A lung Tgf-beta-signaling-mediated endothelial-interstitial macrophage axis prevents age-related abnormalities

3-GSEA analysis comparing TGFbRII-KO to control with bulkRNAseq data

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Abstract

Lung interstitial macrophages (IMs) are monocyte-derived parenchymal macrophages whose homeostatic and tissue-supportive functions remain unclear. While recent progress has been made about the diversity and transcriptional regulation of lung IMs, the microenvironmental signals responsible for their development from monocytes and for their functional specification remain unidentified. Here we found, in mice, that lung endothelial cell-derived Tgf-beta1 specifically triggered a core Tgf-beta receptor-dependent IM signature in bone marrow-derived monocytes and macrophages (Macs). In vivo, myeloid-specific ablation of Tgf-beta receptor signaling severely impaired monocyte-to-IM development, resulting in the accumulation of perivascular monocytes, decreased IM numbers and a loss of IM-intrinsic identity. Of note, monocyte-to-IM development was similarly impaired in the absence of endothelial-specific Tgf-beta1. Functionally, lungs from mice selectively lacking Tgf-beta receptor in IMs exhibited spatial changes in monocyte and IM niche occupancies, a severe disruption in their immunoregulatory environment, and prematurely developed fibrosis, hyperinflation, increased compliance and decreased elastance, changes classically associated with aging. Our work identifies a novel endothelial-IM axis involving Tgf-beta1 - Tgf-beta receptor interactions that shapes IM development and identity and thereby sustains lung tissue integrity, thus providing foundations for IM-targeted interventions in the context of lung aging and other chronic inflammatory disorders.

Contents

1 Description

The fold change between TGFbRII-KO and control for each gene (logFC) calculated in previous step was used to rank the genes from the most up-regulated to the most down-regulated gene in TGFbRII-KO. The ranked genelist was then subjectted to Gene Set Enrichment Analysis (GSEA). The genesets in MSigDB used for GSEA were extracted from msigdbr package *(1)* and then used by `GSEA` function from `clusterProfiler` pakcage *(2)*. `ggplot2` package was used to build plots *(3)*.

2 Prepare data for GSEA analysis

Load packages:

```
suppressMessages(library(ggplot2))
suppressMessages(library(clusterProfiler))
suppressMessages(library(msigdbr))
```

Load data:

```
dds <- readRDS("../1_Bulk_RNAseq_Data_preparation/dds.Rds")

# We will use the logFC calculated in previous step for ranking the genes:
DE.res.KO_vs_WT <- read.table(file = "../2_Differential_Expression_TGFbRII-KO_vs_WT/DE.res.KO_vs_WT.txt",
    sep = "\t")

genelist.ranked.KO_CTL <- DE.res.KO_vs_WT$log2FoldChange.shrunk
names(genelist.ranked.KO_CTL) <- rownames(DE.res.KO_vs_WT)
genelist.ranked.KO_CTL <- sort(genelist.ranked.KO_CTL, decreasing = TRUE)
```

Extract genesets from MSigDB database via `msigdbr` package:

```
gs_c5_bp <- msigdbr(species = "Mus musculus", category = "C5", subcategory = "GO:BP") %>%
    dplyr::select(gs_name, gene_symbol)
gs_c5_cc <- msigdbr(species = "Mus musculus", category = "C5", subcategory = "GO:CC") %>%
    dplyr::select(gs_name, gene_symbol)
gs_c5_mf <- msigdbr(species = "Mus musculus", category = "C5", subcategory = "GO:MF") %>%
    dplyr::select(gs_name, gene_symbol)
gs_h <- msigdbr(species = "Mus musculus", category = "H") %>%
    dplyr::select(gs_name, gene_symbol)
```

2.1 Enrichment with C1-Hallmark genesets

```
gsea_h <- GSEA(geneList = genelist.ranked.KO_CTL, TERM2GENE = gs_h,
   verbose = FALSE,
    seed = TRUE)
gsea_h@result <- gsea_h[order(gsea_h$NES, decreasing = TRUE), ]
write.table(x = gsea_h@result, file = "./gsea_h.txt", sep = "\t")
```

2.2 Enrichment with C5-GO (BP) genesets

```
gsea_c5_bp <- GSEA(geneList = genelist.ranked.KO_CTL, TERM2GENE = gs_c5_bp
    , verbose = FALSE,
     seed = TRUE)
gsea_c5_bp@result <- gsea_c5_bp[order(gsea_c5_bp$NES, decreasing = TRUE),
    ]
```

2.3 Enrichment with C5-GO (MF)

```
gsea_c5_mf <- GSEA(geneList = genelist.ranked.KO_CTL, TERM2GENE = gs_c5_mf
    , verbose = FALSE,
     seed = 123)
gsea_c5_mf@result <- gsea_c5_mf[order(gsea_c5_mf$NES, decreasing = TRUE),
    ]
```

2.4 Enrichment with C5-GO (CC)

```
gsea_c5_cc <- GSEA(geneList = genelist.ranked.KO_CTL, TERM2GENE = gs_c5_cc
    , verbose = FALSE,
     seed = TRUE)
gsea_c5_cc@result <- gsea_c5_cc[order(gsea_c5_cc$NES, decreasing = TRUE),
    ]
```

2.5 C2-Pathway

```
gs_c2 <- msigdbr(species = "Mus musculus", category = "C2") %>%
    dplyr::select(gs_name, gene_symbol)

gsea_c2 <- GSEA(geneList = genelist.ranked.KO_CTL, TERM2GENE = gs_c2,
    verbose = FALSE,
     seed = TRUE)
gsea_c2@result <- gsea_c2[order(gsea_c2$NES, decreasing = TRUE), ]
```

3 Make summarising dotplot

Build dataframe with results:

```
df.pos <- do.call("rbind", list(gsea_c2[1,], # PLASARI_TGFB1_TARGETS_10HR_
    DN
                      gsea_c5_bp[1,],  # GOBP_CELL_CELL_ADHESION_VIA_
                         PLASMA_MEMBRANE_ADHESION_MOLECULES
                      gsea_c5_bp["GOBP_MITOTIC_NUCLEAR_DIVISION",],
                      gsea_h["HALLMARK_INTERFERON_ALPHA_RESPONSE",],
                      gsea_h["HALLMARK_G2M_CHECKPOINT",],
                      gsea_h["HALLMARK_E2F_TARGETS",])
        )
```

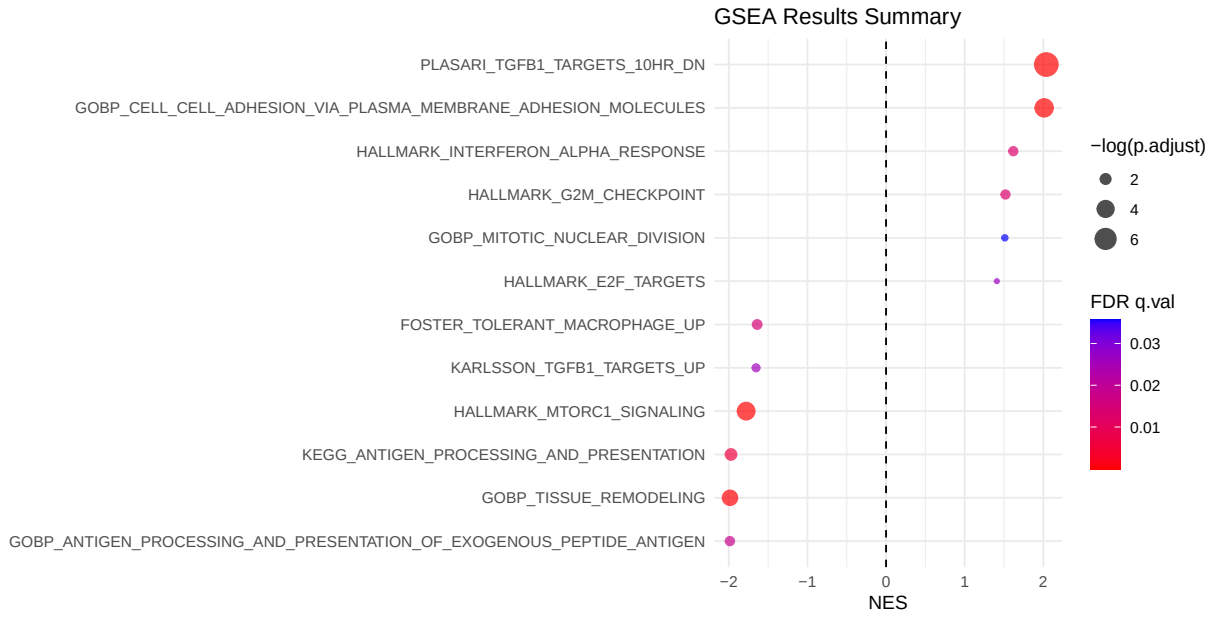
```r


df.neg <- do.call("rbind", list(gsea_c2["KARLSSON_TGFB1_TARGETS_UP",],
                        gsea_c2["KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION"
                            ,],
                        gsea_c5_bp["GOBP_ANTIGEN_PROCESSING_AND_PRESENTATION
                            _OF_EXOGENOUS_PEPTIDE_ANTIGEN",],
                        gsea_c5_bp["GOBP_TISSUE_REMODELING",],
                        gsea_h["HALLMARK_MTORC1_SIGNALING",],
                        gsea_c2["FOSTER_TOLERANT_MACROPHAGE_UP",])
        )

# Order the data frame by NES
df.both <- rbind(df.pos, df.neg)
df.both <- df.both %>% arrange(NES)

# Ensure the Pathway factor levels follow the order in the data frame
df.both$ID <- factor(df.both$ID, levels = df.both$ID)
```

```r
# Create the plot
ggplot(df.both, aes(x = NES, y = ID)) + geom_point(aes(color = qvalue,
    size = -log10(p.adjust)),
     alpha = 0.7) + geom_vline(xintercept = 0, linetype = "dashed") + scale
        _color_gradient(low = "red",
     high = "blue") + labs(x = "NES", y = "", title = "GSEA␣Results␣Summary
        ") + theme_minimal() +
     theme(axis.text.y = element_text(hjust = 1)) + guides(size = guide_
        legend(title = "-log(p.adjust)"),
     color = guide_colorbar(title = "FDR␣q.val"))
```



4 Session information

R sesssion:

```
sessionInfo()
```

```
## R version 4.3.3 (2024-02-29)
## Platform: aarch64-apple-darwin20 (64-bit)
## Running under: macOS 15.1.1
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/lib/libRlapack.dylib;  LAPACK version 3.11.0
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## time zone: Europe/Paris
## tzcode source: internal
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] msigdbr_7.5.1          clusterProfiler_4.10.1 ggplot2_3.5.1
##
## loaded via a namespace (and not attached):
##   [1] DBI_1.2.3               bitops_1.0-9            gson_0.1.0
##   [4] shadowtext_0.1.4        gridExtra_2.3           formatR_1.14
##   [7] rlang_1.1.4             magrittr_2.0.3          DOSE_3.28.2
##  [10] compiler_4.3.3          RSQLite_2.3.9           png_0.1-8
##  [13] vctrs_0.6.5             reshape2_1.4.4          stringr_1.5.1
##  [16] pkgconfig_2.0.3         crayon_1.5.3            fastmap_1.2.0
##  [19] XVector_0.42.0          labeling_0.4.3          ggraph_2.2.1
##  [22] utf8_1.2.4              HDO.db_0.99.1           rmarkdown_2.29
##  [25] enrichplot_1.22.0       purrr_1.0.2             bit_4.5.0.1
##  [28] xfun_0.49               zlibbioc_1.48.2         cachem_1.1.0
##  [31] aplot_0.2.3             jsonlite_1.8.9          GenomeInfoDb_1.38.8
##  [34] blob_1.2.4              BiocParallel_1.36.0     tweenr_2.0.3
##  [37] parallel_4.3.3          R6_2.5.1                stringi_1.8.4
##  [40] RColorBrewer_1.1-3      GOSemSim_2.28.1         Rcpp_1.0.13-1
##  [43] knitr_1.49              IRanges_2.36.0          Matrix_1.6-5
##  [46] splines_4.3.3           igraph_2.1.2            tidyselect_1.2.1
##  [49] qvalue_2.34.0           rstudioapi_0.17.1       yaml_2.3.10
##  [52] viridis_0.6.5           codetools_0.2-20        lattice_0.22-6
##  [55] tibble_3.2.1            plyr_1.8.9              treeio_1.26.0
##  [58] Biobase_2.62.0          withr_3.0.2             KEGGREST_1.42.0
##  [61] evaluate_1.0.1          gridGraphics_0.5-1      scatterpie_0.2.4
##  [64] polyclip_1.10-7         Biostrings_2.70.3       pillar_1.9.0
##  [67] ggtree_3.10.1           stats4_4.3.3            ggfun_0.1.8
##  [70] generics_0.1.3          RCurl_1.98-1.16         S4Vectors_0.40.2
```

```
##  [73] tidytree_0.4.6          munsell_0.5.1           scales_1.3.0       46
##  [76] glue_1.8.0              lazyeval_0.2.2          tools_4.3.3        47
##  [79] data.table_1.16.4       fgsea_1.28.0            babelgene_22.9     48
##  [82] fs_1.6.5                graphlayouts_1.2.1      fastmatch_1.1-4    49
##  [85] tidygraph_1.3.1         cowplot_1.1.3           grid_4.3.3         50
##  [88] ape_5.8                 tidyr_1.3.1             AnnotationDbi_1    51
   .64.1
##  [91] colorspace_2.1-1        nlme_3.1-166                               52
   GenomeInfoDbData_1.2.11
##  [94] patchwork_1.3.0         ggforce_0.4.2           cli_3.6.3          53
##  [97] fansi_1.0.6             viridisLite_0.4.2       dplyr_1.1.4        54
## [100] gtable_0.3.6            yulab.utils_0.1.8       digest_0.6.37      55
## [103] BiocGenerics_0.48.1     ggrepel_0.9.6           ggplotify_0.1.2    56
## [106] farver_2.1.2            memoise_2.0.1           htmltools_0.5.8.1  57
## [109] lifecycle_1.0.4         httr_1.4.7              GO.db_3.18.0       58
## [112] bit64_4.5.2             MASS_7.3-60.0.1                            59
```

References

1. I. Dolgalev, *Msigdbr: MSigDB gene sets for multiple organisms in a tidy data format* (2022; https://CRAN.R-project.org/package=msigdbr).

2. G. Yu, L.-G. Wang, Y. Han, Q.-Y. He, clusterProfiler: An r package for comparing biological themes among gene clusters. *OMICS: A Journal of Integrative Biology* **16**, 284–287 (2012).

3. H. Wickham, *ggplot2: Elegant graphics for data analysis* (Springer-Verlag New York, 2016; https://ggplot2.tidyverse.org).