Journal of Bone Research
ISSN: 2572-4916

Izawa et al., J Bone Res 2017, 5:2
DOI: 10.4172/2572-4916.1000178

Review Open Access

Role of Smad3 and S1P Signaling in Mandibular Condylar Cartilage Homeostasis

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Received Date: July 17, 2017; **Accepted Date:** July 24, 2017; **Published Date:** July 31, 2017

Abstract

Osteoarthritis (OA), the most common degenerative joint disease, results from an imbalance between chondrocyte-controlled anabolic and catabolic processes. OA is characterized by progressive degradation of components of the Extracellular Matrix (ECM) within the articular cartilage, correlated with secondary inflammation. Several studies had investigated the morphological and biochemical changes during OA progression. However, a comprehensive study of the OA pathogenesis still remains to be elucidated to find the best therapy for OA. In this review, recent advances in our understanding of the mechanisms of action of Sphingosine 1-phosphate (S1P) and Smad3 independently and in relation to Temporomandibular Joint Osteoarthritis (TMJ-OA) will be discussed. S1P receptors are expressed on the cell surface and are internalized upon binding of the bioactive lipid, S1P, as part of the migratory response. Meanwhile, Smad3 is an intracellular signaling molecule that mediates signaling from transforming growth factor-β (TGF-β) and activin receptors. Crosstalk between the TGF-β/Smad3 and S1P/$S1P_3$ signaling pathways regulates cell motility and apoptosis in chondrocyte cells. Thus, Smad3/$S1P_3$ signaling in chondrocytes may be responsible for the development of TMJ-OA, and the potential for these proteins to represent targets for the treatment of TMJ-OA warrants further study.

Keywords: Smad3; S1P; Chondrocyte; Migration; TMJ-OA

Introduction

Mandible, a part of the human masticatory system, through contractions of the neuromuscular controls direction of joint loads as dictated by dental eruption and growth of the Temporomandibular Joint (TMJ) eminence [1,2]. The motion of the mandible is relative to the cranial base and distributes the normal stresses of function (speaking and chewing) and parafunction (bruxism and clenching) [3]. A number of clinical orofacial conditions that involve the masticatory musculature, the TMJ, and associated structures are referred to as Temporo Mandibular Disorders (TMD). A severe TMD is osteoarthritis (OA) which often affects the TMJ of patients and involves changes in the subchondral bone and progressive cartilage degradation [4].

In the mandibular condyle, endochondral ossification is the primary process by which subchondral bone is formed and this process is regulated by endogenously expressed factors in chondrocyte. Loss of cartilage integrity caused by (bio)mechanical, biochemical, inflammatory, or immunologic in character disturbs the chondrocyte-controlled balance between synthesis and degradation of the ECM components [5,6]. Increased synthesis and activity of proteases, resulting in an initially degradation of articular cartilage [6,7]. During late stage of OA, severe fibrillated and eroded tissue is may appear and neovascularization of TMJ articular cartilage may be present. Denudation of subchondral bone is frequently seen. Synovial membrane may appear hypervascularization and hyperthropic, or fibrotic and disc displacement and perforation may develop [6].

To date, the relationship between subchondral bone abnormalities and the onset of TMJ-OA has not been determined. It is hypothesized that the accumulation of chondroprogenitor cells at injury sites is due to the migration of these cells from the surrounding matrix [8-12]. Migratory chondroprogenitor cells that are present in cartilage represent a valuable resource for improving cell recruitment into cartilage defects without the need for perforation of the subchondral bone plate. In addition, migratory chondroprogenitor cells have the potential to support the endogenous repair of blunt injured cartilage when traumatic chondrocyte loss has occurred. However, the potential physiologic and/or pathologic functions of chondroprogenitor cells and their migratory effects on healing in TMJ-OA joints remain unknown.

Several studies have reported that a subset of the effects elicited by the TGF-β/Smad3 signaling pathway are transmitted via a pathway that is initiated by activation of Sphingosine Kinase (Sphk), followed by intracellular generation of the bioactive lipid, Sphingosine 1-phosphate (S1P) [13]. Here, we highlight that TGF-β/Smad3 signaling influences cartilage homeostasis by influencing S1P/S1P receptor signaling and chondrocyte migration.

Pathogenesis of TMJ-OA

In elderly adults, chronic disability is most often caused by OA. In the early stages of OA, it has recently been demonstrated that low bone mineral density and increased bone turnover are observed in the knee joint [14-16]. Efficacies of bone resorption inhibitors for the rescue of OA have also been reported [15,17,18]. Taken together, these results suggest that abnormal subchondral bone remodeling is important in the pathogenesis of knee OA.

However, the TMJ is one of the most common sites of OA and TMJ-OA may be part of generalized OA [19-21]. TMJ-OA is present in 70% persons of 73-75-year age group and 89% of patients with or without

reduction of disc displacement [20,22]. TMJ-OA can affect all TMJ tissues to induce anatomical changes and severe pain [4].

It is hypothesized that the subchondral bone has an etiological role in TMJ-OA pathology based on recent observations that increased remodeling of mandibular condylar subchondral bone occurs in the early stages of TMJ-OA [23-25]. Moreover, the relationship between the development of TMJ-OA and the abnormalities in subchondral bone remains to be determined, and this is an area of active study.

The key mediators of cartilage degradation in vivo and in vitro include the Matrix Metalloproteinase (MMPs) and members of the closely related family of a disintegrin and Metalloproteases (ADAMs) with Thrombospondin motifs (ADAM-TS) [26,27]. Roles for matrix MMP-13 and ADAM-TS5 in this degeneration process have been demonstrated [28-33]. Subsequently, key roles have been identified for complement component 5 (C5) [34] and hypoxia-inducible factor-2α (HIF-2α) [35,36]. As OA progresses, articular chondrocytes express interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), runt-related transcription factor 2 (RUNX2), alkaline phosphatase, MMP-13, and type X collagen. Concomitantly, articular cartilage exhibits expanded calcified cartilage zones and low levels of proteoglycans [37-42].

Nitric Oxide (NO) as a free radical play a role in the apoptosis of chondrocytes and inhibits proteoglycan synthesis. These may contribute to the abnormal chondral calcification and osteophyte formation [43,44]. Mitogen-inducible gene-6 (Mig-6), immediate early response gene encoding via threonine kinase receptors, plays an important role in maintaining joint homeostasis. Thus, the involvement of other cells may engage in the pathogenesis of OA [45].

In OA, a primary concern is the degeneration of articular cartilage [33]. The initial and repair stage of OA is characterized biochemically by an increased synthesis of ECM components, DNA, and metabolic activity of the chondrocytes. Accounting for the proliferation, mitoses, and clustering observed histologically [6,42,46]. The repair response is mediated by growth factors (e.g., insulin-like growth factor-1 (IGF-1) and transforming growth factor-β (TGF-β)), and is partially determined by the difussibility of these growth factor through the cartilage matrix to the chondrocytes [47-49]. Moreover, growth factors that are normally bound to the ECM components will be released by cartilage degradation and thereby stimulate the chondrocytes in their repair responses. Balance between repair and degradation established, an increased synthesis of the ECM components equals their degradation due to an increased protease activity [6].

To date, the molecular mechanism responsible for mediating the progression from defective subchondral bone to degeneration of articular cartilage in OA remains largely uncharacterized. Acknowledgment of the imbalance anabolic and inflammatory/catabolic pathways has led to explored interest in treatment of OA with limited side effect that may be able to encourage maintenance of bone turnover and chondral homeostasis [39,44].

The TGF-β/Smad3 signaling system and OA

There are three subfamilies of TGF-β that closely related to the mammalian isoforms, TGF-β1, -β2, and -β3 [50,51]. Proteins of the TGF-β family mediate signaling pathways via serine/ threonine kinase receptors [52]. Specifically, type II serine/threonine kinase receptors (TGFβR II) are activated following their binding of type I serine/threonine kinase receptors (TGFβR I) [53].

Intracellular Smad proteins (50-70 kDa), particularly Smad2 and Smad3, then transmit this activation signal and that of activin to the nucleus. In addition, when a Smad protein is activated by a receptor, its phosphorylated form is able to heterodimerize with Smad4 and translocate to the nucleus where the complex mediates the transactivation of specific target genes. Meanwhile, Smad1, Smad5, and Smad8 transduce signaling from BMP. Conversely, Smad6 and Smad7 provide an inhibitory function whereby phosphorylation of pathway-specific Smads is inhibited and signal transduction is disrupted [53].

When chondrocyte-specific deletion of Smad3 was achieved in mice, OA in the knee joint was induced [54]. Correspondingly, in humans, mutations in Smad3 have been found in the MH2 domain of Smad3 protein, a region that is extremely well conserved among other species and among other Smad proteins that are associated with early onset of OA [55]. More recently, when overexpression of TGF-β1 was achieved in murine subchondral bone, mandibular condyle degradation was observed [56]. In our own study of mandibular condylar subchondral bone, spontaneous abnormalities were found to induce progressive cartilage degradation in Smad3−/− mice [57].

Chondrocyte death is commonly accepted as a hallmark of OA. It has been observed that the extent of chondrocyte death that occurs positively correlates with the severity of osteoarthritic cartilage depletion and destruction [58,59]. In our recent study, cell death in the condylar cartilage of Smad3−/− mice appeared to be progressive since the numbers of both TUNEL+ and active caspase-3+ and caspase-9+ cells did not significantly differ from those detected in 1-month-old Smad3−/− mice, yet they were markedly higher in the 4-month-old Smad3−/− mice [57].

S1P/S1PR system

In both healthy and disease states, the bioactive Sphingolipid metabolite, sphingosine 1-phosphate (S1P), contributes to regulating many cellular processes [60]. For example, the ability of S1P to act via a family of cell surface receptors and to play a critical role in the migration of immune cells throughout the body has been well studied. In addition, control of cell trafficking is a well characterized aspect of the involvement of S1P in disease [61]. To date, there are five G-protein-coupled receptors at the cell surface that have been found to be specific for S1P. They include $S1PR_{1\text{-}5}$, and activation of $S1P_1$ is critical for immune cell trafficking [62]. However, in the glomeruli of rats with diabetic nephropathy, it was recently observed that S1P signals are preferentially transmitted through $S1P_2$, rather than $S1P_1$ [63]. It is possible that this biased delivery of S1P signals may mediate the pathogenesis of endothelial injuries in diabetic nephropathy [63]. $S1P_2$ was also recently shown to be expressed in enteric neurons and migrating cranial crest cells, while expression of $S1P_1$ is significant in the neuroepithelium [64]. Meanwhile, $S1P_4$ and $S1P_5$ are expressed at later stages in neurons [64]. $S1P_3$ primarily localizes to the cell surface on the plasma membrane, and high expression levels of $S1P_3$ have been detected in lung, heart, kidney, spleen, diaphragm, and intestine tissues [65]. Moreover, for neurogenesis and for expression of smooth muscle alpha-actin following arterial injury [66], $S1P_3$ has been found to be essential [67]. $S1P_3$ also contributes to the migration of thyroid cancer cells [68] and VEGF-A secretion induced by S1P [69]. Furthermore, compared with other S1P receptor subtypes, $S1P_3$ receptor antagonists have no effect on S1P-induced Mitogen-Activated Protein Kinase (MAPK) activation [70]. Thus, a role for $S1P_3$ in MAPK signaling has been excluded.

Challenges for regenerative therapy approaches currently include modulation of MMPs, recruitment of chondroprogenitor cells to affected cartilage, and the impact of the extracellular matrix on cell migration [8-10]. Regarding the latter, the regulatory functions of bioactive lysophosholipids, primarily S1P, in cell migration have led to the identification of these proteins as potent mediators of wound healing and tissue repair. Moreover, S1P is released from most cells after they are stimulated by growth factors such as TGF-β. Therefore, S1P receptors should be considered in chondrocyte cell migration, despite a role of S1P receptors in OA being largely uncharacterized. Renal mesangial cells express several S1P receptors (e.g., $S1P_{1-5}$), and these receptors potentially mediate mobilization of intracellular calcium, cell proliferation, and activation of the classic MAPK signaling cascade [71]. In the present study, wild type and Smad3−/− chondrocyte cells derived from condylar cartilage were analyzed [57]. The former expressed higher levels of $S1P_3$ compared with the other S1P receptors assayed. Conversely, expression of $S1P_3$ by the Smad3−/− primary chondrocytes was significantly weaker. This difference in $S1P_3$ expression was further enhanced following stimulation with TGF-β [57]. These results are consistent with the observation that signaling via the Sphk1/$S1P_3$ axis is enhanced during the transdifferentiation of myoblasts into myofibroblasts in response to TGF [72,73]. However, it is important to note that knee hyaline articular cartilage is distinct from mandibular condylar cartilage.

S1P and TGF-β/Smad3 crosstalk in wound healing

It has been observed that TGF-β increases Sphk1 activity and up-regulates mRNA and protein levels of Sphk1 in dermal fibroblasts [74]. Thus, it is hypothesized that crosstalk between TGF-β and S1P regulates MMP expression. S1P utilizes signaling by its receptors to stimulate phosphorylation and activation of TGFβR I kinase, thereby leading to phosphorylation of Smad2 and Smad3 independent of TGF-β ligand, as well as an induction of both proliferation and migration in keratinocytes [75]. Abrogation of Smad3 appears to prevent S1P-mediated effects [57,72,75,76], and this suggests a surprising, and yet essential, role for Smad3 in the signaling cascade of the lysophopholipid, S1P. A role for $S1P_3$ in Smad3 activation was confirmed with the use of small interfering RNA (siRNA) targeting $S1P_3$ and suramin [57,72]. Correspondingly, suramin was reported to be a selective agonist of the $S1P_3$ receptor in vitro [77]. Abrogation of S1P-stimulated Smad3 activation by siRNA targeting TGFβR II further supports the hypothesis that TGFβR II is a component of the S1P signaling cascade [57].

For cell migration, Rho GTPases are critical for coordinating the cellular responses involved [78,79]. In the present study, the Rho GTPases that were assayed exhibited increased levels of activity following stimulation by TGF-β. However, when primary chondrocyte cells were transfected with siRNA targeting $S1P_3$ and then were stimulated with TGF-β, the activity levels of GTP-Rac1, GTP-RhoA, and GTP-Cdc42 decreased [57].

Conclusion

Overall, these findings suggest a model in which chondrocyte cells are maintained via crosstalk between the TGF-β/Smad3 and S1P/$S1P_3$ signaling pathways. The crosstalk between these pathways also regulates cell motility and apoptosis in these cells. Thus, Smad3/$S1P_3$ signaling in chondrocytes may be responsible for the development of TMJ-OA, and the potential for these proteins to represent targets for the treatment of TMJ-OA warrants further study (Figure 1).

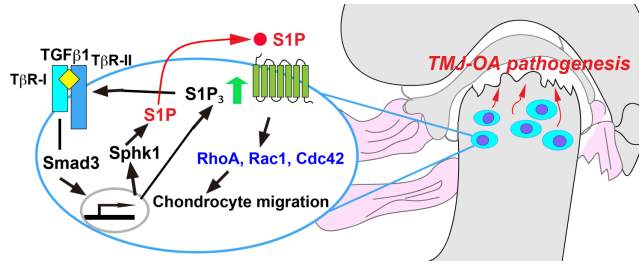

Figure 1: A proposed model for the mechanisms by which crosstalk between the TGF-β/Smad3 and S1P/$S1P_3$ signaling pathways regulates chondrocyte migration. The maintenance of chondrocyte cells, which includes the processes of cell motility and apoptosis, is regulated by crosstalk between the TGF-β/Smad3 and S1P/$S1P_3$ signaling pathways. Furthermore, Smad3/$S1P_3$ signaling in chondrocytes may play a crucial role in the cause of TMJ-OA.

Acknowledgments

This work was supported by the grants provided by JSPS KAKENHI (Grant Numbers. 25713063, 15K15757, 17K19758 to T.I. 16H07019 to H.M.), Otsuka Toshimi Scholarship Foundation (to I.H.), The Ichiro Kanehara Foundation, Suzuken Memorial Foundation, The Nakatomi Foundation, and Smoking Research Foundation (to T.I.).

Conflicts of Interest

The authors declare no conflict of interest.

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