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Requirement of phosphatidylinositol 3-kinase/Akt signaling pathway for regulation of tissue inhibitor of metalloproteinases-3 gene expression by TGF-β in human chondrocytes

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Abstract

Transforming growth factor beta (TGF-β1) induces cartilage extracellular matrix synthesis and tissue inhibitor of metalloproteinases-3 (TIMP-3), an important natural inhibitor of matrix metalloproteinases, aggrecanases and TNF-alpha-converting enzyme, which are implicated in cartilage degradation and joint inflammation. This study tested the hypothesis that Akt/protein kinase B signaling pathway could mediate TGF-β1 induction of TIMP-3 in human articular chondrocytes. TGF-β activated phosphorylation of Akt in a delayed and sustained fashion that correlated with TIMP-3 mRNA induction. Phosphatidylinositol kinase (PI3K) inhibitors, Wortmannin and LY294002 and Akt inhibitor (NL-71-101) significantly inhibited TGF-β-induced Akt phosphorylation, TIMP-3 expression, TIMP-3 promoter (−940 to +376)-driven luciferase activity and Sp1 transcription factor binding. PI3K p85, Akt and Sp1 small interfering RNA (siRNA)-driven knockdown of the respective gene products significantly suppressed TGF-β-induced TIMP-3 gene expression. TGF-β-stimulated phosphorylation of p70S6 Kinase and TIMP-3 protein induction was inhibited by rapamycin. Thus TGF-β induces TIMP-3 gene expression in human chondrocytes partly through PI3K/Akt pathway and Sp1 transcription factor and by translational mechanisms via mammalian target of rapamycin (mTOR) signaling. TGF-β induction of pro-survival Akt cascade and TIMP-3 may be related to strengthening of cartilage extracellular matrix, increased chondrocyte viability and maintenance of joint tissue integrity.

Keywords: Arthritis; Cartilage; Transforming growth factor beta; Signal transduction; Akt; Transcription factors; TIMP-3; Gene regulation

1. Introduction

A hallmark of rheumatoid arthritis (RA) and osteoarthritis (OA) is resorption of cartilage extracellular matrix (ECM). This is partly due to impaired endogenous repair processes induced by an imbalance between anabolic growth factors and catabolic proinflammatory cytokines, interleukin-1 (IL-1), IL-17 and tumor necrosis factor (TNF-α), which inhibit the ECM synthesis and induce matrix metalloproteinases (MMPs) production [1,2]. Adult cartilage has limited capacity to regenerate and transforming growth factor beta (TGF-β) family members have the potential to stimulate its repair. Human OA cartilage responds poorly to TGF-β due to decreased receptor II [3]. Inhibition of endogenous TGF-β causes impaired cartilage repair and excessive TGF-β leads to the formation of osteophytes in OA [4]. TGF-β, a multi-functional factor produced by monocytes–macrophages, platelets and chondrocytes, induces chondrogenesis and ECM synthesis [5]. TGF-β1 is elevated in human RA synovial fluid and tissue, has immunosuppressive properties, [6] and is a major growth factor for maintaining chondrocyte phenotype and homeostasis [7]. It suppresses inflammatory cell infiltration, pannus formation and joint erosion during acute and chronic arthritis by counteracting the effects of IL-1 [8].

MMPs and aggrecanases (ADAMTS, a disintegrin and metalloproteinase with thrombospondin motif) digest major cartilage ECM components including type II collagen and aggrecan as well as several non-ECM substrates during physiological and pathological remodeling [9,10]. Tissue inhibitors

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of metalloproteinases (TIMPs) are 4 natural inhibitors of MMPs with growth promoting, pro-apoptotic, anti-apoptotic and anti-angiogenic functions [11,12]. Excessive MMPs and ADAMTSs over TIMPs cause loss of articular cartilage. TGF-β inhibits the expression of most MMPs but induces TIMP-1 and TIMP-3 in chondrocytes [7]. TIMP-3 is uniquely located in ECM where its N-terminal domain binds to chondroitin- and heparan sulfate [13] and also inhibits MMP-13, ADAMTS4 and ADAMTS5, the principal cartilage-degrading enzymes [14,15]. It blocks aggrecan degradation in cartilage explants [16] and inhibits pro-inflammatory, TNF-α converting enzyme (TACE/ADAM-17) activity [17]. TIMP-3 can thus reduce inflammation in arthritis. TIMP-3 inhibits angiogenesis by blocking the binding of VEGF to its receptor and could reduce rheumatoid pannus formation [reviewed in [11]]. Such unique features make TIMP-3 a potentially therapeutic protein in arthritis [11,17]. Indeed, TIMP-3 overexpression in proliferating rheumatoid synovial fibroblasts induces apoptosis [18] and prevents invasion of cartilage by pannus [19]. TIMP-3 knockout mice display an increased initial inflammation and serum TNF-α level in antigen-induced arthritis, supporting its protective function against inflammatory arthritis [20]. In other systems, TGF-β binding to cell surface associates types I and II receptors leading to phosphorylation of type I receptor kinase domain, transmission of signal via stimulatory Smads and transcription of the target genes [21]. In chondrocytes, Smad, PKA, PKC and Wnt pathways are induced by TGF-β relative to various cartilage functions [22]. We previously showed the involvement of Smad and extracellular-signal-regulated kinase (ERK1/2)-mitogen-activated protein pathways in TGF-β-induced TIMP-3 in chondrocytes [23,24], however, role of phosphoinositide 3-kinase (PI3K/Akt) pathway and its target transcription factors implicated in this induction are unknown. PI3K-Akt/protein kinase B (PKB) pathway is stimulated by insulin-like growth factor leading to cell proliferation, survival and inhibition of apoptosis [25]. Although PI3K/Akt pathway is activated by TGF-β in human rheumatoid synovial fibroblasts in association with their proliferation [26], its role in chondrocytes and regulation of specific genes is not known. Here, we show the previously unknown and critical role of PI3K/Akt pathway and Sp1 transcription factor in TGF-β-stimulated increase of TIMP-3 in human knee articular chondrocytes.

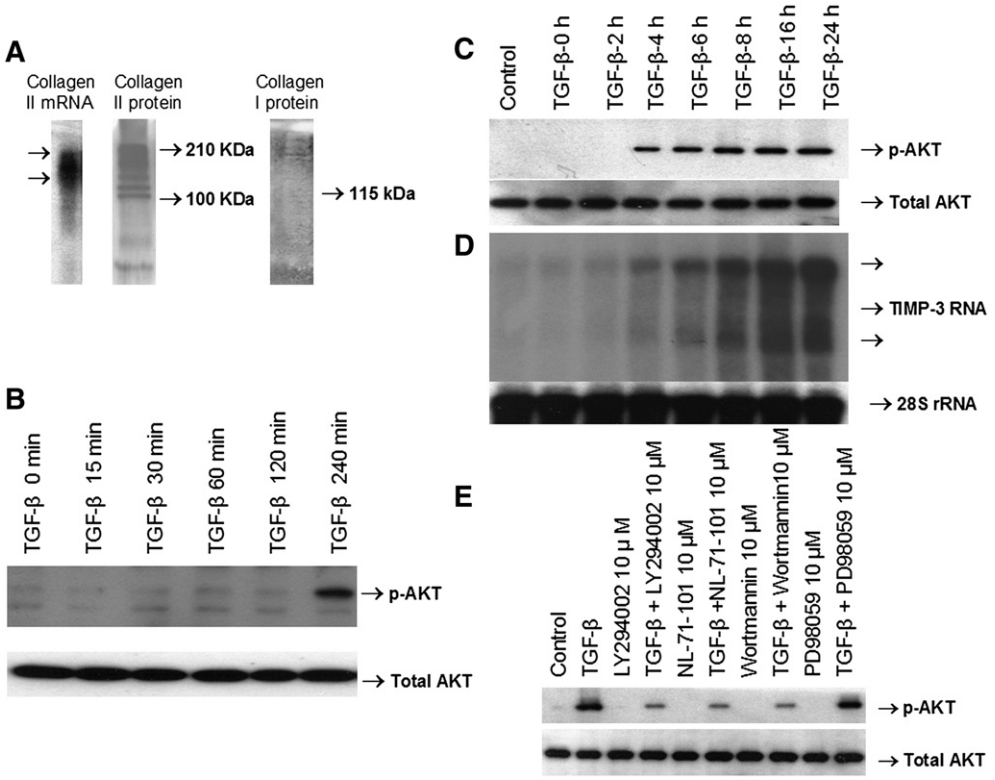

Fig. 1. Monitoring the phenotype of human articular chondrocytes and time-dependent induction of Akt phosphorylation followed by TIMP-3 gene expression in response to TGF-β. In (A), RNA (left panel) and protein (middle and right panels) from passage 3 confluent chondrocytes was extracted and analyzed by Northern or Western blotting for type II and type I collagen expression. Arrows indicate the positions of bands. They do not express 115 kilodalton (kDa) type I collagen but do express 100–210 kDa type II collagen protein and its mRNA. Confluent chondrocytes maintained in serum-free medium were left untreated (Control) or stimulated with TGF-β1 (10 ng/ml) for different time periods up to 4 h (B) or 24 h (C and D). Equal amounts of protein extracts were analyzed by Western blotting (B and C) with anti-phospho Akt (upper panels) and Akt (lower panels) antibodies. Duplicate samples were also analyzed by Northern hybridization of 5 μg of RNA with human TIMP-3 (D, upper panel) and 28S ribosomal RNA (D, lower panel) probes. The three arrows in the Northern blot indicate 3 TIMP-3 transcripts produced due to alternative usage of polyadenylation sites. E) Downregulation of TGF-β-induced Akt phosphorylation by different pharmacological inhibitors. Human chondrocytes were either treated with vehicle (DMSO and PBS with 0.1% BSA and 4 mM HCl) as control or pretreated with 10 μM of the indicated inhibitors and then stimulated with TGF-β for 24 h. Western blots show the levels of phosphorylated and total Akt proteins in cell extracts. PI3K/Akt inhibitors suppress Akt phosphorylation while PD98059 has no effect.