Substrate Stiffness Promotes Chondrocyte Differentiation through Coordinated Activation of the TGFβ Pathway

INTRODUCTION:
The extracellular microenvironment is rich in physical cues that, like biochemical cues, are powerful regulators of cell behavior. Specifically, the physical cue of extracellular matrix (ECM) stiffness acts as a powerful regulator of mesenchymal stem cell differentiation and lineage selection (1). Accordingly, distortion of normal ECM stiffness has been implicated in many diseases, including cancer and liver fibrosis (2, 3). Cellular mechanosensing of ECM stiffness is tightly controlled, with a biochemical cue intersecting with that activated by ECM stiffness not known, in chondrocytes or in other cell types. Therefore, the objective of this study is to determine whether ECM stiffness and TGFβ cooperate to control chondrocyte differentiation.

METHODS:
To examine the effect of ECM stiffness on chondrocyte differentiation, we designed polyacrylamide gels with a range of stiffnesses (0.2–1.1 MPa) that includes the stiffness of articular cartilage, as measured by nanindentation (4). Acidic acrylamide and N-hydroxysuccinimide ester were added to facilitate collagen II coating and cell attachment (5), with collagen-coated plastic acting as a control. Collagen II density on different substrates was determined not to be significantly different through an indirect ELISA assay. ATDC5 mouse chondroprogenitor cells were cultured on gels with different stiffnesses in the presence or absence of TGFβ. Sox9, Col2a1, and aggrecan were induced by 5, 19, and 16-fold, respectively, relative to cells on plastic (Fig. 1). Mouse mesenchymal stem cells and primary articular chondrocytes performed similarly, with the greatest chondrocyte gene expression found on 0.5 MPa substrates. Like other mechanosensitive responses, this induction was sensitive to inhibition of ROCK. At short time points (24h), culture of ATDC5 cells on a 0.5 MPa substrate induces mRNA expression and endogenous secretion of TGFβ, along with a concurrent stiffness-specific increase in phosphorylation of Smad3. This endogenous TGFβ expression seems to be responsible for promoting chondrocyte differentiation on 0.5 MPa substrates, as Col2a1 up-regulation on a gel is sensitive to TβRI inhibitors.

RESULTS:
The substrates tested, we found maximal chondrocyte gene expression on gels with a stiffness similar to cartilage (0.5 MPa). After 7 days of culture on this substrate, expression of chondrocyte differentiation genes, Sox9, Col2a1, and aggrecan was induced 5, 19, and 16-fold, respectively, relative to cells on plastic (Fig. 1). Mouse mesenchymal stem cells and primary articular chondrocytes performed similarly, with the greatest chondrocyte gene expression found on 0.5 MPa substrates. Like other mechanosensitive responses, this induction was sensitive to inhibition of ROCK. At short time points (24h), culture of ATDC5 cells on a 0.5 MPa substrate induces mRNA expression and endogenous secretion of TGFβ, along with a concurrent stiffness-specific increase in phosphorylation of Smad3. This endogenous TGFβ expression seems to be responsible for promoting chondrocyte differentiation on 0.5 MPa substrates, as Col2a1 up-regulation on a gel is sensitive to TβRI inhibitors.

DISCUSSION:
In summary, we found that chondrocyte differentiation is induced by culture on a stiffness that mimics articular cartilage. ATDC5s are stimulated to produce TGFβ on 0.5 MPa gels, which in turn leads to specific activation of the TGFβ pathway and induction of chondrocyte differentiation genes. Exogenously added TGFβ can rescue cells on sub-optimal stiffness. However, when TGFβ and a stiffness of 0.5 MPa combine, chondrocyte differentiation is synergistically enhanced, through Smad3 and p38 dependent mechanisms.

SIGNIFICANCE:
These findings illustrate how cells integrate physical and biochemical cues to regulate differentiation—in this case through hierarchical regulation of TGFβ signaling, a finding that has relevance to cartilage and many other tissues. Understanding these mechanisms may bring insight into how chondrocyte differentiation is regulated in healthy cartilage and disrupted in OA, as well as elucidate new methods for regenerative articular cartilage.

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