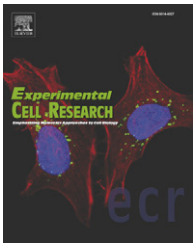
EXPERIMENTAL CELL RESEARCH 315 (2009) 2386–2398

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/yexcr



Research Article

Smurf2 induces degradation of GSK-3β and upregulates β-catenin in chondrocytes: A potential mechanism for Smurf2-induced degeneration of articular cartilage

Qiuqian Wu[a,*,1], *Jason H. Huang*[b], *Erik R. Sampson*[a], *Kyung-OK Kim*[a], *Michael J. Zuscik*[a], *Regis J. O'Keefe*[a], *Di Chen*[a], *Randy N. Rosier*[a]

[a]*Center for Musculoskeletal Research, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY 14642, USA*
[b]*Department of Neurosurgery, University of Rochester Medical Center, 601 Elmwood Avenue, Box 645, Rochester, NY 14642, USA*

ARTICLE INFORMATION

Article Chronology:
Received 15 November 2008
Revised version received
19 May 2009
Accepted 20 May 2009
Available online 27 May 2009

Keywords:
Smurf2
GSK-3β
β-catenin
Articular chondrocytes
Osteoarthritis

ABSTRACT

We have previously demonstrated that Smurf2 is highly expressed in human osteoarthritis (OA) tissue, and overexpression of Smurf2 under the control of the *type II collagen promoter* (*Col2a1*) induces an OA-like phenotype in aged *Col2a1-Smurf2* transgenic mice, suggesting that Smurf2 is located upstream of a signal cascade which initiates OA development. However, the factors downstream of Smurf2 in this signal cascade and how Smurf2-induced OA is initiated are largely unknown. In this study, we further characterized the phenotypic changes in *Col2a1-Smurf2* transgenic and WT articular cartilage from the postnatal stage to adulthood. We found that the articular cartilage degeneration occurring at the cartilage surface in 6 month-old *Col2a1-Smurf2* transgenic mice progressed from an expanded hypertrophic domain in the basal layer of the deep articular cartilage at 2.5 weeks of age, which may lead to an accelerated calcification and ectopic ossification of this region at 1 month of age, and aggregation and maturation of articular chondrocytes in the middle and deep zones at 2 months and 4.5 months of age, respectively. Furthermore, we discovered that ectopically expressed Smurf2 interacted with GSK-3β and induced its ubiquitination and subsequent proteasomal degradation, and hence upregulated β-catenin in *Col2a1-Smurf2* transgenic chondrocytes *ex vivo*. It is therefore likely that Smurf2-mediated upregulation of β-catenin through induction of proteasomal degradation of GSK-β in chondrocytes may activate articular chondrocyte maturation and associated alteration of gene expression, the early events of OA.

* *Corresponding author.* Fax: +1 585 276 1947.
E-mail addresses: Qiuqian_wu@urmc.rochester.edu, qwu1@yahoo.com (Q. Wu), Jason_huang@urmc.rochester.edu (J.H. Huang), Erik_sampson@urmc.rochester.edu (E.R. Sampson), kokim67@yahoo.co.kr (K.-O.K. Kim), michael_zuscik@urmc.rochester.edu (M.J. Zuscik), regis_okeefe@urmc.rochester.edu (R.J. O'Keefe), di_chen@urmc.rochester.edu (D. Chen), randy_rosier@urmc.rochester.edu (R.N. Rosier).

Abbreviations: Smurf2, Smad ubiquitination-related factor 2; TGF-β, transforming growth factor β; BMP, bone morphogenetic protein; GSK-3β, glycogen synthase kinase 3β; APC, adenomatous polyposis coli; OA, osteoarthritis; WT, wild type; Col2, type II collagen; ColX, type X collagen; MMP, matrix metalloproteinase

[1] Current address: Department of Neurosurgery, University of Rochester Medical Center, Rochester, NY 14642, USA.

0014-4827/$ – see front matter
doi:10.1016/j.yexcr.2009.05.019

Introduction

The process of endochondral ossification consists of multiple stages. First, mesenchymal cells aggregate to form condensations, which subsequently differentiate into two types of cells: chondrocytes that form cartilage elements and osteoblast lineage cells that form the perichondrium surrounding the cartilage rudiment. Second, the differentiated chondrocytes undergo proliferation to form orderly parallel columns. The proliferating chondrocytes synthesize and deposit cartilage matrix such as *type II collagen* (*Col2*), and proteoglycans. Third, chondrocytes exit the cell cycle and mature into hypertrophic chondrocytes, and express the stage-specific marker *type X collagen* (*ColX*). Finally, terminally differentiated hypertrophic chondrocytes express *matrix metalloproteinase* (*MMP*) *13*, and the cartilage matrix is degraded and replaced by bone.

Chondrocyte differentiation and maturation during endochondral ossification are tightly regulated by several key growth factors and transcription factors. Members of the TGF-β superfamily such as TGF-β isoforms and BMPs play important roles in the regulation of chondrocyte differentiation and maturation during this process. For example, TGF-β induces chondrogenesis including chondrocyte differentiation and cartilage matrix synthesis in developing limb buds [1,2]. On the other hand, TGF-β signaling inhibits chondrocyte maturation and *ColX* expression during long bone lengthening, evidenced by loss of TGF-β signaling in the growth plate resulting in increased thickness of hypertrophic zone and upregulation of *ColX* expression [3,4]. Similar to the stimulatory role of TGF-β signaling in chondrogenesis, BMP signaling is critical for mesenchymal condensation and chondrocyte differentiation during limb bud development [5–9]. However, in contrast to the inhibitory effect of TGF-β signaling on chondrocyte maturation, BMP signaling promotes this process [6,10,11]. TGF-β/BMP signaling is initiated by binding of these ligands to their receptors, which induces receptor phosphorylation and phosphorylation of receptor regulated Smads including Smad1, Smad5, Smad8 (BMP receptor), Smad2, and Smad3 (TGF-β receptor) [12–15]. Recently, accumulating evidence suggests that canonical Wnt signaling, which is transduced via β-catenin, is implicated in multiple-steps of endochondral bone formation [16–20]. For example, ectopic β-catenin in cells of the chondrogenic lineage inhibits chondrocyte differentiation but stimulates chondrocyte maturation and ossification during embryonic development [16,20].

In general, β-catenin levels are upregulated by Wnt ligands. β-catenin levels are normally limited through continuous proteasome-mediated degradation of phosphorylated β-catenin, which is catalyzed by the enzyme GSK-3β in a "destruction complex" brought together by Axin and APC [21,22]. Upon Wnt ligand binding to its receptors, the complex is disassociated, and the kinase activity of GSK-3β is suppressed, and hence non-phosphorylated β-catenin accumulates in the cytoplasm and translocates into the nucleus to activate expression of Wnt target genes [23]. The β-catenin protein levels are therefore tightly regulated by the Wnt-mediated activity of the "destruction complex", and any mechanism by which normal β-catenin regulation is uncoupled from Wnt signaling control could result in dysregulation of osteoblast/chondrocyte function, leading to alterations in bone mass or degenerative joints [24–26]. However, our understanding of the abnormal regulation of β-catenin in these diseases and animal models remains incomplete.

Osteoarthritis (OA) is a degenerative joint disease, and its developmental process recapitulates many of the events that occur during endochondral ossification. The primary feature in OA-affected joints is articular cartilage degeneration. Unlike the cartilage in the developing growth plate, in which chondrocytes mature and matrix turns over rapidly, the articular cartilage is a permanent tissue with minimal turnover of its cells and matrix [27–29]. The function of articular cartilage that ensures the frictionless movement of joints depends on the unique architecture of the extracellular matrix and zonal articular chondrocytes. The matrix is a highly hydrated network of collagen fibrils embedded in a gel of negatively charged proteoglycan molecules. The articular chondrocytes, which are sparsely distributed in the matrix, are responsible for maintenance of the matrix. Articular cartilage is developed postnatally and cross-link maturation of the collagen network is completed by adulthood. Appearance of articular cartilage is at postnatal 2 weeks when a small domain of secondary ossification occurs in the center of the epiphysis, which separates the articular cartilage from the growth plate cartilage [9,30]. At this stage, the deep zone of the newly formed articular cartilage is a growth plate-like tissue and contains a thin layer of enlarged chondrocytes expressing *ColX*, which may be associated with formation of the calcified zone seen in adult articular cartilage [31–33]. By 1 month of age, the secondary ossification center is expanded, and the zonal articular cartilage formation is completed [33]. Articular chondrocytes normally do not undergo maturation. However, during OA development, articular chondrocytes undergo maturation, and express maturational marker genes such as *ColX* [34–37], and *MMP-13* [37,38–40], which in turn degrades matrix and leads to articular cartilage degeneration [39,41–43]. As OA progresses, osteophytes formed via endochondral ossification are often seen at the margins of the joint and bridge the joint cavity. While OA is a leading cause of disability in older people [44,45], the underlying molecular mechanism is largely unknown. Although loss of TGFβ/Smad3 signaling in skeletal cells via genetic approaches results in OA in mice such as in the dominant-negative *TGFβ type II receptor* transgenic mice and in the *Smad3* KO mice [3,4], or local administration/overexpression of TGF-β in the knee joints induces osteophyte formation [46–48], these events do not occur in humans with OA. Recently, several lines of evidence suggest a role for increased Wnt/β-catenin signaling in OA development. For example, mutations in FRZB, a soluble Wnt antagonist, are OA risk factors, and increase hip OA susceptibility in women [26,49,50], which are further confirmed by the phenotype of *Frzb* KO mice, which are more susceptible to chemically-induced OA [51]. Consistently, high β-catenin protein levels were detected in human OA chondrocytes, but not in normal articular cartilage [52–54], indicating that increased β-catenin signaling in articular chondrocytes may be responsible for a conversion of normal cells to OA cells. However, the upstream signaling pathway(s) that dictates upregulation of β-catenin in articular chondrocytes during OA initiation is not clear.

We have recently demonstrated that Smurf2 is highly expressed in human OA tissue, and that overexpression of Smurf2 under the control of the *type II collagen promoter* (*Col2a1*) induces OA in mice, suggesting that Smurf2 is located upstream of a signaling cascade that is responsible for OA initiation in humans [37]. In some cell lines, ectopically overexpressed Smurf2 directly interacts with Smad1 and Smad2, and indirectly interacts with the type I receptor of TGF-β and β-catenin via an adaptor Smad7 for ubiquitination and