Data in Brief

Gene expression profiling of human fibrocytic myeloid-derived suppressor cells (f-MDSCs)

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A B S T R A C T

Myeloid-derived suppressor cells (MDSCs) have been shown to control self-reactive and anti-graft effector T-cells in autoimmunity and transplantation, but their therapeutic use is limited by their scarce availability in the peripheral blood of tumor-free donors. We isolated and characterized a novel population of myeloid suppressor cells, named fibrocytic MDSC (f-MDSC), which are differentiated from umbilical cord blood (UCB) precursors (Zoso et al., 2014). This MDSC subset promotes regulatory T-cell expansion and induces normoglycemia in a xenogeneic model of type 1 diabetes. Here we describe in details the experimental design and the bioinformatics analyses of the gene expression dataset used to investigate the molecular mechanisms at the base of MDSC tolerogenic and suppressive properties. We also provide an R code to easily access the data and perform the quality controls and basic analyses relevant to this dataset. Raw and pre-processed data are available at Gene Expression Omnibus under accession GSE52376.

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Direct link to deposited data

Deposited data are publicly available at Gene Expression Omnibus (GEO) under GSE52376: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52376.

Experimental design, materials and methods

Cell culture

Fibrocytic myeloid-derived suppressor cells (f-MDSCs) were differentiated from umbilical cord blood (UCB) precursors by a 4-day culture in the presence of recombinant human (rh)-GM-CSF and rh-G-CSF after Ficoll-Paque gradient separation and red cell lysis [1]. Cultures in the presence of both cytokines result in the differentiation of MDSC precursors to MDSCs. Differentiation of MDSCs was confirmed by flow cytometry analysis, which showed the expression of the CD33+IL4Rα+ subset (f-MDSCs) as well as the expression of CD11b and CD14.

Sample source location

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0.6%, Gibco) activated, carboxyfluorescein-succinimidyl-ester (CFSE, Molecular Probe) labeled CD3⁺ T-cells negatively isolated with human Pan-T-cell magnetic beads and an LS column (Miltenyi-Biotec). Suppressive assays were performed in either conventional dishes (contact) or using trans-well membranes (Corning) separating f-MDSC and CD3⁺ T-cells. HEK-293 human fibroblasts were used as negative controls.

Microarray study design

We extracted RNA from f-MDSC CD33⁺IL4Rα⁺ cells under the following conditions: 1) before co-culture with CD3⁺ T-cells (before contact; n = 7), 2) after 3-day co-culture with T-cells in conventional dishes (contact; n = 7), and 3) after 3-day co-culture with T-cells using trans-well membranes (transwell; n = 7). RNA was also prepared from two replicates of HEK-293 cells maintained in complete RPMI-140 10% FBS complete media (Gibco). The RNA samples were subjected to microarray analysis using a Human Gene 2.0 ST Array (Affymetrix, Santa Clara, CA).

RNA labeling and hybridization

RNA samples were stored in Trizol (Invitrogen), purified using the miRNeasy mini kit (Qiagen), analyzed with an Agilent Bioanalyzer 6000 (Agilent Technologies), and hybridized by the Gene Expression Core Facility at the University of Miami using Affymetrix GeneChip WT Terminal Labeling and Hybridization, Wash, and Stain kits. Briefly, sense-strand cDNA was fragmented with a combination of uracil DNA glycosylase and apurinic/apyrimidinic endonuclease 1 and its yield further labeled by terminal deoxynucleotidyl transferase (TdT) with the Affymetrix proprietary DNA Labeling Reagent that is covalently linked to biotin. Following labeling, 10 μg of cRNA was hybridized for 16 h at 45 °C on GeneChip Human Gene 2.0 ST arrays. Arrays were washed and stained (streptavidin, R-phycocerythrin conjugate and biotinylated anti-streptavidin) in the Affymetrix Fluidics Station 400 and scanned using standard Affymetrix scanning procedures on a Scanner 3000 7G. Raw intensity data (CEL files) were generated using Affymetrix GeneChip Command Console Software (AGCC). CEL files and sample annotation data were deposited in GEO under accession number GSE52376.

Quality controls, normalization, and signal quantification

Quality controls were performed using the oligo and AffyQCControl Bioconductor packages on an ExpressionFeatureSet object generated from CEL files. As can be seen from the distribution of all PM intensities (Fig. 1A), from the heat map of the array–array Spearman rank correlation coefficients (Fig. 1B) and from Relative Log Expression (RLE; Fig. 1C) and Normalized Unscaled Standard Error (NUSE; Fig. 1D)
plots, all arrays in the dataset have good and reproducible quality metrics according to standard guidelines.

CEL file fluorescence signals were converted to expression values using the robust multi-array average procedure RMA of the Bioconductor affy package [2]. Specifically, probe fluorescence intensities were background adjusted, normalized using quantile normalization, and log2 expression values for a total of 23,786 custom probe sets calculated using median polish summarization and the custom chip definition files for Human Gene 2.0 ST arrays based on Entrez genes (hugene20st_Hs_ENTREZG version 17.1.0; [3]). All data analyses were performed in R version 3.0.2 using Bioconductor libraries of BioC 2.13 and R statistical packages.

Unsupervised analysis

To identify samples that display similar gene expression profiles in an unbiased manner, we used unsupervised hierarchical clustering on the subset of probe sets that change their expression level among the various samples. Global unsupervised clustering was performed using the function hclust of the R stats package with the Pearson correlation as distance metric and average agglomeration method (Fig. 2A). In order to reduce the effect of noise from non-varying genes, those probe sets with a coefficient of variation smaller than the 90th percentile of the coefficients of variation in the entire dataset, were removed before the unsupervised clustering. The filter retained 2509 probe sets that are more variable across samples. To assess cluster-specific reproducibility, we calculated p-values for sample clusters using the multiscale bootstrap resampling method coded in the R pvclust package. Briefly, hierarchical clustering was first performed, using the correlation matrix of row-wise standardized expression values and the average agglomeration method, on the original data and on B = 1000 replications of different resampling of the given dataset (bootstrapped data). Then, p-values were computed for all clusters of the original data as the frequency that any cluster appears in the bootstrap replicates (Bootstrap Probability). To confirm the relationship between sample clusters, we used Principal Component Analysis (PCA) coded by the prcomp function of the R stats package. PCA is a mathematical technique to reduce data dimensionality while retaining most of its variation. Data reduction is accomplished by identifying directions, named principal components, along which the data variability is maximal. By using a few components, each sample can be projected in a space of reduced dimension making it possible to determine if samples form clusters or groups. Considering the filtered data matrix (2509 probe sets), the first 3 principal components accounted for 45.73% (PC1), 29.93% (PC2) and 16.28% (PC3) of the total variance (Fig. 2B). Both unsupervised clustering and PCA indicate that f-MDSCs are completely different from HEK-293 fibroblasts and that f-MDSCs co-cultured with CD3+T-cell in contact condition (contact) slightly segregate from both before contact and transwell samples.

Discussion

Here we described the characteristics of the gene expression dataset used to investigate a novel population of myeloid suppressor cells, named fibrocytic MDSC (f-MDSC), that are differentiated from umbilical cord blood (UCB) precursors [1]. In particular, this dataset provided clues to elucidate the molecular mechanisms at the base of f-MDSC tolerogenic and suppressive properties, indicating that fibrocytic MDSCs require direct contact with activated T-cells to exert their protolerogenic function. This new myeloid subset may represent an important tool for the in vitro and in vivo production of T regulatory cells for the treatment of autoimmune diseases and the prevention or control of allograft rejection.

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Appendix A. Supplementary data

Supplementary data to this article include a Sweave file to reproduce the analysis and the Sweave report generated using R version 3.0.2 and Bioconductor libraries of BioC 2.13. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.gdata.2014.10.018.
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