The Involvement of CCR3-CCL24 Axis in Endothelial Mesenchymal Transition Process and in Pulmonary Arterial Hypertension in Systemic Sclerosis Patients

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CCL24 is involved in the pathogenesis of Systemic Sclerosis. Our results highlight the involvement of CCL24 in EndMT resulting in disease complications such as PAH.

Introduction

1

Systemic sclerosis (SSc) is an autoimmune disease characterized by vascular injury and extensive tissue fibrosis of the skin and internal organs. Endothelial cells (ECs), a predominant target of autoimmune attack, may undergo proliferation arrest, apoptosis, or differentiation to myofibroblasts, leading to complications including pulmonary arterial hypertension (PAH). The differentiation of ECs into myofibroblasts through endothelial mesenchymal transition (EndMT) represents a critical step in the blood vessels remodeling[1]. The pro-fibrotic chemokine CCL24 has been implicated in several fibrotic-inflammatory diseases[2-4]. ECs in the dermal microvascular tissue of patients with SSc exhibit robust expression of CCR3, the cognate receptor of CCL24, which has been found to be elevated in the serum and skin biopsies of patients with SSc[2]. Here we aim to evaluate the association between serum CCL24 levels and clinical characteristics of SSc patients, and the effect of CCL24-CCR3 axis on ECs phenotype and EndMT process.

Method

2

Clinical samples: CCL24 levels were evaluated in the sera of 75 patients with SSc using ELISA. In this cohort, Pearson correlation tests were conducted to determine the relationship between CCL24 levels and performance measures such as the 6-minute walk test (6MW test).

in-vitro system: We established a system to assess EndMT in human umbilical vein endothelial cells (HUVECs). Combination of TGFβ and TNFα were tested, alone, with CCL24, and with CM-101, a CCL24-neutralizing antibody. Studies included functional assessment of proliferation, cell death migration as well as evaluation of multiple markers of EndMT processes: αSMA, SNAIL, CD31 and VE-cadherin. Protein load was tested by Ponceau and served to normalize the results.

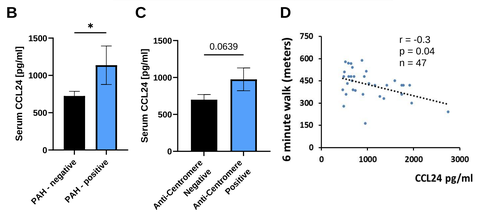
Results

3

SSc Patients with PAH have increased blood levels of CCL24 compared to SSc patients without PAH

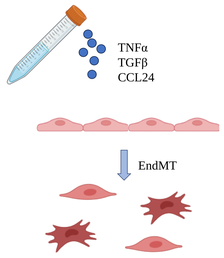
A

	Cohort	Expected #
N	75	
Female, N (%)	62 (87)	80
Age, mean	54	40-50
Limited/Diffuse, N (%)	22 (29.3)	50-60
ILD, N (%)	24 (32)	35–52
DU, N (%)	35 (46.6)	30-58
PAH, N (%)	9 (12)	7.5–12



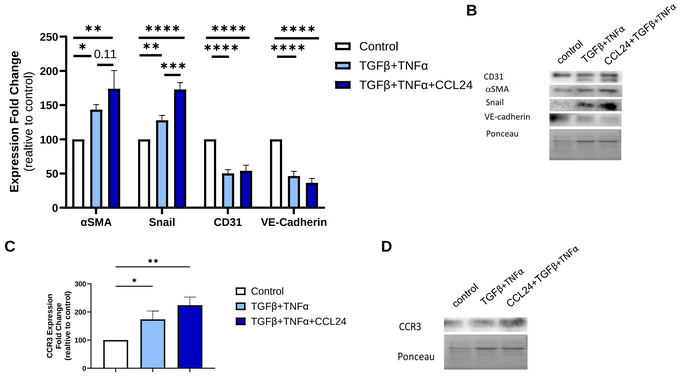
(A) Patient characteristics table. (**B-C**) CCL24 serum levels are increased in SSc patients with PAH (**B**) and patients positive for anti-Centromere antibodies (**C**). (**D**) Negative correlations were found between CCL24 levels to 6-minute-walk test. Unpaired T test *, p<0.05. # expected based on literature[5].

Characterization of in-vitro induced EndMT



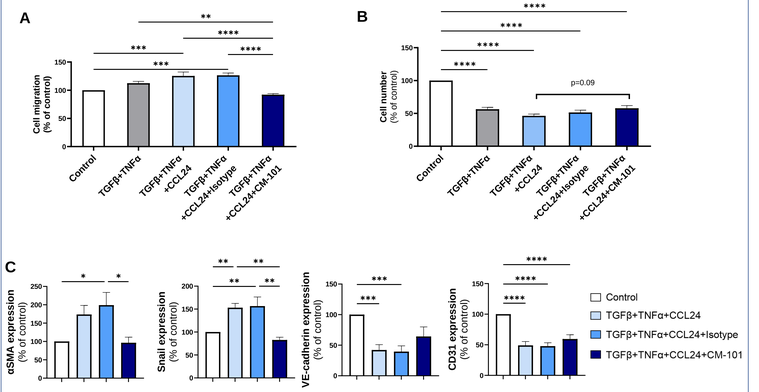
- Functional: proliferation, viability, migration
- Endothelial/mesenchymal marker expression: CD31, VE-cadherin, Snail, αSMA,
- CCR3 expression

CCL24 has an additive effect on in-vitro induced EndMT



(**A-B**) in-vitro induced EndMT by TGFβ (10 ng/mi) + TNFα (10 ng/mi) was increased with CCL24 addition (50 ng/ml). EndMT was examined by protein expression of mesenchymal and endothelial markers **(C-D)** in-vitro induced EndMT is characterized by increased CCR3 expression. one-way ANOVA followed by multiple comparisons tests (Dunnetts or Holm-Sidak). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

CCL24 blockade in-vitro attenuates cell migration and increases cell number



(**A**) Addition of Anti-CCL24 antibody (CM-101) to the cells restored the increase in migration capability caused by in-vitro induced EndMT. (**B**) CM-101 addition slightly recovered the cells number. (**C**) CM-101 addition reduced expression of mesenchymal markers. one-way ANOVA followed by Holm-Sidak's multiple comparisons test . *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Conclusions

4

- CCL24 is associated PAH in systemic sclerosis.
- SSc microenvironmental factors (TGFβ and TNFα) induce EndMT and increase CCR3 expression in ECs.
- CCL24 increases the mesenchymal characteristics of ECs.
- Blocking CCL24 attenuates EndMT related markers and cell migration.

References

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Disclosures

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HL and IV are employees of Chemomab Ltd.