Diabetic Complications Consortium

Application Title: Iterative Integration of Single Cell RNA-seq and ATAC-seq Data for Inferring Transcription Factor Regulatory Networks in Macrophages

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1. Project Accomplishments:

Macrophages are capable of performing a diverse array of tasks during wound healing, ranging from destructive killing functions to pro-healing and homeostatic duties. Recent studies especially those based on the single-cell RNA-seq approach have demonstrated that macrophages adopt a spectrum of phenotypes during skin wound healing in both healthy (ref) and diabetic mice (unpublished data). To identify the transcriptional regulatory mechanisms potentially driving the heterogeneity in macrophage phenotypes in during healing of skin diabetic wounds, we applied our recently developed tool to the time-course scRNAseq datasets obtained from the skin wounds of non-diabetic and diabetic db/db mice on days 3,6 and 10 post-injury to infer the activity of transcription factors (TF) in individual cells. BITFAM leverages all published TF ChIP-seq data in scRNAseq data analysis to predict activities of TFs in individual cells and provides a rank list of targeted genes for each transcription factor[1]. We further performed the differential activity analysis to identify TFs that exhibit distinct activity in each macrophage subpopulation in 1) non-diabetic and diabetic mice, respectively, and 2) between non-diabetic and diabetic mice. For each TF, using individual cell transcriptomes we computed AUCell scores[2] as the proxy of the activity of its target gene set inferred from the BITFAM. These results will be combined with the scATACseq data for additional information about TF binding sites in the target genes, which were generated from Dr. Koh's lab under R35GM136228.

2. Specific Aim:

Specific Aim 1A. Infer TF activity using the scRNAseq data of macrophages in skin wounds obtained from non-diabetic (GSE154400)[3] and db mice (data unpublished) on Day 3, 6, and 10 post-injuries.

Results: We performed the BITFAM analysis to identify the TF activity profiles of 39 TFs using scRNAseq data obtained from Dr. Koh's lab. The Seurat analysis on preprocessed transcriptomes (Table 1) revealed 8 macrophage subpopulations (Fig.1).

Table 1 Number of cells

	non-diabetic	diabetic
D3	894	1121
D6	585	1214
D10	1334	961
total	2813	3296

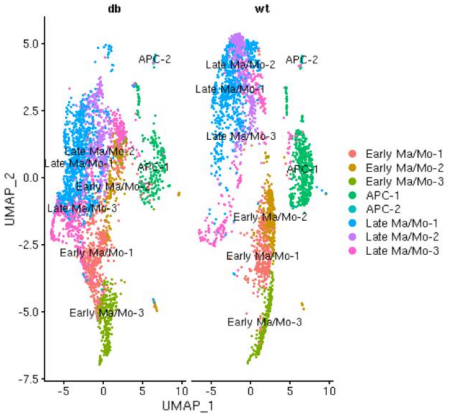

Fig.1 The clustering of the macrophage subpopulations. Left: wt (diabetic) mice; Right: wt (non-diabetic) mice. The Early Macro-1,2,3 subclusters consists of cells from D3 and D5; Later Macro 1,2,3 subclusters consist of cells from D10; and The clusters APC-1 and 2 are mixed with cells from all the three days.

The heatmaps of the differential activities in each macrophage subpopulation are shown in Fig. 2. The differential analysis was performed using the Wilcoxon rank-sum test with the threshold of 0.05 for the adjusted p-value. For each of the TF in Fig.2, the activity of their target genes is shown in Fig. 3.

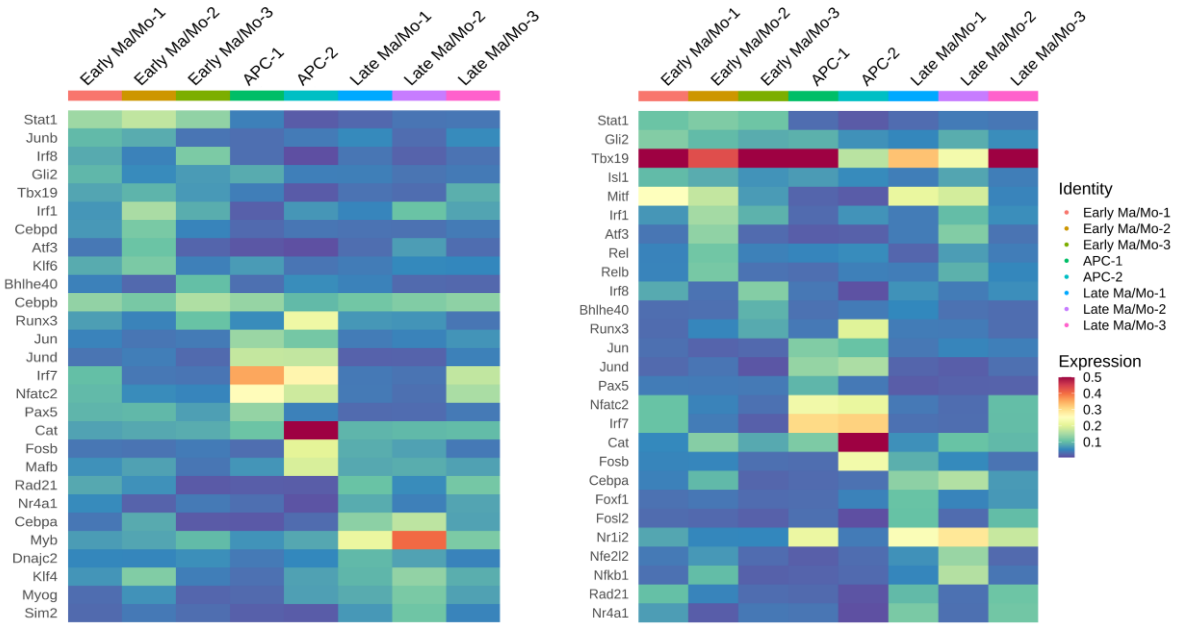

Fig 2. TFs with differential activities in macrophage subpopulations based on the inferred activity profiles from BITFAM. Left: wt mice; Right: diabetic mice.

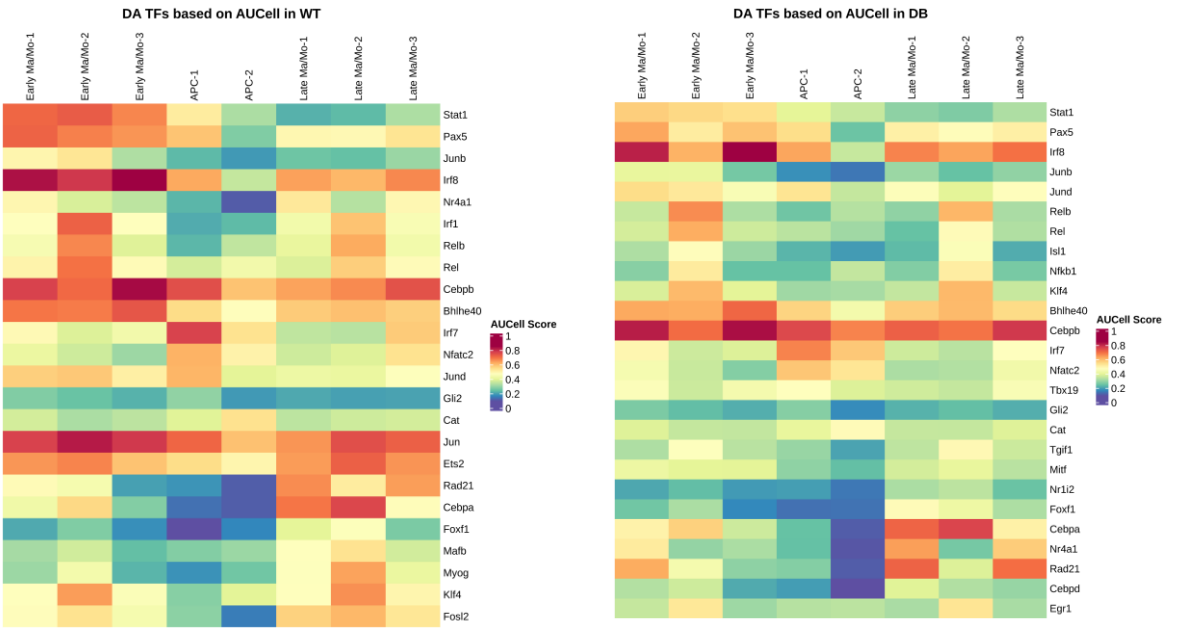

Fig. 3. The activities of the top 10 target genes are inferred from BITFAM. The activity of the target genes was calculated using ACUcell scores from the ranked gene list in ease cell based on the single cell transcriptome. The average AUCell scores of each cluster was used in the heatmaps. Left: non-diabetic mice; Right: diabetic mice.

The additional differential analysis identified the TFs displayed differential activity between diabetic and nondiabetic mice (Fig. 4). An example of TF Nrii2 and the AUCell scores of the top 10 target genes were also calculated in wt and db conditions are shown in Fig.5.

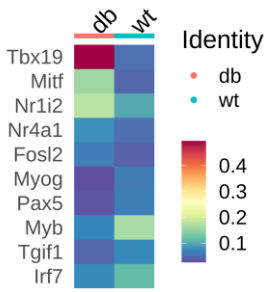

Fig. 4. The TFs with differential activities in each macrophage subpopulations. TFs with differential activities in diabetic and non-diabetic mice based on the Wilcoxon rank-sum analysis of the BITFAM inferred TF activity profiles (adj. Pvalue<0.05).

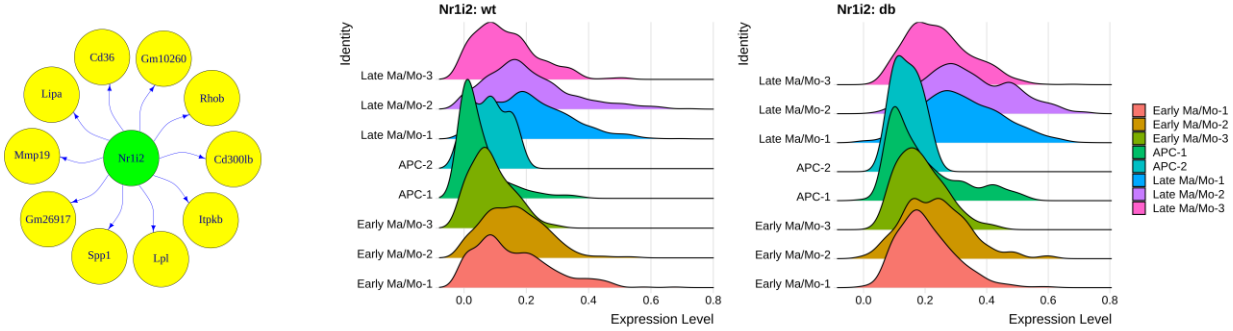

Fig. 5. The TFs with differential activities in each macrophage subpopulations. Left: The predicted top gene targets of Nr1i2 from BITFAM; Middle: The AUCell scores of the top 10 target genes of Nr1i2 in the macrophage subpopulations in non-diabetic mice; Right: The AUCell scores of the top 10 target genes of the Nr1i2 in the macrophage subpopulations in non-diabetic mice

Specific Aim 1B. Iterative application of BITFAM using the predicted TF-targets from scATAC-seq data.

Results: We are currently in the process of analyzing the scATAC-seq data from db and non-db skin wounds on D2 and D6 post-injury.

3. Publications:

We plan to submit a paper to combine the scATACseq data.

References

1. Gao, S., Dai, Y., and Rehman, J. (2021). A Bayesian Inference Transcription Factor Activity Model for the Analysis of Single Cell Transcriptomes. Genome Res *(second revision)*.
2. Aibar, S., González-Blas, C.B., Moerman, T., Huynh-Thu, V.A., Imrichova, H., Hulselmans, G., Rambow, F., Marine, J.-C., Geurts, P., Aerts, J., et al. (2017). SCENIC: single-cell regulatory network inference and clustering. Nature Methods *14*, 1083-1086. 10.1038/nmeth.4463.

3. Pang, J., Maienschein-Cline, M., and Koh, T.J. (2021). Enhanced Proliferation of Ly6C(+) Monocytes/Macrophages Contributes to Chronic Inflammation in Skin Wounds of Diabetic Mice. J Immunol *206*, 621-630. 10.4049/jimmunol.2000935.