions.

V. Conclusion

This project thus predicts the interactions between CD8+ T-cells and cDCs in the context of melanoma using a computational tool: NicheNet. This is very important to understand how the signaling molecules from cDCs interact with the receptor genes from CD8+ T-cells in order to manipulate these communication pathways for prevention against Melanoma using methods like immunotherapy.

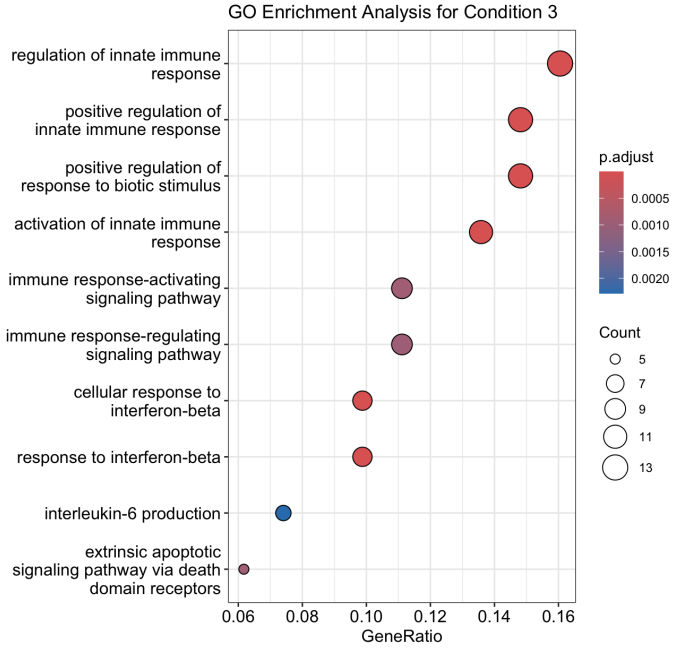

Fig. 5. Heatmap visualizing ligand receptor interactions.

References

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