The Epigenetic Foundation of Knee Arthrofibrosis

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INTRODUCTION: Arthrofibrosis (AF) is a common complication after total knee arthroplasty (TKA) that leads to clinically relevant 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 surgical 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 (TGFβ) which induces fibroblast 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 myofibroblastogenesis in the knee. Using a holistic approach, we investigate the epigenetic shift observed in arthrofibrotic knee tissue and during myofibroblastogenesis in primary patient knee fibroblasts *in vitro*. We functionally correlate cellular and molecular markers to decipher the role of epigenetic gene regulation in the etiology of knee arthrofibrosis.

METHODS: We collected knee AF tissue (n=14; 5 males & 9 females) from patients undergoing a revision TKA for arthrofibrosis and, as controls, suprapatellar pouch tissue (SP, n=11; 5 males & 6 females) from patients who received a primary TKA for osteoarthritis. All patients included in this study were consented according to our approved Institutional Review Board (IRB) protocol prior to enrollment. Post collection, RNA was extracted and submitted for RNA-Seq to acquire transcriptomic data. Further, primary knee fibroblasts were isolated from SP (n=10) and AF (n=19) tissues via collagenase I digestion and put in culture using Advanced MEM media supplemented with 5% human platelet lysate, heparin, GlutaMAX, and antibiotic/antimycotic. Myofibroblastogenesis was induced with TGFβ1 (10 ng/mL), and protein and RNA were collected after 3 days of culture. RNA-Seq files were analyzed using our established RNA-Seq data analysis pipelines (including packages like RNA STAR, FeatureCounts, DESeq2 and others).

RESULTS: Analysis of all known epigenetic regulators revealed that a substantial fraction are differentially expressed between SP and AF knee tissues (Fig. 1A). Notably, regulators of DNA methylation were most prominently altered, followed by those involved in H3K27 methylation and histone acetylation/deacetylation (Fig. 1B), highlighting broad epigenetic divergence between fibrotic and non-fibrotic tissues. To further explore the role of epigenetic mechanisms in myofibroblast differentiation, we (i) examined the impact of TGFβ1 on the expression of these regulators in vitro and (ii) compared the results with our tissue RNA-Seq data. These analyses identified a consistent set of epigenetic regulators, particularly those linked to DNA methylation, H3K27 methylation, and histone acetylation that are altered in SP vs. AF tissues and in TGFβ1-treated vs. control SP or AF knee fibroblasts (Fig. 2). Western blot analyses demonstrated a significant upregulation of the H3K27-related factors SMYD2 and EZH2 in AF tissues (Fig. 3A). Pearson correlation analysis revealed a strong positive correlation between SMYD2 and EZH2 protein levels in AF tissues but not in SP tissues (Fig. 3B). Moreover, TGFβ1 modulated SMYD2 expression in a cell-type specific manner between SP- and AF-derived fibroblasts (Fig. 3C).

DISCUSSION: Our data reveal extensive epigenetic alterations between non-fibrotic and fibrotic knee tissues as well as between control and TGFβ1 treated knee fibroblasts suggesting that epigenetic mechanisms control initiation and progression of arthrofibrosis in the knee.

SIGNIFICANCE/CLINICAL RELEVANCE: Our data indicates a primary role of epigenetic gene regulation in arthrofibrosis.

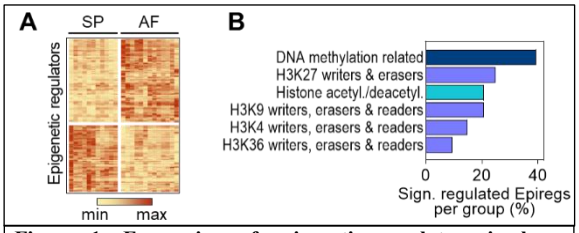

Figure 1: Expression of epigenetic regulators in knee arthrofibrosis. (**A**) Heat map analysis of differentially expressed epigenetic regulators in suprapatellar pouch (SP, control) versus arthrofibrotic (AF) knee tissues. (**B**) Functional classification of differentially expressed epigenetic regulators between SP and AF patient tissues.

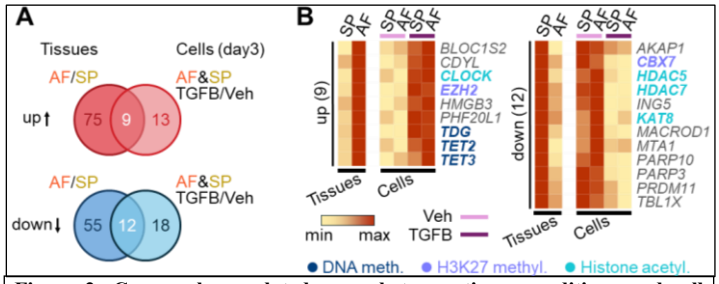

Figure 2: Commonly regulated genes between tissue conditions and cell treatments. (**A**) Venn diagram for commonly regulated epigenetic regulators. (**B**) Heat map analysis of by tissue condition or by TGFβ1 treatment commonly regulated epigenetic regulators.

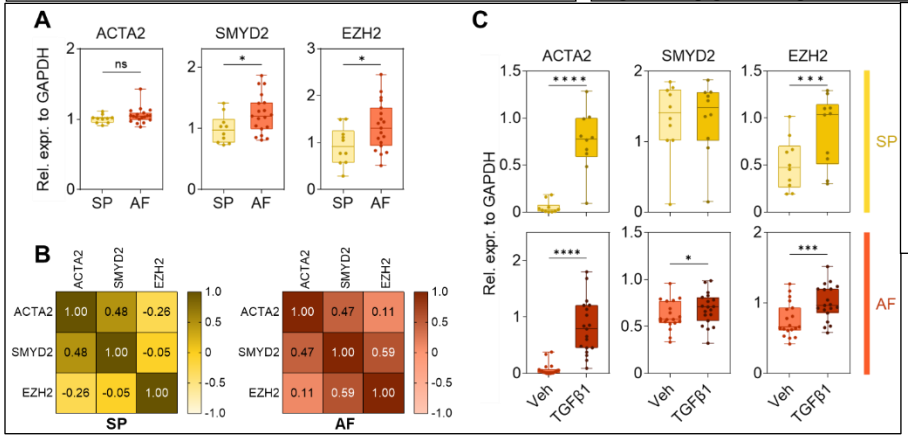

Figure 3: Protein expression analyses. (**A**) ACTA2, SMYD2 and EZH2 expression in SP versus AF tissues. (**B**) Pearson correlation analysis for data shown in (A). (**C**) Comparison for ACTA2, SMYD2 and EZH2 protein expression response upon TGFβ1 treatment in SP or AF patient tissue derived fibroblasts. *p<0.05; ***p<0.001, ****p<0.0001 as measured by two-tailed paired t-test.