Diabetic Complications Consortium

Application Title: **Proteomic and Transcriptomic Single Cell Analysis In DFU Patient**.

Principal Investigator: Aristidis Veves and Manoj Bhasin (MPI application)

1. Project Accomplishments:

There is very satisfactory progress in the project. We have collected a large number of samples and we are in the final stages of single-cell RNAseq and proteomic analysis. The most exciting fighting is the identification of the specific cell type of fibroblasts that is associated with wound healing of diabetic foot ulceration. We have already study procedures the patent this finding. In addition, we have identified additional pathways that are associated with wound healing.

We have regular monthly teleconferences with the team from Yale that is working on the second grant of the same call. This has allowed us to compare findings and also develop strategies for further data analysis.

Our next step is to publish the data, something we hope will be done over the next few months. We also hope that the collected data will allow us to pursue further funding that will enable continuation of our studies

2. Specific Aims:

Specific Aim 1. ***Evaluate single cell level Transcriptome and protein expression changes in foot and forearm skin specimens in patients with healed and non-healed DFU, DM patients with no DFU and healthy, non-DM patients.***

Results: As shown in the following figure, we have performed comparative analysis of single cell transcriptome profiles of DFU, forearm, and peripheral blood mononuclear cells (PBMCs), delineating gene signatures and biological pathways associated with DFU-specific cell types.

A. Split Uniform Manifold Approximation and Projection (UMAP) of Foot, Forearm, and PBMC samples. The clusters were annotated manually to various canonical and novel cell types based on expression of specific markers.

B. Stacked bar plots showing the proportion of cells from Foot, Forearm, and PBMC in different clusters (Dark brown: Foot, Beige: Forearm, Red: PBMCs).

C. Heatmap showing significantly differentially expressed genes at the wound site (i.e., foot) as compared to non-wounded site (i.e., forearm.). Relative gene expression is shown in pseudocolor, where green represents down regulation, and red represents up regulation.

D. Comparative analysis of pathways dysregulation across Foot, Forearm and PBMCs. The pathways dysregulation was measured based on Z score: with Z score >2 and <-2 indicate activated and inhibited pathways respectively.

Scatterplot showing top differentially expressed genes in the Fibroblast (E), smooth muscle cell (F) and keratinocyte (G) clusters at the wound site (i.e., foot) as compared to non-wounded site (i.e., forearm.)

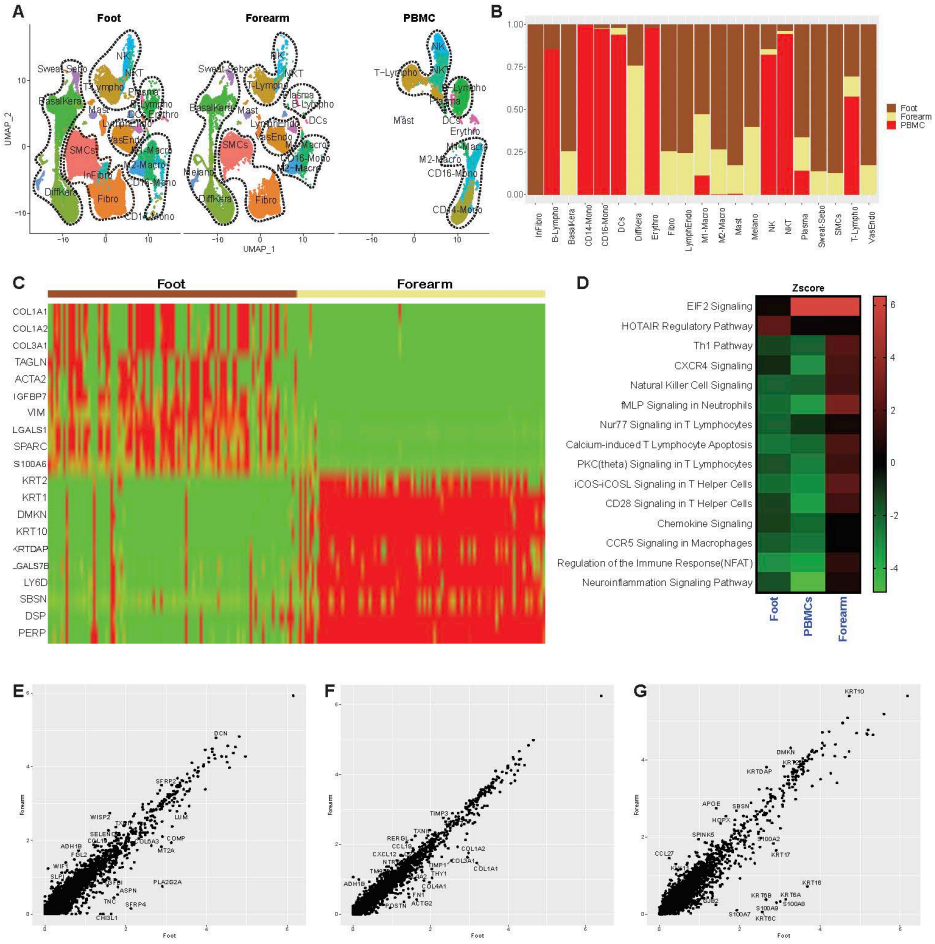

Figure 2

Specific Aim 2. ***Evaluate the single cell level gene of PBMCs from the same patients to identify circulatory markers associated with wound healing at individual cell level.***

Results: The following figure indicated the performed comparative analysis of transcriptome profiles of PBMCs in different clinical groups, uncovering differences in systemic immunological landscape associated with wound healing response in DFUs.

A. UMAP dimensionality reduction embedding of peripheral blood mononuclear cells (PBMCs) from Healers, Non-healers, non-diabetic Control, and non-DFU Diabetic patients.
B. Stacked bar plots showing the cluster wise proportion of different cell types across clinical groups (ie., Green: non-diabetic Controls, Orange: Healers, Red: Non-healers, Purple: non-DFU Diabetic patients).
C. Heatmap showing significantly differentially expressed genes in PBMCs of Healers as compared to Non-healers.
D. Biological pathways that significantly activated (Z score >1.5) /inhibited (Z score <-1.5) in PBMCs of healers as compared to Non-healers.
E. Upstream regulatory molecules significantly activated (orange) or inhibited (blue) in the PBMCs of healers as compared to non-healers. This analysis helps to identify upstream regulators that are predicted to be significantly activated and upstream of the gene expression alterations in the healer group.

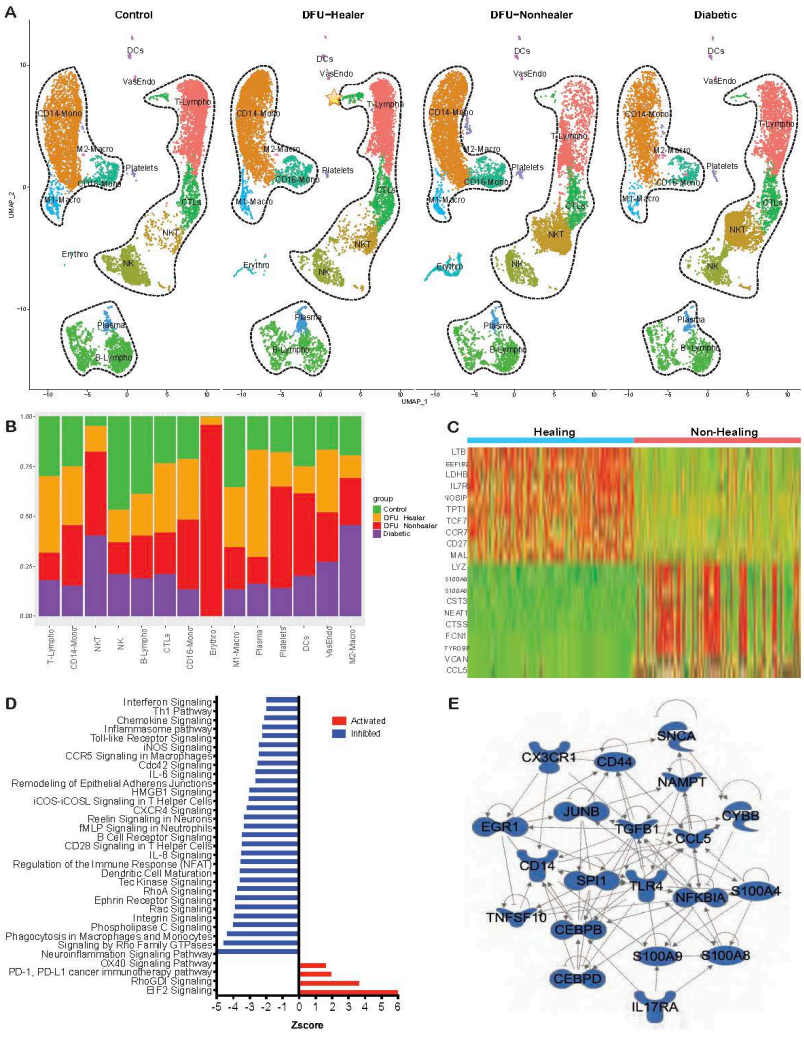

Figure 3

3. Publications:

No publications as yet