The GSK3β-MAFB axis controls the pro-fibrotic gene profile of pathogenic monocyte-derived macrophages in severe COVID-19

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ABSTRACT

MAF and MAFB are members of the "large MAF" transcription factor family that shape the transcriptome of anti-inflammatory and pro-tumoral human macrophages. We have now determined the MAF- and MAFB-dependent gene profile of M-CSF-dependent monocyte derived macrophages (M-MØ) and found that both factors exhibit overlapping transcriptional outcomes during monocyte-to-M-MØ differentiation, but differentially affect macrophage effector functions like production of monocyte-recruiting chemokines, T-cell activation and immunosuppression. Remarkably, MAFB was found to positively regulate the expression of the genesets that define the pathogenic monocyte-derived pulmonary macrophage subsets in COVID-19, as evidenced through siRNA-mediated silencing and analysis of MAFB overexpressing M-MØ from a Multicentric Carpotarsal Osteolysis (MCTO) patient. MAFB silencing downregulated the expression of genes coding for biomarkers of COVID-19 severity, and genome-wide mapping of MAFB-binding elements in M-MØ identified biomarkers of COVID-19 severity (CD163, IL10 and CCL2) as direct MAFB targets. Further, and in line with the GSK3β-dependent expression of MAFB, GSK3β inhibition in M-MØ significantly boosted the expression of genes that characterize pathogenic macrophage subsets in severe COVID-19, an effect that was primarily dependent on MAFB. Indeed, SARS-CoV-2 infection was found to significantly upregulate the expression of GSK3β- and MAFB-dependent pro-fibrotic genes in human monocyte-derived M-MØ. Globally, our results demonstrate that the GSK3β-MAFB axis controls the transcriptome of pathogenic pulmonary macrophages in COVID-19, and positively regulates the expression of biomarkers for COVID-19 severity. Thus, macrophage reprogramming through modulation of GSK3β-MAFB axis has potential therapeutic strategy for COVID-19 and inflammatory diseases.

RESULTS

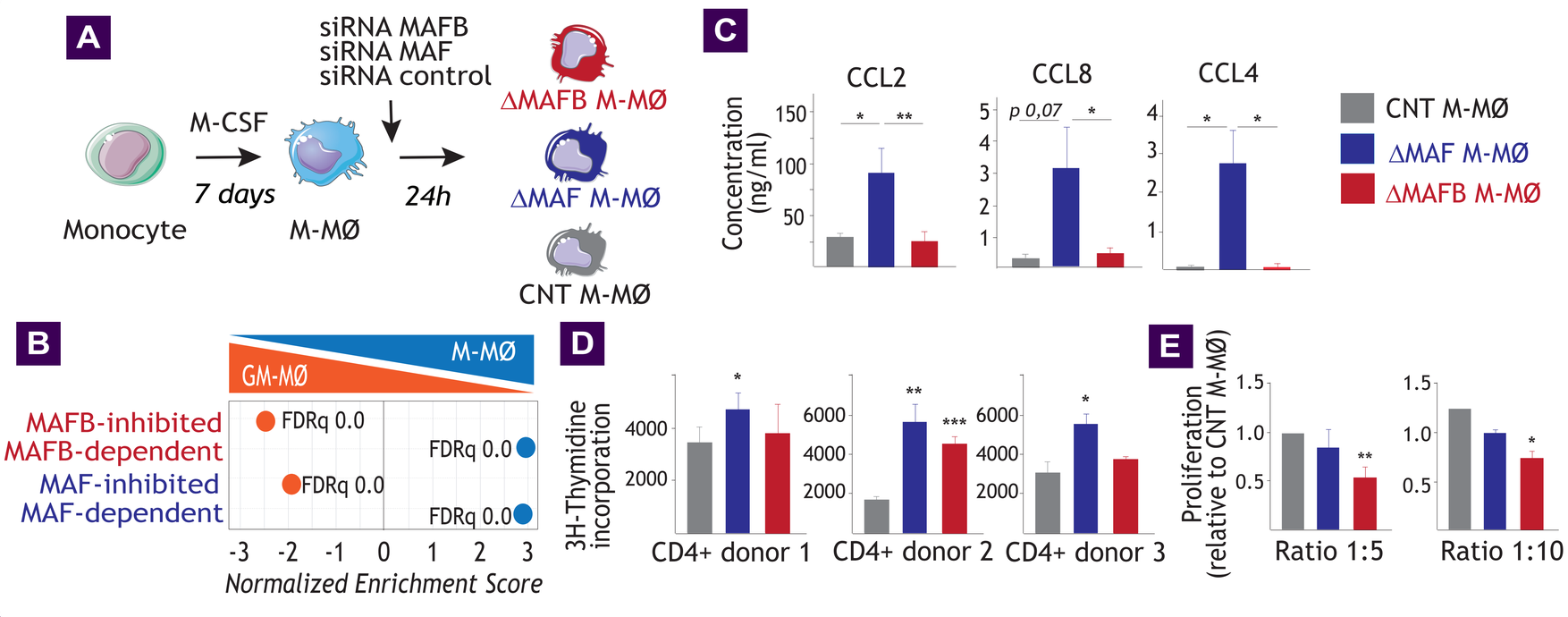


Figure 1. MAFB and MAF regulate shared and disparate genes and functions in anti-inflammatory monocyte-derived M-MØ. A. Schematic representation of the generation of ΔMAFB M-MØ, ΔMAF M-MØ and control M-MØ (CNT M-MØ) before RNA isolation and RNA sequencing (GSE155719). **B.** Summary of GSEA of the top 150 MAF/MAFB dependent or MAF/MAFB-inhibited genes on the ranked comparisons of the M-MØ and GM-MØ transcriptomes (GSE68061). **C.** Production of the indicated chemokines in ΔMAFB M-MØ, ΔMAF M-MØ and CNT M-MØ, as determined by ELISA. Mean ± SEM of 9 independent donors are shown (*, p<0.05; **, p<0.01). **D.** Allogeneic CD3+ T lymphocyte proliferation promoted by ΔMAFB M-MØ, ΔMAF M-MØ or CNT M-MØ. Mean ± SEM of the 4 replicates performed in each experiment are shown (*, p<0.05; **, p<0.01). **E.** Proliferation of CD3/CD28-stimulated CD4+ T lymphocytes in the presence of ΔMAFB M-MØ, ΔMAF M-MØ or CNT M-MØ, using 2 different M-MØ:T cell ratios. Mean ± SEM of 7 (ratio 1:5) or 4 (ratio 1:10) independent experiments are shown (*, p<0.05; **, p<0.01).

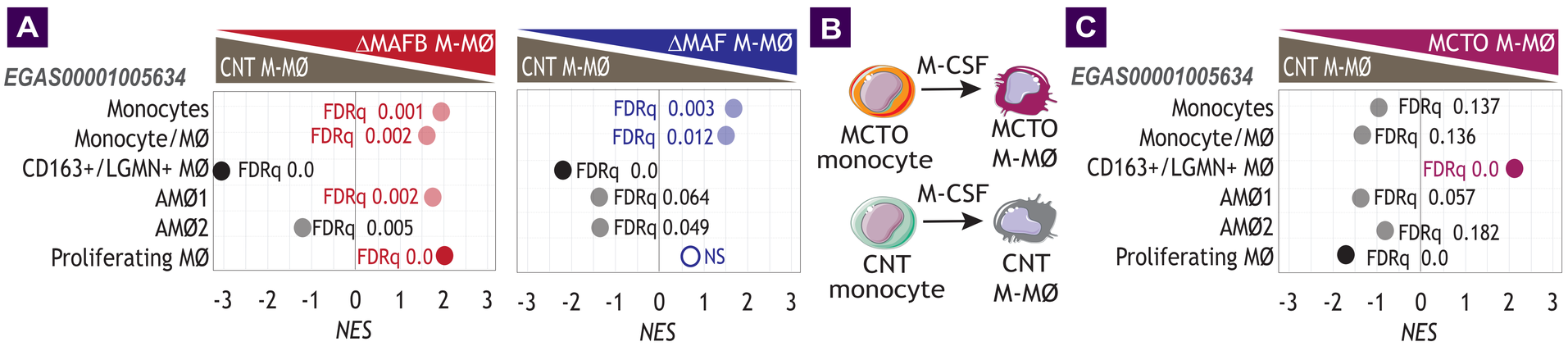


Figure 2. MAFB shapes the transcriptome of pathogenic macrophages in severe COVID-19. A & C. Summary of GSEA of the gene sets that characterize the macrophage subsets identified in severe COVID-19 on the ranked comparison of the transcriptomes of ΔMAFB M-MØ vs. CNT M-MØ (**A** left panel), ΔMAF M-MØ vs. CNT M-MØ (**A** right panel) or MCTO M-MØ vs. CNT M-MØ (**C**). **B.** Schematic representation of the in vitro generation of M-MØ from a MCTO patient (MCTO M-MØ) or healthy controls (CNT M-MØ) before RNA isolation and RNA-sequencing (GSE155883).

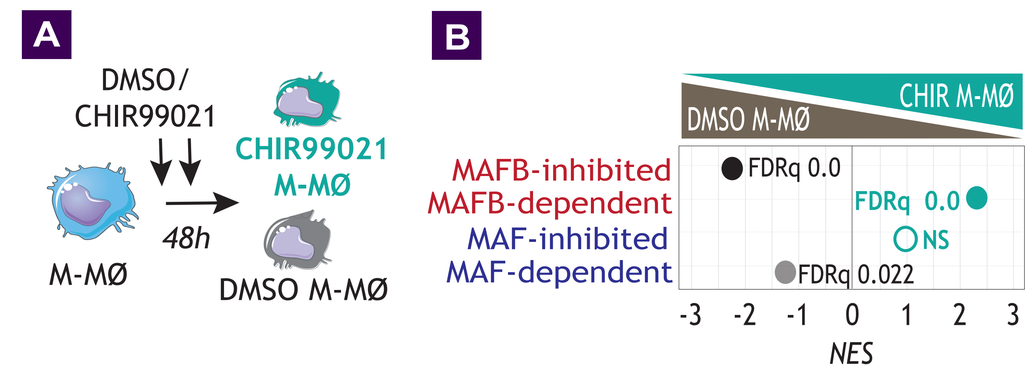
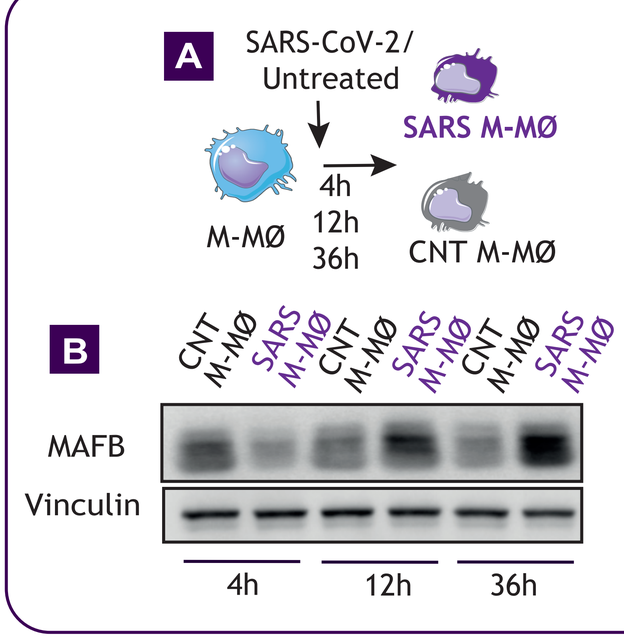


Figure 3. GSK3β inhibition prompts the acquisition of the pro-fibrotic gene profile that identifies pathogenic macrophages in severe COVID-19 primarily via MAFB. A. Schematic representation of the treatment of M-MØ with the GSK3β inhibitor CHIR99021 (CHIR99021 M-MØ) or DMSO (DMSO M-MØ) as a control before RNA isolation and RNA-sequencing (GSE185872). **B.** Summary of GSEA of MAF/MAFB-dependent gene sets and MAF/MAFB-inhibited gene sets on the ranked comparison of the CHIR99021 M-MØ and DMSO M-MØ transcriptomes. **C.** Summary of GSEA of the gene sets that characterize the macrophage subsets identified in severe COVID-19 on the ranked comparison of the transcriptomes of CHIR99021 M-MØ vs. DMSO M-MØ.



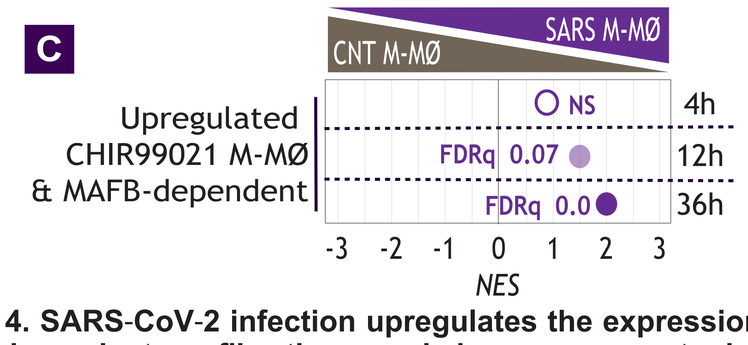


Figure 4. SARS-CoV-2 infection upregulates the expression of GSK3β- and MAFB-dependent pro-fibrotic genes in human monocyte-derived macrophages. A. Schematic representation of the generation of SARS-CoV-2 infected M-MØ (SARS M-Ø) and uninfected M-MØ (CNT M-MØ) at different times before RNA isolation and RNA-sequencing (GSE207840). **B.** MAFB protein levels in M-MØ subjected to the indicated treatments, as determined by Western blot and densitometry. Vinculin protein levels were determined as protein loading control. **C.** Summary of GSEA of the MAFB-dependent and upregulated by GSK3β inhibitor CHIR99021 gene set on the ranked comparison of the SARSCoV-2 infected M-MØ (SARS-M-MØ) relative to uninfected M-MØ (CNT M-MØ) transcriptomes at indicated times.

CONCLUSIONS

- MAFB IS RELEVANT IN THE ADQUISITION OF THE PRO-FIBROTIC PROFILE OF PATHOGENIC MACROPHAGES IN COVID-19.
- THE GSK3β-MAFB AXIS IS A POTENTIAL THERAPEUTIC TARGET IN SEVERE SARS-CoV-2 INFECTION.

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