Identification of molecular biomarkers for response to immune checkpoint inhibitors

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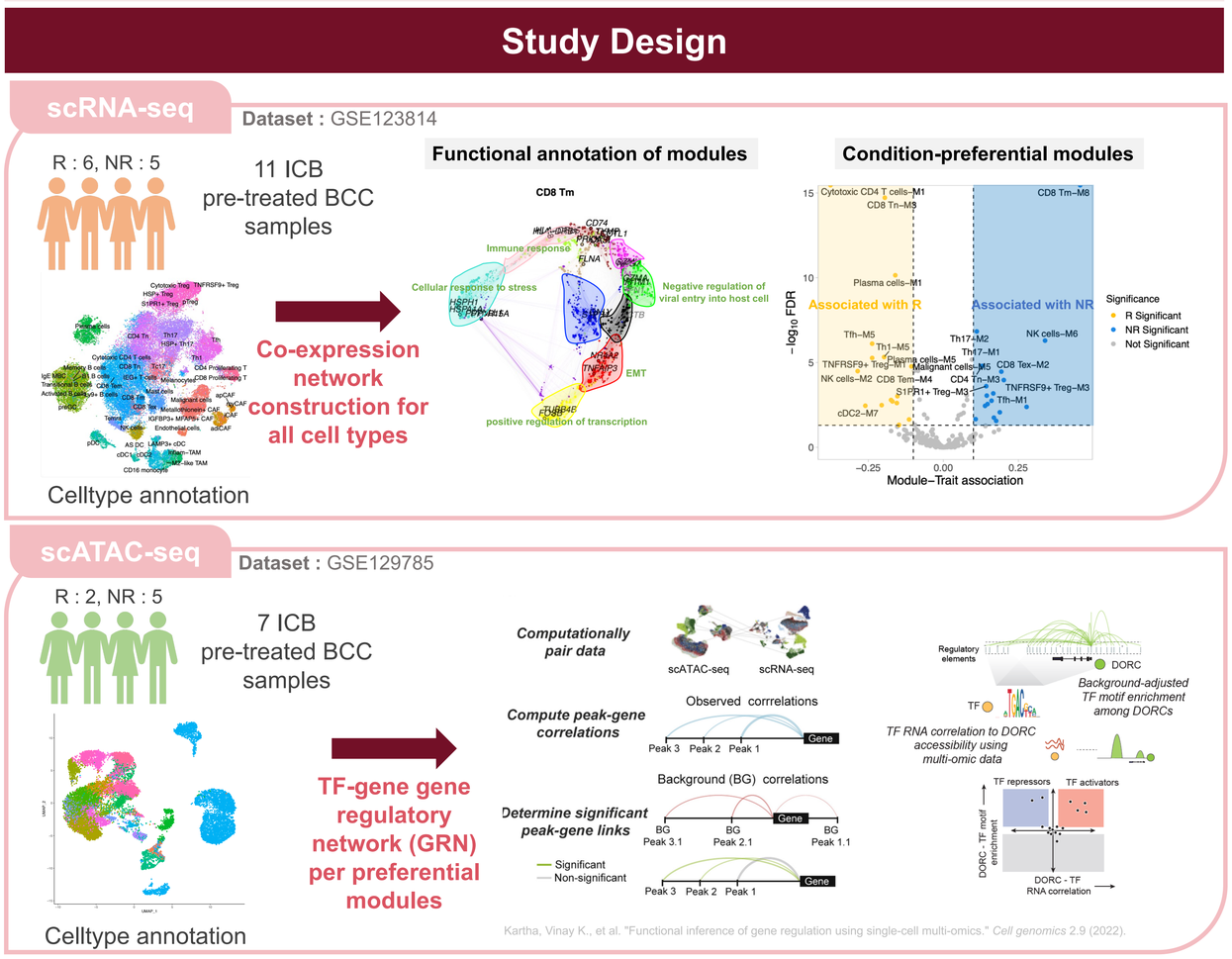
Introduction

The advent of **Immune checkpoint blockade (ICB)** has substantially enhanced the survival of cancer patients. However, not all individuals respond to ICB treatment, as the objective response rate with anti-PD-1 or anti-PD-L1 therapy ranges from 10% to 30% across different tumor types (Yarchoan et al., 2017). Elucidating the **mechanisms underlying this variability in response** is critical for overcoming this hurdle and maximizing the potential of ICB therapy.

This study focuses on **unraveling intricate biological networks associated with ICB response at a single-cell level in the tumor microenvironment**. Leveraging publicly available multi-omics data, including single-cell RNA-sequencing (**scRNA-seq**) and single-cell ATAC-sequencing (**scATAC-seq**) data from immunotherapy-treated basal cell carcinoma (BCC) patients, we utilize Weighted Gene Co-expression Network Analysis (WGCNA) and **gene regulatory networks (GRNs)** to integrate gene expression and epigenetic information.

This enables the identification of molecular biomarkers linked to ICB resistance at a single-cell level, providing resolution in understanding ICB response. Furthermore, we systematically explored hub genes with extensive connections to other genes and upstream regulators within response-associated modules.

Study Design



Kartha, Vinay K., et al. "Functional inference of gene regulation using single-cell multi-omics." *Cell genomics* 2.9 (2022).

Results

A total of 50 cell types identified from ICB-treated BCC scRNA-seq data

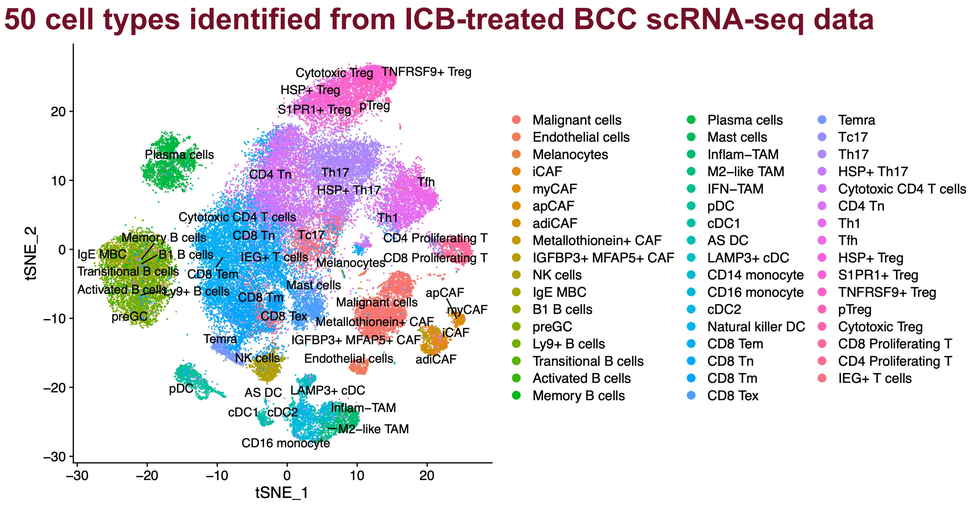


Figure 1. A total of 50 cell types were identified from ICB-treated BCC patients. We obtained scRNA-seq profiles from malignant, lymphocyes, myeloid, and stromal cells.

Of the 228 modules identified from 50 cell types, 33 are associated with the response

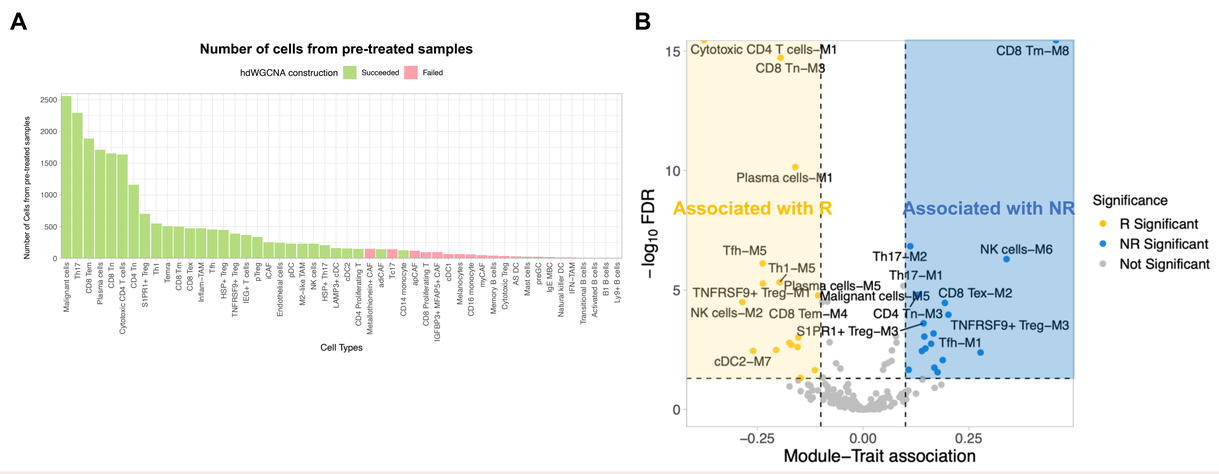


Figure 2. (A) Out of 50 cell types, 29 cell types were able to construct hdWGCNA modules. **(B)** Module-Trait associations were computed for 228 modules across these 29 cell types, and a total of 33 modules were found to display a significant correlation with the response, meeting the criteria of |Module-Trait association| > 0.1 and FDR < 0.05 (Fig. 2B).

Results

Among the 16 responders-associated modules, Cytotoxic CD4 T cells-M1 displayed the strongest association

Ten upstream regulators were identified to control the genes within this module by regulating the immune response.

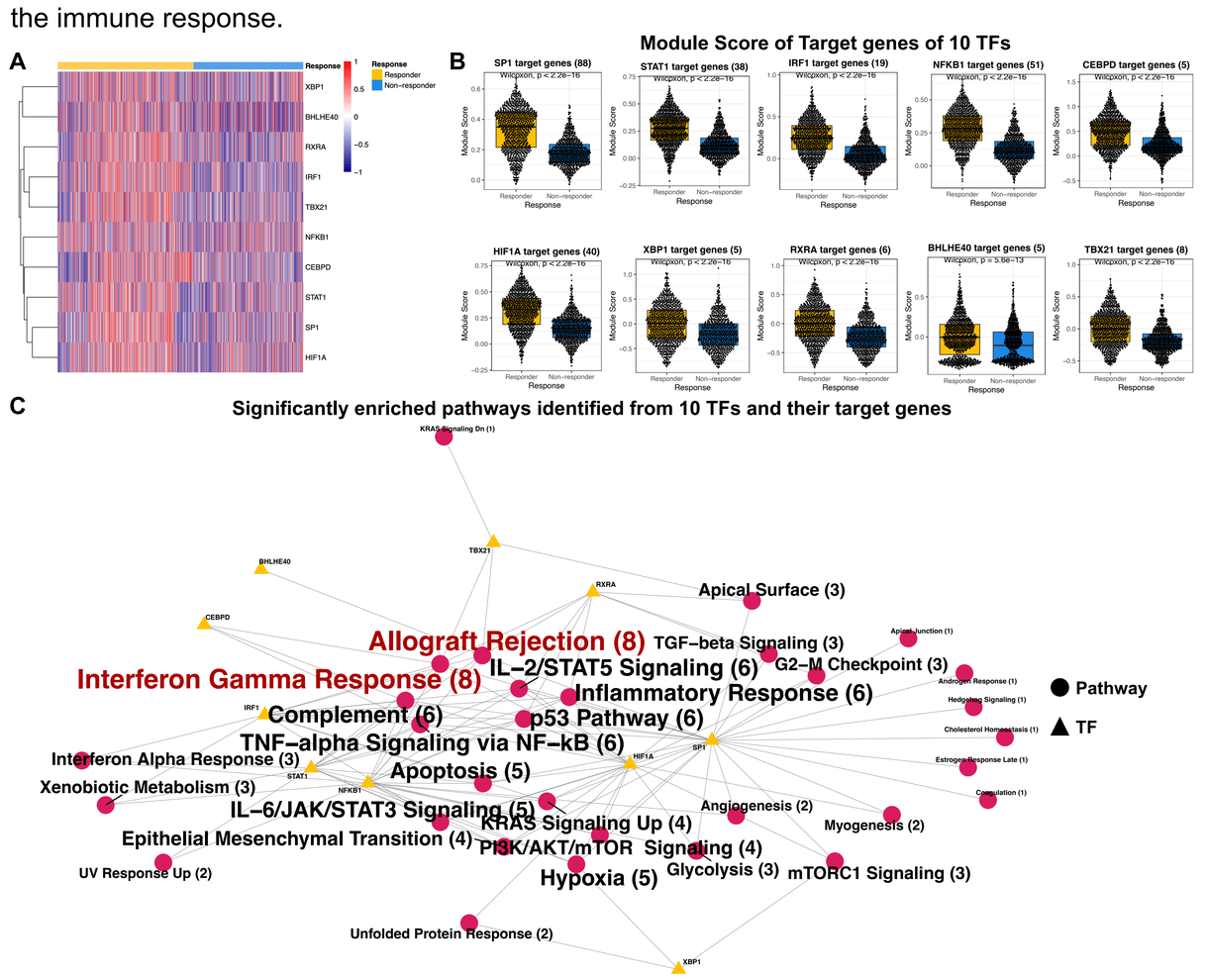


Figure 3. (A) 10 upstream transcription factors exhibited higher TF activities in responders which were members of a Cytotoxic CD4 Tcells-M1. **(B)** The module scores of the target genes for each of these TFs were significantly elevated in responders. **(C)** The responder group exhibited enrichment in INF-γ response and allograft rejection processes, both of which play a role in inducing the recruitment of immune cells through pro-inflammatory cytokines and directly killing tumor cells.

[Limitation of GRN inferred with the scRNA-seq data]

However, TF activities inferred from gene expression data and TF-target databases do not provide direct insights into the regulatory processes of target genes controlled by accessible promoter regions. To overcome this limitation, GRNs were constructed by integrating scRNA-seq data and scATAC-seq data.

BHLHE40 was found to have opposite functions depending on the condition

Constructing GRNs for response-associated modules identified recurrent and exclusive activators and repressors.

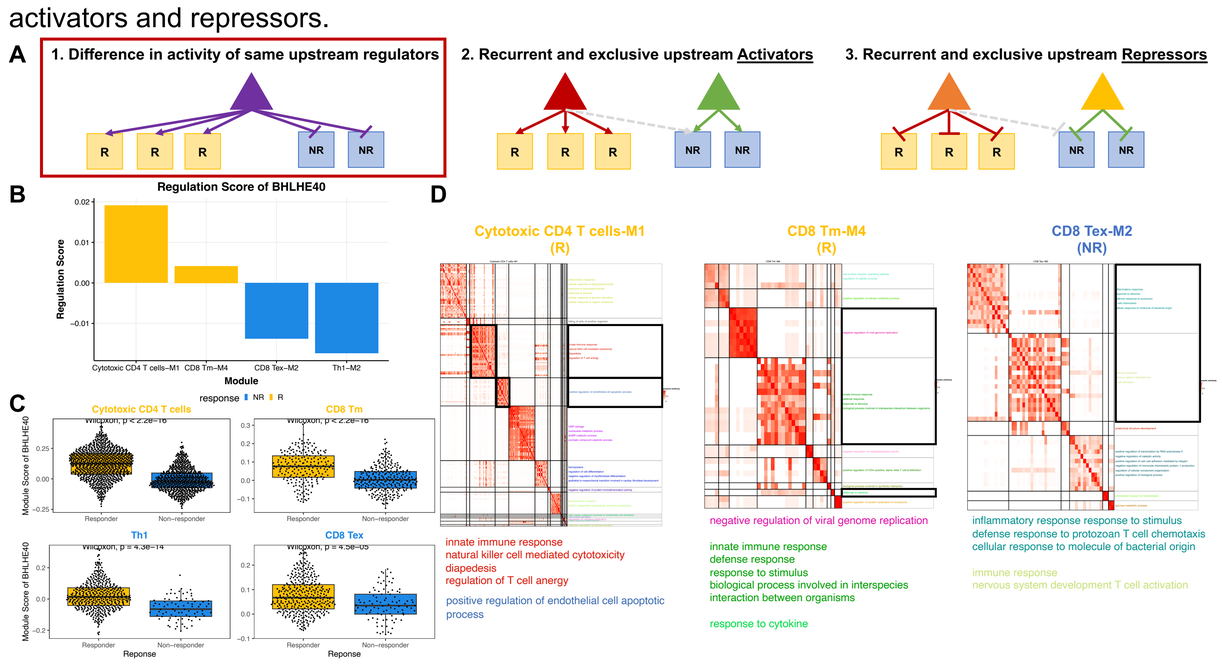


Figure 4. (A) Three possible scenarios of TF functions inferred from the GRNs constructed by integrating scRNA-seq and scATAC-seq data and first scenarios were focused first. **(B)** Regulation score of *BHLHE40*, indicating opposite functions depending on the condition. **(C)** Gene expression of target genes of *BHLHE40* by the cell types from Fig. 4B. **(D)** Biological function of target genes regulated by *BHLHE40* in each module associated with immune response and T cell functionality.

Conclusion

From the scRNA-seq data analysis, 33 modules associated with response outcomes were identified. Each module had upstream transcription regulators, with some exclusively regulating condition-specific modules. Notably, 10 TFs emerged as upstream regulators for the responders-associated Cytotoxic CD4 Tcell-M1 module, and their biological functions were related to the **immune responses which are responsible for the immunotherapy response**. To enhance precision, **GRNs were refined by integrating scATAC-seq data**, enabling the identification of recurrent and exclusive activators and repressors within response-associated modules. ***BHLHE40* exhibited dual functions depending on the condition** and was linked to **immune response and T cell functionality**. These findings provide insights that *BHLHE40* may contribute to immunotherapy resistance in BCC.