# Inflammatory Keloid Fibroblasts Promote CCL2-Dependent Monocyte Recruitment and Polarization Toward M2-like Macrophages

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**Running Title**
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## BACKGROUND

Beyond Beyond being a simple fibroproliferative disorder, keloids should be regardedas a chronic inflammatory disease in which inflammation, together with mechanical cues and genetic susceptibility, contributes to the initiation and persistence of tissue fibrosis. This complexity calls for new clinical paradigms to improve keloid management, prevent recurrence, and overcome treatment resistance. At the cellular level, increasing evidence highlights the importance of crosstalk between keloid fibroblasts (KFs) and immune cells, particularly those of the innate immune system, such as mast cells, dendritic cells, and macrophages. Our group previously demonstrated that the accumulation of type 2 macrophages in keloid tissue promotes the acquisition of a more fibrogenic phenotype by keloid fibroblasts (Dirand et al., 2024). In the present study, we aimed to determine how KFs influence monocytes and macrophages during keloid fibrogenesis.

## METHODS

Keloid fibroblasts were collected and banked from tissue collected during our clinical trial 'Scar Wars' (NCT03312166). Using bulk RNA sequencing, we first identified differentially expressed genes between KFs (n=3) and non-keloid fibroblast cell lines (NKFs; n=3). KFs were also phenotypically compared to NKFs by flow cytometry (CD34, CD90, CD138, CD140a, FAP, and podoplanin), and their secretory profiles were analysed using proteome arrays (cytokines and proteases). We then focused on CCL2, a key cytokine involved in monocyte chemoattraction and immune regulation. CCL2 expression in KFs (vs NKFs) was assessed by RT–PCR and ELISA. Then, KFs secretome was used to evaluate the role of KF-derived CCL2 in monocyte chemoattraction (Transwell assay, Incucyte S3 live-cell imaging system) and macrophage polarization.

## RESULTS

Specific membrane markers analysis showed that CD34, FAP, CD90, CD138, and CD140a were equally expressed in KFs and NKFs. Interestingly, Podoplanin expression was statistically higher in KFs compared to NFKs, and was upregulated after TGF-β1 treatment. In addition, we characterized protein-level differences between KFs and NKFs regarding inflammatory and remodeling-associated secreted factors. We determined that KFs synthesise higher levels of cytokines commonly associated with inflammatory and pro- fibrotic fibroblasts (CCL2, Pentraxin 3, THBS1, IGFBP-3 and Angiogenin).

On the proteases side, we observed a strong downregulation of major remodelling-associated proteinases in KF (MMP1, MMP2, MMP3 and Cathepsins) and a loss of expression of MMP7, MMP8, MMP9 and MMP12. From our transcriptomic analysis, we detected 787 downregulated and 505 upregulated genes in KF compared to normal cells. Among modified signalling pathways, we particularly observed that the chemokine signalling pathway, and particularly CCL2 expression, was strongly upregulated in our fibrotic cells. In correlation with RNA sequencing and proteome array, we validated CCL2 overexpression in KF by RT-PCR and ELISA. In a transwell migration assay, we then demonstrated that KFs were able to recruit more THP1 cells by chemotaxis than NKFs, specifically thanks to CCL2 overexpression. Moreover, we have also shown that such secretome was also able to polarize macrophage towards an M2-like phenotype.

## CONCLUSİON

As recently reviewed by Guo et al., the CCL2/CCR2 signalling pathway is a major axis involved in the development of fibrotic disorders across multiple organ systems. Here we further contribute to deciphering the role of this pathway in keloid (Liao et al., 2010; Chen et al., 2025). In line with the literature and our previously published data, our work highlights the central role of fibroblast-macrophage crosstalk in keloid fibrosis. Indeed, we suggest that keloid fibroblasts may contribute to sustaining a vicious cycle in which persistent macrophage recruitment and activation continuously amplify tissue fibrosis. In conclusion, CCL2 could be a promising therapeutic target to treat or prevent keloid by disrupting the pathological inflammation/fibrosis loop.

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## DİSCLOSURE DECLARATİON

The authors declare that there are no conflicts of interest.