

# The Role of the Epigenetic Regulator SMYD2 in Knee Arthrofibrosis

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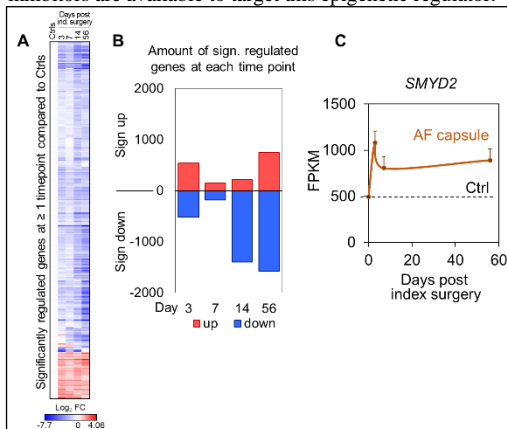
**INTRODUCTION:** Arthrofibrosis is a common complication after total knee arthroplasties (TKAs) and leads to decreased range of motion in around 5% of patients. The excessive scarring found in the joint is postulated to be triggered by an overreaction of the immune system from surgery insult in conjunction with patient-specific genetic and environmental risk factors. In this context, immune-related factors increase the synthesis of transforming growth factor beta (TGF $\beta$ ) which induces fibroblasts to myofibroblast differentiation and excessive extracellular matrix deposition, two main hallmarks of arthrofibrosis. Little is known, however, about the cellular and molecular mechanisms causing arthrofibrotic tissue development and whether epigenetic mechanisms contribute to driving myofibroblastogenesis. Using our rabbit model for arthrofibrosis and our ex-vivo patient cell culture system, here we applied an integrative approach to investigate the molecular basis of knee arthrofibrosis.

**METHODS:** In the first part of our study, mature New Zealand White female rabbits underwent a contracture-inducing surgery that immobilized their right knees. Rabbits were then sacrificed at 3, 7, 14 and 56 days post-surgery and the experimental posterior capsule tissues were collected from 3 rabbits at each time point. Prior to surgery, capsules from 3 unaffected rabbits were also collected as controls. Total RNA was then extracted from all collected tissues and next generation bulk RNA sequencing was performed. In the second part of our study, we collected control tissues from three patients undergoing primary TKA for osteoarthritis from two distinct anatomical locations within the knee – quadriceps tendon (TKA-QT) and suprapatellar pouch (TKA-SP). Fibrotic tissues were collected from three patients undergoing revision TKA for arthrofibrosis (RTKA-AF). For the use of human tissues and cells, all patients included in this study were consented according to our approved Institutional Review Board (IRB) protocol prior to enrollment. Post collection, tissues were digested with collagenase type I and the resulting cell suspensions were placed in culture to select for plate-adhering fibroblasts. Fibroblast to myofibroblast differentiation was induced using TGF $\beta$ 1 (10 ng/ml) for three days. In addition, cells were treated with distinct SMYD2 inhibitors or siRNAs to inhibit the expression of selected genes. Thereafter, RNA, protein or cell matrices were collected for cellular and molecular analyses.

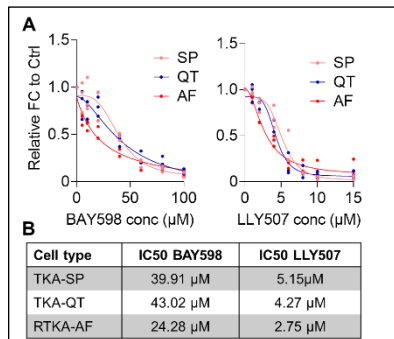
**RESULTS:** Temporal RNA-Seq analysis of our rabbit model for arthrofibrosis revealed that specific gene groups (e.g., collagens) were transiently regulated in the arthrofibrotic knee capsules, reflecting the molecular dynamics during fibrotic tissue development (**Figure 1**). However, we also found a small number of genes which were persistently down- or up-regulated in the arthrofibrotic rabbit knees when compared to control tissues. Of note, genes which remained continuously suppressed include the collagen crosslinkers *LOXL4* and *P3H4*. Among the small number of genes which were constantly upregulated in the arthrofibrotic knees include SMYD2, an epigenetic enzyme which regulates gene expression via histone modifications and by stabilizing other epigenetic regulators such as EZH2. When assessing for the effects of two selective SMYD2 inhibitors (BAY598 & LLY507) on cell proliferation of patient derived cells ex-vivo, our data showed that RTKA-AF cells are approximately twice as sensitive when compared to TKA-QT and TKA-SP control cells (**Figure 2**). Upon induction of myofibrogenesis by TGF $\beta$ 1, western blot analyses showed an upregulation of SMYD2 and EZH2 in patient derived knee cells (**Figure 3**). Furthermore, selective knock down of SMYD2 by siRNA decreased TGF $\beta$ 1 mediated extra cellular matrix deposition and protein expression of EZH2 as well as of ACTA2, a major myofibroblastic marker.

**DISCUSSION:** Our data suggest that upregulation of the epigenetic regulator SMYD2 plays a central role in myofibroblastic differentiation of knee fibroblasts during the development of knee arthrofibrosis. SMYD2 dependent epigenetic mechanisms may control initiation and progression of arthrofibrosis in the knee.

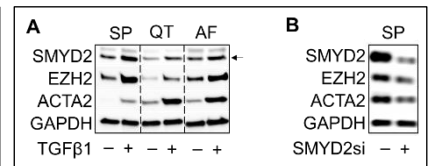
**SIGNIFICANCE/CLINICAL RELEVANCE:** Our data indicates a specific role of an epigenetic enzyme in arthrofibrosis. Most importantly, selective inhibitors are available to target this epigenetic regulator.



**Figure 1: SMYD2 is among the few genes upregulated in our rabbit model for arthrofibrosis.** (A) Gene expression heat map of up (red) or down (blue) regulated genes and (B) number of genes significantly up and down regulated during arthrofibrogenesis (AF) of rabbit knee capsules vs control capsules. (C) mRNA expression profile for *SMYD2* in AF (red) vs control capsules (dotted line). N=3 for all samples.



**Figure 2: Distinct sensitivity of human arthrofibrotic versus control knee fibroblasts to SMYD2 inhibitors.** (A) Effect of increasing BAY598 or LLY507 concentrations on cell proliferation in patient derived TKA-SP (SP), TKA-QT (QT) and RTKA-AF (AF) cells. (B) Half-maximal inhibitory concentrations (IC<sub>50</sub>) for each cell type are shown. N=3 for all samples.



**Figure 3: SMYD2 expression is linked to TGF $\beta$ 1 induced myofibrogenesis ex-vivo.** (A) Expression of SMYD2, EZH2, ACTA2, and GAPDH in TKA-SP (SP), TKA-QT (QT) and RTKA-AF (AF) cells with or without TGF $\beta$ 1 as shown by western blot analysis. (B) Effects of SMYD2 knock down by siRNA on the expression of epigenetic regulator EZH2 and the myofibrogenic marker ACTA2.