"*Sticky*" tenocytes as a potential therapeutic tool for patellar tendinopathy

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INTRODUCTION: Patellar tendinopathy (PT), commonly referred to as "jumper's knee," is a chronic and often career-limiting disorder characterized by persistent pain, inflammation, tissue degeneration, and impaired mobility[1]. In advanced cases, degenerative PT manifests as focal structural breakdown and tenocyte loss, conditions under which the intrinsic capacity of the tendon to regenerate is profoundly limited. Current management strategies—ranging from physical therapy and injections to surgical debridement—offer variable outcomes and are associated with lengthy rehabilitation, high recurrence rates, and substantial healthcare costs[2]. Emerging cell-based therapies represent a transformative opportunity in tendon repair by addressing the root cause of degeneration: cellular depletion and impaired regenerative signaling[3-4]. A critical axis in tendon healing involves stromal cell-derived factor 1 (SDF-1) and its receptor CXCR4, which orchestrate stem and progenitor cell homing to sites of injury[5]. While acute tendon injuries benefit from this signaling cascade, degenerative tendinopathy is marked by inadequate tenocyte recruitment and survival, resulting in incomplete or failed repair. We hypothesize that human tenocytes genetically engineered to constitutively express CXCR4 (hTNCL-CXCR4, or "*sticky*" tenocytes) will exhibit enhanced retention (*stick* to the injury site) and migratory response toward SDF-1 secreted. By injured tissue, thereby restoring regenerative capacity and driving more efficient tendon healing. This strategy not only holds promise for PT but also has broad translational potential for other tendon and soft tissue injuries.

METHODS: *Creation of "sticky" tenocytes*: To generate "*sticky*" tenocytes, primary human tenocytes were first stabilized using SV40, producing expandable human tenocyte cell lines (hTNCL). These stabilized tenocytes were then transduced with a third-generation lentiviral vector encoding the CXCR4 transgene under a constitutive promoter, with Green Fluorescent Protein incorporated as a reporter downstream to CXCR4. Control cells were transduced with a vector containing red fluorescent protein (RFP) to allow dual fluorescence and enable direct comparison. *Transcriptomic profiling*: For transcriptomic analysis, RNA was isolated from four groups: (i) primary tenocytes, (ii) stabilized hTNCL, (iii) vector-control hTNCL, and (iv) "*sticky tenocytes*". RNA sequencing was performed (GeneWiz platform) and analyzed using Ingenuity Pathway Analysis (IPA). *Cell migration assay*: Cell migration was evaluated in vitro using a modified Boyden chamber system. A 3 µm culture insert allowed the free diffusion of soluble factors like SDF-1, while "*sticky*" tenocytes were seeded in an 8 µm insert. Migration toward SDF-1 gradients was quantified after 48 hours using an MTT assay (n=4). Proliferation and viability were assessed by MTT to rule out adverse effects of immortalization and CXCR4 overexpression. *Statistical analyses* were performed using the non-parametric Kruskal–Wallis test.

RESULT: We successfully established stabilized human tenocyte lines (hTNCL), enabling scalable expansion and cryopreservation **(Fig.1A)**. CXCR4 overexpression was achieved via lentiviral transduction, confirmed by fluorescence (dual fluorescence in case of vector controls) and quantitative RNA sequencing **(Fig.1B-C)**. MTT cell proliferation assay revealed that neither the stabilization nor CXCR4 overexpression adversely affects the viability of the cells **(Fig.1D)**. Transcriptomic analysis further confirmed CXCR4 overexpression by ~9-fold **(Fig.2)** and revealed broad modulation of regenerative pathways, including those regulating inflammation, extracellular matrix production, differentiation, and cell migration **(Fig.2)**. Approximately 850 genes were significantly altered, highlighting a strong regenerative signaling shift induced by CXCR4 overexpression. Functional migration assays demonstrated that hTNCL-CXCR4 exhibited greater migration in response to SDF-1 compared with controls, with a clear dose-dependent effect **(Fig.3A-B)**. These results strongly support the hypothesis that CXCR4-engineered tenocytes are more likely to localize to SDF-1–rich injury sites and promote efficient repair.

DISCUSSION: Our findings demonstrate that CXCR4-engineered "*sticky*" tenocytes can be generated safely and effectively, with enhanced migratory capacity and activation of regenerative molecular pathways. Importantly, CXCR4 overexpression did not compromise cell viability or proliferation, mitigating concerns regarding lentiviral modification and long-term stability. Transcriptomic profiling confirmed broad activation of pathways central to tissue regeneration, suggesting that these cells not only migrate to injury sites but may also actively remodel the local microenvironment. This study establishes the foundation for a regenerative platform capable of addressing the critical limitations of current tendon therapies. By engineering cells to overcome poor retention and survival in degenerative tissue, our approach provides a novel and potentially durable solution to chronic tendinopathy. The translational trajectory of this work will involve validating tenocyte retention and repair capacity in human tendon explants and tendon-on-chip models, followed by preclinical studies in rodent and large animal models. Ultimately, this strategy could shift the paradigm of care for tendon disorders, reducing reliance on invasive surgery and enabling faster, more complete recovery for patients.

CLINICAL RELEVANCE: Patellar tendinopathy is a debilitating condition with limited effective treatment options once degeneration sets in. Current conservative and surgical approaches often fail to fully restore tendon structure and function, leaving patients with persistent pain, impaired mobility, and prolonged recovery times. Our work introduces a novel cell-based therapeutic strategy using CXCR4-engineered "*sticky*" tenocytes designed to home to injury sites, survive, and promote tissue repair. This approach directly addresses the critical unmet need for targeted regenerative therapies in tendon disease. By enhancing cell retention and functional integration at the site of injury, this therapy has the potential to accelerate healing, reduce rehabilitation time, and lower the risk of surgical complications. Importantly, the principles underlying this strategy can be extended beyond the patellar tendon to other major tendons, ligaments, and soft tissues, laying the foundation for a broadly applicable regenerative platform in musculoskeletal medicine.

REFERENCES: **[1]** Charles et al., *Front Immunol*. 2023 Aug 16:14:1193835. **[2]** Sugrañes et al., *JBJS Rev.* 2023 Aug 17;11(8). **[3]** Am J Sports Med. 2025 Feb;53(2):396-405. **[4]** Kader et al., Expert Opin Biol Ther. 2021 Aug;21(8):1035-1047. **[5]** Sun et al., *Biomaterials*. 2018 Apr:162:22-33.

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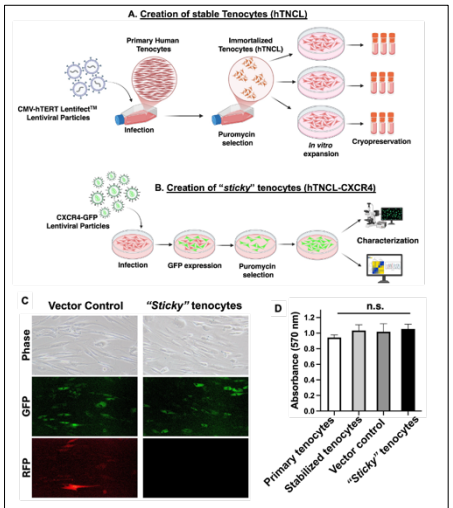

Figure 1: Establishment of CXCR4-hTNCL. ***(A-B)*** Schematic representation of the establishment of hTNCL constitutively overexpressing CXCR4. ***(C)*** Representative fluorescent microscopy images of control hTNCL (bearing dual reporter) and hTNCL-CXCR4. Images were taken at 10X magnification ***(D)*** MTT cell proliferation assay (n=4) n.s. indicates not significant.

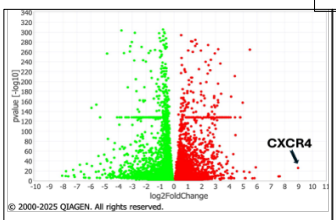

Fig.2: CXCR4 overexpression modulates the transcriptome landscape of hTNCL. Volcano Plot represents differentially expressed genes in hTNCL-CXCR4 as compared to unmodified control cells. Red dots represent upregulated genes, whereas green dots represent downregulated genes. Analysis was performed using Ingenuity Pathway Analysis software (Qiagen, USA).

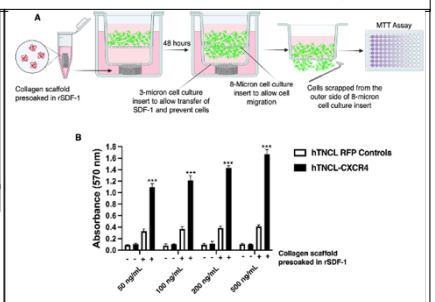

Figure 3: hTNCL-CXCR4 migrates in response to SDF-1. ***(A)*** Schematic representation of 3D cell migration assay. ***(B)*** MTT assay to quantify the migrated cells from the outer side of the 8-micron cell culture insert. Wells without the scaffold were used as controls. *** = p value < 0.001.