

# Intraarticular injection of transgenic CXCR4 overexpressed human CPCs significantly improve meniscus healing and protects articular cartilage from PTOA mediated erosion in rabbits

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**INTRODUCTION:** Meniscus injuries that fail to heal can instigate catabolic changes in the knee microenvironment, posing a high-risk for the development of post-traumatic osteoarthritis (PTOA). We have established human articular cartilage-derived progenitor cell lines (CPCs) as a potential therapeutic tool in our continuous efforts to develop novel cell-based approaches for accelerating meniscus healing. Further, characterization of these cell lines revealed that they have several pro anabolic and anti-catabolic properties in comparison to marrow-derived stromal cells (BM-MSCs)<sup>1-3</sup>. Veritably, our most recent *in vivo* data suggest that treating meniscus tears with a human CPC cell line promotes meniscal tear healing compared to either the BM-MSC-treated group or untreated control group in athymic rats (**Fig. 1**)<sup>5</sup>. Additionally, we had demonstrated that Stromal Cell-Derived Factor-1 (SDF-1) pathway signaling is necessary for stimulating the directional migration of CPCs to stimulate meniscal fibrocartilage repair<sup>1,2</sup>. SDF-1 is highly expressed by injured meniscus tissue<sup>4</sup>, making it crucially important how cells used in biologic therapies react to the presence of this chemokine. In this study, we demonstrate that CPCs exhibit reduced NF- $\kappa$ B catabolic pathway signaling in response to SDF-1. Hence, we **hypothesized** that constitutively increasing the expression of CXCR4 (an SDF-1 receptor) in CPCs may further improve their efficacy in meniscus injury repair. Our findings below show that administering CXCR4 overexpressing CPCs via intraarticular injection significantly improved meniscus tear healing and protects against cartilage erosion in rabbits.

**METHODS:** Cell Culture: Healthy (non-arthritic) human CPC cell lines were established as previously described<sup>1</sup>. CPCs were then infected with lentivirus bearing CXCR4 followed by Puromycin selection and expansion. Immunoblotting: Cells were lysed, and the protein lysate was resolved on polyacrylamide gel in equal concentration, which was then transferred onto a PVDF membrane, followed by blocking step. The proteins of interest were probed with respective antibodies, and the blots were then analyzed using ECL substrate. Animal Studies: A medial parapatellar arthrotomy was performed on the right knees of skeletally mature New Zealand White rabbits. A 2.0 mm longitudinal bucket handle tear that spans the entire thickness of the meniscus was created. The patella was then returned to the natural position and the joint capsule, fascia, and skin were closed by suturing, respectively.  $5.0 \times 10^6$  cells were injected in respective groups at Day 7 and Day 28, post-op. Animals were divided in three experimental groups (6 animals/group): (1) CXCR4 overexpressing CPCs (CXCR4-OE CPC) injected; (2) Non targeting control CPCs (unmodified-CPCs) injected; (3) Saline injected controls. Animals were sacrificed 60 days post-op for analysis. Histology Analysis: The knee joint was harvested and fixed in 10% Neutral Buffered Formalin, followed by decalcification and processing. Samples were sectioned and stained with Safranin Orange (Saf-O)/Fastgreen. Modified double blinded OARSIS Scoring was used to evaluate articular cartilage of tibial plateau. Statistical analysis was performed by Kruskal–Wallis test, which is a one-way analysis of variance.

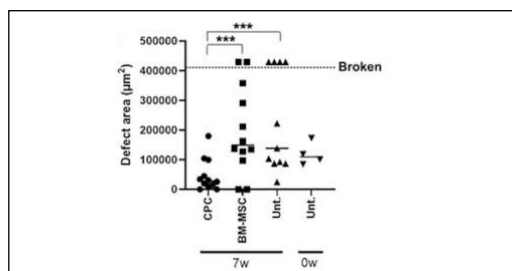
**RESULTS:** SDF-1/CXCR4 pathway regulation was investigated in CPCs. To investigate the mechanistic details, we examined the expression of downstream targets of these canonical pathways in response to SDF-1. We analyzed the NF- $\kappa$ B pathway by measuring the protein expression of I $\kappa$ B- $\alpha$  and NF- $\kappa$ B p65. The Erk and MAPK pathway were analyzed by measuring Erk and p38 along with their phosphorylation states (pErk) and (p-p38). Our results indicate that SDF-1 treatment in CPCs leads to significant inhibition of canonical NF- $\kappa$ B (**Fig.2A**) Erk and MAPK (**Fig.2B**), which are upregulated in response to SDF-1 in other cell types. Our results from the rabbit study demonstrate that CXCR4-OE CPCs significantly improves meniscus tear healing as compared to the unmodified-CPC or saline controls (**Fig. 3A**) (see circled region of interest). Additionally, CXCR4-OE CPCs promote restoration of proteoglycan content as can be seen by Saf-O (red) staining (**Fig. 3A**). We carefully examined all the menisci (18 total; 6 per experimental group) for healing (**Fig.3C**), which indicates that 83% animals showed fully healed menisci in CXCR4 OE CPCs treated group, which was significantly high as compared to Unmodified-CPC treated (50%) and saline control treated animals (33%). Additionally, CXCR4-OE CPCs significantly prevents articular cartilage degradation in tibial plateau, as compared to the saline controls (**Fig.3 B, D**).

**DISCUSSION:** Our results demonstrate that intra-articular injection of CPCs following meniscus tearing stimulates fibrocartilage restoration and healing in athymic rats<sup>5</sup>. Previously, we have demonstrated that SDF1/CXCR4 signaling axis is crucial for migration of CPCs to the injury site *in vivo*<sup>2</sup>. To investigate the mechanistic details, we examined the SDF-1/CXCR4 downstream signaling pathway by analyzing different catabolic branches of the pathway including NF- $\kappa$ B, Erk and MAPK. Our results revealed that these catabolic signaling is inhibited in CPCs in response to SDF-1 treatment. These findings are consistent with our previous observation that matrix metalloproteinase 13 (MMP13), a downstream target of NF- $\kappa$ B, is maintained at a lower expression level in CPCs than in BM-MSCs<sup>5</sup>. Additionally, CPCs that overexpress CXCR4 chemokine significantly promotes meniscus tear healing, restores proteoglycan content, and prevents cartilage degeneration in rabbits. Collectively these findings support our hypothesis that CXCR4 plays a vital role in CPCs mediated meniscus and cartilage repair through inhibiting downstream, catabolic, and inflammatory signaling.

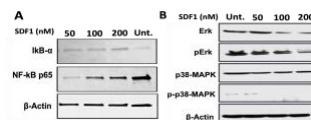
**SIGNIFICANCE/CLINICAL RELEVANCE:** This study demonstrates that stable human CPCs made to overexpress CXCR4 can be used as an injectable cell therapy to stimulate meniscus healing and prevent PTOA in rabbits.

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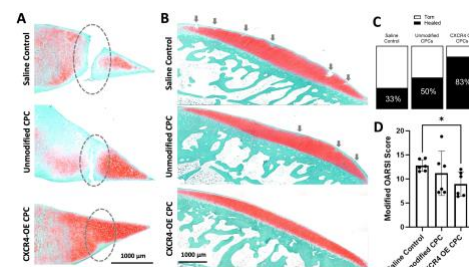
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**Fig – 1:** Intra-articular injection of CPC stimulates fibrocartilage tear healing. Open areas remaining within the meniscal tear were quantified by image analysis. Data points above the dotted line indicate menisci broken in two at the harvest time. A 0-week control group was used to determine the open space of the meniscal tear before healing.  $N \geq 8$  per group. **\*\*\***,  $P \leq 0.005$ .



**Fig – 2:** SDF-1 inhibits inflammatory and catabolic pathways in CPCs. The protein level expressions downstream targets of NF- $\kappa$ B pathway (**A**) and Erk and MAPK pathway (**B**) is shown. SDF-1 inhibits catabolic pathways in a dose dependent manner.



**Fig – 3:** Overexpression of CXCR4 promotes meniscus tear filling and prevents cartilage degeneration. Histology of the meniscus (**A**) and the tibial plateau (**B**) of Saline controls, Unmodified-CPCs treated, and CXCR4-OE CPCs treated animals is shown. Grey arrows represent areas of cartilage damage. The percentage of animals with healed vs torn menisci (**C**). Modified OARSIS scoring of tibial plateaus (**D**).  $N = 6$  animals per group. **\*** =  $P \leq 0.05$ .