OVEREXPRESSION OF TGF-beta ACTIVATED KINASE-1 (TAK1) BY ADENOVIRAL TRANSDUCTION STIMULATES CHONDROCYTE PROLIFERATION

Introduction: TGF-β is an important regulator of chondrocyte metabolism, and is capable of stimulating collagen, proteoglycan and DNA synthesis. The extracellular presence and concentration of TGF-β is communicated through the cell by two distinct pathways, one involving Smad proteins, and the other involving a cascade mediated by TAK-1 (TGF-β Activated Kinase-1) (1). However, the individual contributions of these pathways to each of the diverse TGF-β responses in cells, particularly chondrocytes, have not been determined. We have focused our attention on the TAK pathway, with the expectation that a greater understanding of its contributions to TGF-β responses may be used to enhance chondrogenesis and cartilage repair.

Cellular proliferation is a complex event regulated by a variety of kinase signaling pathways downstream from growth factor/cytokine interactions with their cognate cell surface receptors. The resultant expression of immediate early genes and suppression of cell cycle inhibitors orchestrate replication. Consequently, the endpoint of proliferation is not measurable by monitoring expression of any single responsive gene. We have therefore constructed adenoviral vectors containing expression cassettes for human TAK1a and its endogenous activator, TAB1 (TAK1a Binding Protein-1) (2) so that 85-95% of chondrocytes can be transduced and proliferation can be monitored by ³H-thymidine incorporation (DNA synthesis). The response of primary and secondary chondrocytes to overexpressed TAK1a and TAB1 has been evaluated in a variety of basal media and in the presence of TGF-β (to provide costimulation of the Smad pathway).

Methods: Adenoviral vectors were constructed by homologous recombination using viral DNA/TPC complexes (TakaRa) and shuttle vectors containing expression cassettes for hTAK1a and hTAB1 driven by the matched promoters, minimal CMV (pC) and intron-less beta-actin (pCEA3), respectively (3). In addition, a tandem vector containing both cassettes (Tdm) and one containing the constitutively active, N-terminal deletion mutant (AN) were used.

Articular chondrocytes were released from 1.5 kg NZW rabbits and plated in 48-well plates in primary or secondary culture. Secondary cells were plated to be confluent in 18h. Confluent cultures were washed and infected with adenoviral constructs in DMEM/ITS+ for 20h. Cells were washed and fed with DMEM/ITS+ or DMEM/10% heat inactivated FCS and were labeled for 24h without medium exchange by addition of tritiated thymidine 24 or 48h post-infection. DNA synthesis was measured as TCA insoluble tritium and DNA content, as PicoGreen fluorescence. During and following infection some cells were treated with blocking pan- or isoform-specific anti-TGF-β antibodies or blocking antibodies to PDGF and its receptors (R&D Systems).

Results: Infection of confluent secondary chondrocytes with Ad-TAK1a or Ad-Tdm (tandem cassette expressing both TAK1a and TAB1) in ITS+ led to an 8-fold stimulation of thymidine incorporation over that in ITS+ alone. In contrast, infection with Ad-TAB alone or promoter-only cassettes, or culture in 10% serum, produced less than 2-fold stimulation (Figure 1). The response to Ad-TAK1a was dose-dependent, yielding 8-fold incorporation with moi=10 and 18-fold with moi=20. When Ad-TAK1a infected chondrocytes were cultured in 10% serum, incorporation was 14-fold the basal level (DMEM/IT+) and 7-fold that of serum alone. In contrast, stimulation by TGF-β in ITS+ produced only a 3-fold increase in incorporation.

Costimulation of Ad-TAK1a responses with TGF-β in general led to only slight additional stimulation, suggesting near saturation of the post-confluent proliferative response by Ad-TAK1a. Importantly, Ad-TAK1a stimulation could be partially mimicked by addition of conditioned medium (CM) from Ad-TAK1a infected cells. Two-fold diluted CM produced a 2-fold stimulation in virally naïve cells, suggesting that part of the response was due to induction of an autocrine growth factor/s, in addition to the expected stimulation/activation of transcription factors.

The effects of Ad-TAK1a and Ad-AN infection were persistent and produced up to a 5-fold enhancement of DNA content above that of confluent monolayers in ITS+ when infected cells were culture for five days in 10% serum; but only a 60% increase when cultured in ITS+.

The involvement of autocrine TGF-β production in TAK1a-dependent stimulation of DNA synthesis was evaluated using blocking antibodies. Over a range of concentrations, pan- or isoform-specific TGF-β antibodies did not block thymidine incorporation stimulated by Ad-TAK1a, while completely blocking the additional incorporation caused by costimulation with TGF-β. Ad-hTAK1a stimulation was also not blocked by simultaneous treatment with anti-PDGF and anti-PDG receptor blocking antibodies.

Primary cultures of chondrocytes demonstrated enhanced Ad-TAK1a thymidine incorporation in the presence of costimulators. When cultured in ITS+, TGF-β stimulated incorporation 4-fold at either 0.1 or 5 ng/ml, while Ad-hTAK1a alone stimulated 10-fold. Costimulation by TAK1a and TGF-β resulted in 40 and 60-fold stimulation with 0.1 and 5 ng/ml TGF-β. Serum (2%) stimulated 6-fold, and simultaneous treatment with 0.1 or 5 ng/ml TGF-β produced 12 and 18-fold stimulation. Ad-hTAK1a infected cells cultured in 2% serum resulted in 50-fold stimulation. Proliferation in ITS+ was reduced by 50% when the insulin concentration was decreased from 300 to 10 nM.

Discussion: Overexpression of hTAK1a, or hTAK1a and TAB, by transduction with adenoviral vectors caused substantial stimulation of thymidine incorporation in confluent rabbit articular chondrocytes that surpassed that caused by TGF-β or 10% serum. Blocking antibodies demonstrated that this was not due to activation of endogenous TGF-β or PDGF, which suggests an important response to hTAK1a that appears independent of Smad signaling. The profile of enhancement of the hTAK1a response by costimulators (including TGF-β) suggests that hTAK1a causes progression of the cell cycle in competent chondrocytes. This is at least partly caused by induction of growth (CM) factor expression, in addition to its role in transcription factor activation. Elucidation of the mechanism of Ad-TAK1a mediated proliferation may facilitate cartilage repair under conditions where chondrocytes are in short supply, if their phenotype is not adversely affected.

References:
*Contributions equal to those of the presenting author.