High-Throughput Screen of 100 Epigenetic Compounds in a TGF-β1-Mediated Model of Primary Human Knee Fibroblast Differentiation

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INTRODUCTION: Arthrofibrosis is a complication following total knee arthroplasty (TKA) characterized by aberrant scar tissue deposition and debilitating limitations in knee range of motion. Currently, there are no well-established therapies for arthrofibrosis, and contemporary surgical treatments come with significant costs and risks. There is a paucity of literature characterizing the epigenetic mechanisms underlying arthrofibrosis. As such, the purpose of this study was to perform a high-throughput screen of epigenetic compounds in a TGF-β1-mediated model of arthrofibrosis in primary human knee fibroblasts.

METHODS: Following Institutional Review Board approval, fibrotic knee tissue was harvested intraoperatively from a consenting patient undergoing revision TKA for arthrofibrosis. Knee fibroblasts were isolated via collagenase type I digestion. Adherent fibroblasts were cultured in Advanced MEM medium supplemented with 5% human platelet lysate, heparin, GlutaMAX™, and antibiotic/antimycotic. At confluence (Day 0), cells were treated with TGF-β1 (T; 10 ng/mL) and either an epigenetic drug at varying concentrations (0.3, 1.3, 5, or 20 μM) or vehicle control (V), in the presence of ascorbic acid (50 μg/mL). On Day 2, Hoechst staining was performed as a proxy for cell number, and picrosirius red (PSR) staining was performed to assess extracellular matrix (ECM) deposition of collagen types I and III. ECM deposition per cell was quantified by calculating the ratio of PSR fluorescent signal to Hoechst staining intensity. ECM deposition per cell values for each epigenetic drug were subjected to clustering analysis to identify data trends.

RESULTS: Among the 100 epigenetic compounds screened, clustering analysis revealed eight compounds that demonstrated a concentration-dependent decrease in ECM deposition per cell (**Figures 1A&B**). Of these, JIB-04, a pan-selective histone demethylase inhibitor, and 6-thioguanine, a purine analog that indirectly inhibits DNA methyltransferase 1 through DNA incorporation, were selected because they produced the greatest reductions in collagen deposition per cell (**Figures 1B&C**). Both JIB-04 and 6-thioguanine induced a mild reduction in cell number at 1.3 μM, with progressively larger reductions at higher treatment concentrations (**Figure 2A**). Further, there were significant reductions in ECM deposition, as measured by PSR staining, at concentrations of 1.3 μM and above (**Figure 2B**). A significant reduction in ECM deposition per cell was observed with 5 μM JIB-04 and 1.3 μM 6-thioguanine (**Figure 2C**).

DISCUSSION: Of the 100 epigenetic drugs screened, eight compounds induced a concentration-dependent reduction in TGF-β1-mediated ECM deposition in human knee fibroblasts. Importantly, the present study identified epigenetic compounds targeting histone demethylation (JIB-04) and DNA methylation (6-thioguanine) pathways as modulators of TGF-β1-stimulated collagen deposition. Future studies should optimize *in vitro* treatment concentrations and incorporate additional patient-derived cell lines to enable more-targeted downstream assays and draw more definitive conclusions.

SIGNIFICANCE/CLINICAL RELEVANCE: These data suggest that targeting histone demethylation and DNA methylation pathways may represent a potential therapeutic strategy for arthrofibrosis following TKA.

IMAGES AND TABLES:

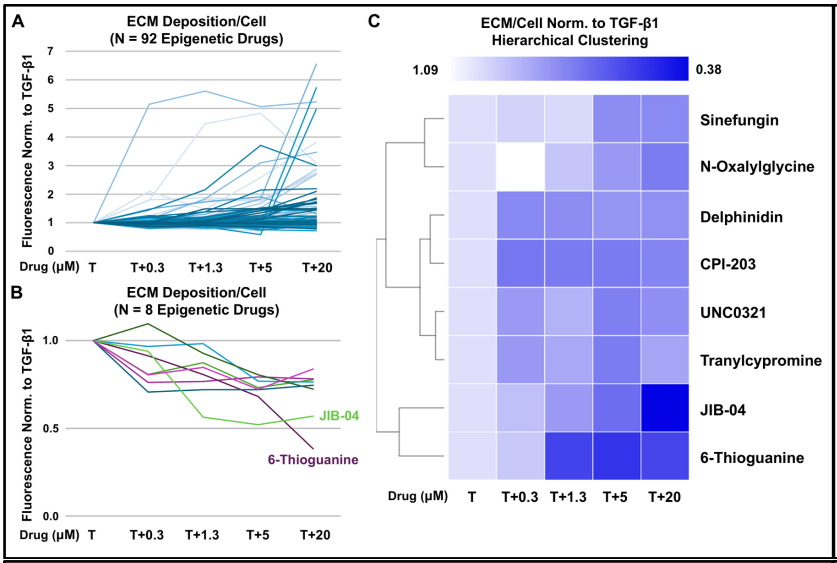

Figure 1. Eight epigenetic drugs induced a concentration-dependent reduction in TGF-β1-mediated extracellular matrix (ECM) deposition per cell. Cluster of 92 epigenetic drugs that did not suppress ECM deposition per cell, as measured by the ratio of picrosirius red (PSR) to Hoechst staining intensity (**A**). Cluster of eight epigenetic drugs that modulated TGF-β1-mediated ECM deposition per cell in a concentration-dependent fashion (**B**). Hierarchical clustering of the eight epigenetic drugs that reduced ECM deposition per cell, based on Euclidean distance (**C**). T = TGF-β1.

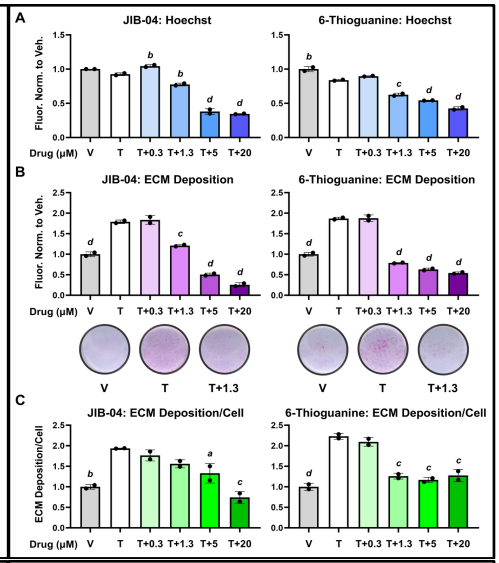

Figure 2. JIB-04 and 6-thioguanine suppressed TGF-β1-induced extracellular matrix (ECM) deposition. Hoechst staining for cell number (**A**) and ECM deposition measured by picrosirius red (PSR) staining (top = quantification, bottom = staining example) (**B**). ECM deposition per cell was measured by the ratio of PSR to Hoechst fluorescent signal intensity (**C**). Levels of significance, relative to TGF-β1, are as follows: *a* = $p \leq 0.05$, *b* = $p \leq 0.01$, *c* = $p \leq 0.001$, and *d* = $p \leq 0.0001$. T = TGF-β1, V = vehicle control.