The peri-ductular CCL24 rich niche promotes bile duct fibrosis related liver damage in primary sclerosing cholangitis

A. AHARON [1], D. THORBURN [2], N. BARASHI[1], M. SEGAL-SALTO [1] and A.MOR [1]

1 Chemomab Ltd., Tel Aviv, Israel

2 Sheila Sherlock Liver Centre & UCL Institute of Liver and Digestive Health, Royal Free Hospital, London, UK.

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1 Introduction

The C-C motif chemokine ligand 24 (CCL24 or Eotaxin-2) is a chemokine that signals through CCR3, a G-protein coupled receptor (GPCR) that is selectively found on the surface of various target cells. Recent studies have shown that CCL24 has an immune-metabolic role driving the Th2/M2 immune response which is responsible for continuous tissue injury and fibrosis.

PSC is a chronic cholestatic liver disease characterized by peri-biliary inflammation and fibrosis. Using liver biopsies from PSC patients, overexpression of CCL24 was shown in immune cells and cholangiocytes centered around the damaged bile duct areas.

2 Aim

This study aims to establish CCL24's expression under profibrotic conditions and its pivotal role as a driver of fibrosis in PSC related pathophysiology.

3 Method

Expression of CCL24 in primary human biliary epithelial cell (BEC) and M2 Macrophages was quantified using real time PCR and ELISA.

Human primary hepatic stellate cells (pHSC) and LX2 cells proliferation was assessed by CFSE staining and FACS. Serum samples from PSC patients were analysed for CCL24 levels and ELF score. Multivariate linear regression analysis was used to test the dependence between CCL24 and ELF with respect to disease severity.

5 Conclusions

The immune response that drives biliary fibrosis is characterized by generation of a CCL24 rich niche. Cholangiocytes (BEC) and M2 macrophages secrete CCL24 and drive a vicious circle that perpetuate liver inflammatory and fibrotic damage. This localized CCL24 rich environment set the stage for peri-ductular ongoing fibroblast proliferation and activation as well as inflammatory cells recruitment. The clinical relevance of CCL24 in human PSC pathophysiology is further supported by the bidirectional dependency of CCL24, ELF and bilirubin, in PSC patients' sera, that is increased with disease progression. CM-101, a monoclonal antibody neutralizing CCL24, is currently studied in a phase 2 study as a potential novel treatment for PSC.

6 Contact information

Arnon@chemomab.com

4 Results

CCL24 expression is upregulated in macrophages and cholangiocytes under profibrotic conditions

M2 macrophages are important promotors of fibrosis through the maintenance of an immune environment enriched by pro-fibrotic factors. M2 polarization of freshly isolated human macrophages induced a 4-fold increase in CCL24 gene expression that was also reflected by elevated CCL24 secretion compared to M0 unpolarized cells (Fig 1A-B).

Under normal conditions pHSC do not secrete CCL24. Co-culturing of pHSC with M2 macrophages show a significant elevation in CCL24 secretion compared to M2 macrophages alone. To further test whether the elevated CCL24 secretion is derived from the M2 macrophage or the pHSC, pHSC were Incubated with conditional medium (CM) from either M0 or M2 macrophages (Fig. 2B; M0-CM or M2-CM respectively). Culturing of pHSC with CM from M2 macrophages resulted in elevated expression of CCL24 compared to similar culturing with CM-M0, thus suggesting that the M2-macrophages induce CCL24 secretion from pHSC (Fig 2A-B).

Staining of PSC patients' liver biopsies demonstrated robust CCL24 expression in cholangiocytes. To further understand what drives cholangiocytes to secret CCL24, we cultured primary bile epithelial cells (BEC, cholangiocytes) in the presence of several profibrotic and proinflammatory cytokines and evaluated their effect on CCL24 expression. Similar to the effects seen on macrophages, we found that the Th2 cytokines (i.e. IL-4 and IL-13), induced a substantial, 12 and 13-fold, increase in CCL24 expression respectively, compared to untreated controls (Fig 3A-B).

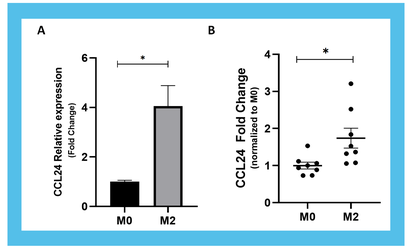

Figure 1: CCL24 elevated expression in M2 macrophages. *upregulation of CCL24 expression in M2 polarized macrophage (A). Increased secretion of CCL24 to the media by M2 (polarized by IL4) compared to M0 non-polarized macrophages (B).*

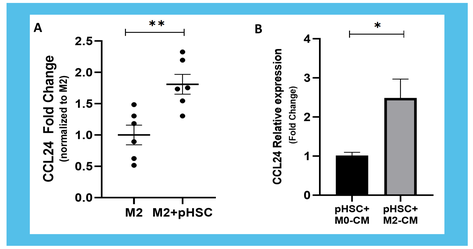

Figure 2: Co-culturing of M2 macrophages with pHSC increase CCL24 expression. *CCL24 concentration in cell media increase by 70% following M2 and pHSC co-culturing (A). Conditional medium from M2 induced a 2-fold increase in CCL24 gene expression compared to culturing with conditional medium from unpolarized M0 macrophages (B).*

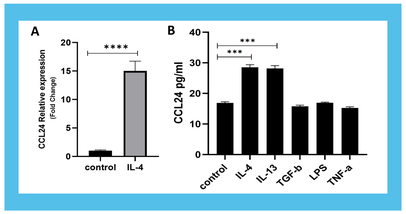

Figure 3: Culturing of BEC with Th2 cytokine increased CCL24 expression. *Culturing of human primary BEC in the presence of IL-4 increased CCL24 expression (A). Secretion of CCL24 to the medium by human primary BEC is induced by pre incubation with IL-4 and IL-13 (B).*

CCL24 induces hepatic stellate cell proliferation *in-vitro*

The extensive expression of CCL24 by immune cells and cholangiocytes induced by pro-fibrotic IL-4-/L-13 cytokines, suggest that under the fibrosis supportive Th2/M2 conditions the peri-ductal space may be saturated with CCL24. This over secretion can potentially activate CCR3 positive myofibroblasts. Incubation of pHSC with CCL24 resulted in a significant 50% increase their proliferation measured by cell count and CFSE staining (Fig 4A). Supporting these results, culturing LX2 cells (immortal HSC cell line) with escalating levels of CCL24 resulted in a dose dependent increase in proliferation with matching increase in proliferation index (Fig. 4B). CM-101, a monoclonal antibody that specifically blocks CCL24, inhibited pHSC and LX2 proliferation and reduced cell count back to baseline levels (Fig 4A-B).

CCL24 correlates with serum fibrotic biomarkers in PSC patients

In a cohort of PCS patients, Enhanced Liver Fibrosis (ELF™) score was found to be associated with CCL24 serum levels, more profoundly in the subpopulation of patients that had Alkaline phosphatase (ALP) levels >1.5 ULN. These data support the involvement of CCL24 in fibrotic PSC related damage and more significantly in active disease. Multi-variate analysis demonstrated a correlation between CCL24, ELF and bilirubin. This correlation reflects the ability to predict elevated CCL24 levels by ELF and bilirubin and vice a versa. This positive association increased with disease severity (Fig. 5; r=68%, p=0.017 for patients with ALP>1.5 ULN).

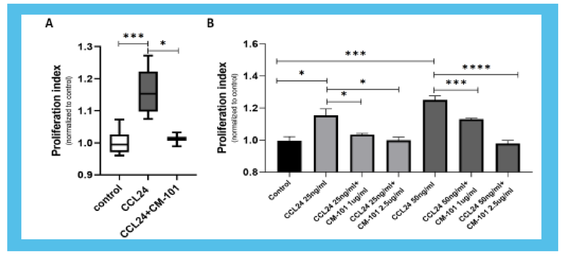

Figure 4: CCL24 induced hepatic fibroblast proliferation. *Culturing of human pHSC (A) or LX2 cells (B) with CCL24 increased the cells proliferation index. This effect was reversed back to baseline levels following treatment with CM-101, a CCL24 neutralizing monoclonal antibody.*

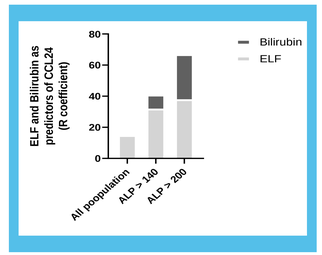

Figure 5: CCL24, ELF and bilirubin measured in PSC patients' sera are strong predictors of each other. *Multi-variant analysis revealed that for patients presented with higher ALP levels, CCL24, ELF and bilirubin become better predictors of each other.*