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FULL LENGTH ARTICLE

Aberrant expression of Twist1 in diseased articular cartilage and a potential role in the modulation of osteoarthritis severity

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Abstract The bHLH transcription factor Twist1 has emerged as a negative regulator of chondrogenesis in skeletal progenitor cells and as an inhibitor of maturation in growth plate chondrocytes. However, its role in articular cartilage remains obscure. Here we examine Twist1 expression during re-differentiation of expanded human articular chondrocytes, the distribution of Twist1 proteins in normal versus OA human articular cartilage, and its role in modulating OA development in mice. High levels of Twist1 transcripts were detected by qPCR analyses of expanded de-differentiated human articular chondrocytes that had acquired mesenchymal-like features. The induction of hallmark cartilage genes by Bmp-2 mediated chondrogenic differentiation was paralleled by the dramatic suppression of Twist1 in vitro. In normal human articular cartilage, Twist1-expressing chondrocytes were most abundant in the superficial zone with little to no expression in the middle and deep zones. However, our analyses revealed a higher proportion of deep zone articular chondrocytes expressing Twist1 in human OA cartilage as compared to normal articular cartilage. Moreover, Twist1 expression was prominent within proliferative cell clusters near fissure sites in more severely affected OA samples. To assess the role of Twist1 in OA pathophysiology, we subjected wild type mice and transgenic mice with gain of Twist1 function in cartilage to surgical destabilization of the medial meniscus. At 12 weeks post-surgery, micro-CT and histological analyses revealed attenuation of the OA phenotype in Twist1 transgenic mice compared to wild type mice.

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Collectively, the data reveal a role for Twist in articular cartilage maintenance and the attenuation of cartilage degeneration.

List of abbreviations

AC	articular chondrocytes
bHLH	basic helix loop helix
Bmp-2	bone morphogenetic protein-2
DMEM	Dulbecco's modified Eagle's medium
DMM	destabilization of the medial meniscus
IHC	immunohistochemistry
OA	osteoarthritis
TGFb	transforming growth factor-beta
TG	transgenic

Introduction

Osteoarthritis (OA), the most common form of arthritis in middle aged and older individuals, is characterized by the progressive degeneration of articular cartilage, joint space narrowing, subchondral bone sclerosis, and the formation of bony outgrowths at the joint margins.[1] During the development of OA, the articular chondrocytes undergo distinct phenotypic changes, including the activation of hypertrophy and maturation, aberrant induction of cartilage matrix degrading enzymes, and cell death.[2,3] The signaling pathways and molecular mechanisms that play an essential role in maintaining chondrocyte homeostasis and integrity of the hyaline cartilage matrix are becoming increasingly better understood. Aberrant signaling through developmental pathways controlling chondrocyte differentiation, such as the transforming growth factor-beta (TGF-β) pathway and the wingless MMTV integration (Wnt) pathway, are recognized as key contributing factors to the progressive degeneration of articular cartilage in OA (1—5). Thus, functional characterization of the common transcriptional regulators downstream of these fundamental signaling pathways may offer new insights into the pathophysiology of OA. In this regard, we previously identified the transcription factor Twist1 as a negative regulator of chondrocyte progression toward hypertrophy and terminal maturation in response to TGF-β and canonical Wnt signals.[4]

Twist1 is an evolutionarily conserved basic helix loop helix (bHLH) transcription factor that exerts pleiotropic effects as a mesoderm-determining factor, an epithelial-mesenchymal transitional regulator, and a critical regulator of the gene regulatory network in mesenchymal cell lineage allocation during skeletal development.[5—13] Expression of Twist1 is abundant throughout the condensed mesenchyme that gives rise to both chondrocytes and osteoblasts of the vertebrate skeleton.[6] In humans, haploinsufficiency of Twist1 results in an autosomal dominant inherited disorder Saethre-Chotzen syndrome, characterized by craniosynostosis, short stature, and craniofacial defects.[14] Moreover, several genetic studies examining the role of Twist1 in limb development previously demonstrated the requirement for Twist1 in mediating the outgrowth and patterning of the limb via modulation of the fibroblast growth factor (FGF) and sonic hedgehog (SHH) signaling pathways.[8,15—18] Krawchuk et al further demonstrated significant forelimb patterning defects, tibial aplasia, and highly disorganized cartilage elements in mice with inactivation of Twist1 in Prx1-expressing mesenchyme.[8] Collectively, these studies have provided substantial evidence of the requirement for Twist1 activity in defining multiple functions during limb development.

Through a combination of functional and mechanistic studies we, and others, previously examined the specific role of Twist1 in chondrogenesis and chondrocyte maturation.[4,19,20] Using chondrocyte precursor cells, Reinhold et al demonstrated that Twist1 functions as a potent inhibitor of chondrogenesis[19] and the suppressive effects of Twist1 are mediated, in part, through binding of the carboxyl-terminal Twist box to the DNA-binding domain of the master chondrogenic factor Sox9.[19] Importantly, this direct physical interaction led to inhibition of Sox9-dependent activation of chondrocyte marker gene expression.[19,21] Association of Twist1 to the 3′UTR of Sox9 has also been found to negatively regulate the chondrogenic initiation program in skeletogenic and mesenchymal murine cell lines.[22] We previously determined that Twist1 expression is maintained in the proliferating, immature chondrocytes of the postnatal growth plate, whereas its expression is repressed in hypertrophic chondrocytes both in vitro and in vivo.[4,23] Bialek et al had previously demonstrated that the direct interaction between Twist box with the DNA-binding domain of the transcriptional factor Runx2 led to inhibition of Runx2 transcriptional activity.[6] Using in vitro models of chondrocyte maturation, we demonstrated that Twist1 acts as a potent repressor of hypertrophy in growth plate chondrocytes downstream of TGF-beta and canonical Wnt signaling[4] and speculated that the inhibition of chondrocyte maturation is mediated through its repressive effects on Runx2, the master regulator of chondrocyte hypertrophy.[24] Thus, through its interaction with transcriptional regulators, Twist1 has the ability to regulate not only its direct targets, but also other transcription factor-mediated gene expression pathways involved in cartilage development and maturation. When we generated Twist1 transgenic (TG) mice with persistent expression in growth plate chondrocytes (Col2a1 expressing cells and progeny), we observed abnormal growth plate organization and postnatal longitudinal growth retardation attributed to impaired endochondral ossification.[23] While these collective studies