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Review Article

Roles of CCN2 as a mechano-sensing regulator of chondrocyte differentiation

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ABSTRACT

Cellular communication network factor 2 (CCN2) is a cysteine-rich secreted matricellular protein that regulates various cellular functions including cell differentiation. CCN2 is highly expressed under several types of mechanical stress, such as stretch, compression, and shear stress, in mesenchymal cells including chondrocytes, osteoblasts, and fibroblasts. In particular, CCN2 not only promotes cell proliferation and differentiation of various cells but also regulates the stability of mRNA of TRPV4, a mechanosensitive ion channel in chondrocytes. Of note, CCN2 behaves like a biomarker to sense suitable mechanical stress, because CCN2 expression is down-regulated when chondrocytes are subjected to excessive mechanical stress. These findings suggest that CCN2 is a mechano-sensing regulator. CCN2 expression is regulated by the activation of various mechano-sensing signaling pathways, e.g., mechanosensitive ion channels, integrin-focal adhesion-actin dynamics, Rho GTPase family members, Hippo-YAP signaling, and G protein-coupled receptors. This review summarizes the characterization of mechanoreceptors involved in CCN2 gene regulation and discusses the role of CCN2 as a mechano-sensing regulator of mesenchymal cell differentiation, with particular focus on chondrocytes.

1. Introduction

It is well known that gravitational force plays an important role in the maintenance of our body. In fact, when an astronaut comes back to earth, or aged persons are bedridden for long time, their muscle atrophies and bone fragility becomes evident [1]. In addition to gravitation, we are subject to many and various mechanical stimuli, including stretch, compression, fluid shear stress, hydrostatic pressure, and so on [2]. Mechanical stimuli maintain the homeostasis of our tissues and organs and regulate several cell functions including proliferation, differentiation, and cell death in the cells constituting our body [2]. Therefore, mechanical stress plays an essential role in our activities; and so its regulatory role and mechanism have been examined and clarified by many studies.

Especially, cellular communication network factor 2 (CCN2) is induced by mechanical stress, such as stretch, compression, shear stress, hydrostatic pressure, and ultrasound, and is a unique factor to regulate important cellular functions [3–8]. CCN2 belongs to the CCN family of structurally related polypeptides, which comprise 6 distinct proteins, namely, CCN1 to CCN6 (Fig. 1). It has been reported that the expression of CCN1 as well as that of CCN2 is markedly up-regulated by mechanical stress, but that the expression of other members of the CCN family is barely detectable, in fibroblasts subjected to mechanical stress [9]. Because data related to the effect of mechanical stress on CCN2 are the best available, we focused on CCN2 in this review article. As shown in Fig. 1, CCN2 is a secretory protein, composed in 349 amino acids with a molecular weight of 36–38 kDa; and it is expressed in various mesenchymal cells including fibroblasts, osteoblasts, osteocytes, osteoclasts, chondrocytes, and vascular endothelial cells [3]. Furthermore, CCN2 is a multifunctional matricellular protein that is characterized by 4 modules, i.e., IGFBP (insulin-like growth factor binding protein)-like, VWC (von Willebrand factor type C), TSP1 (thrombospondin 1 type 1) repeat, and CT (C-terminal cystine knot) module [3]. As all 4 of these modules comprising CCN2 are highly interactive with a variety of other molecules, such as growth factors [10–13], heparan sulfate proteoglycan [14], and integrins [15,16], this factor promotes cell differentiation, migration, adhesion, and extracellular matrix formation depending on

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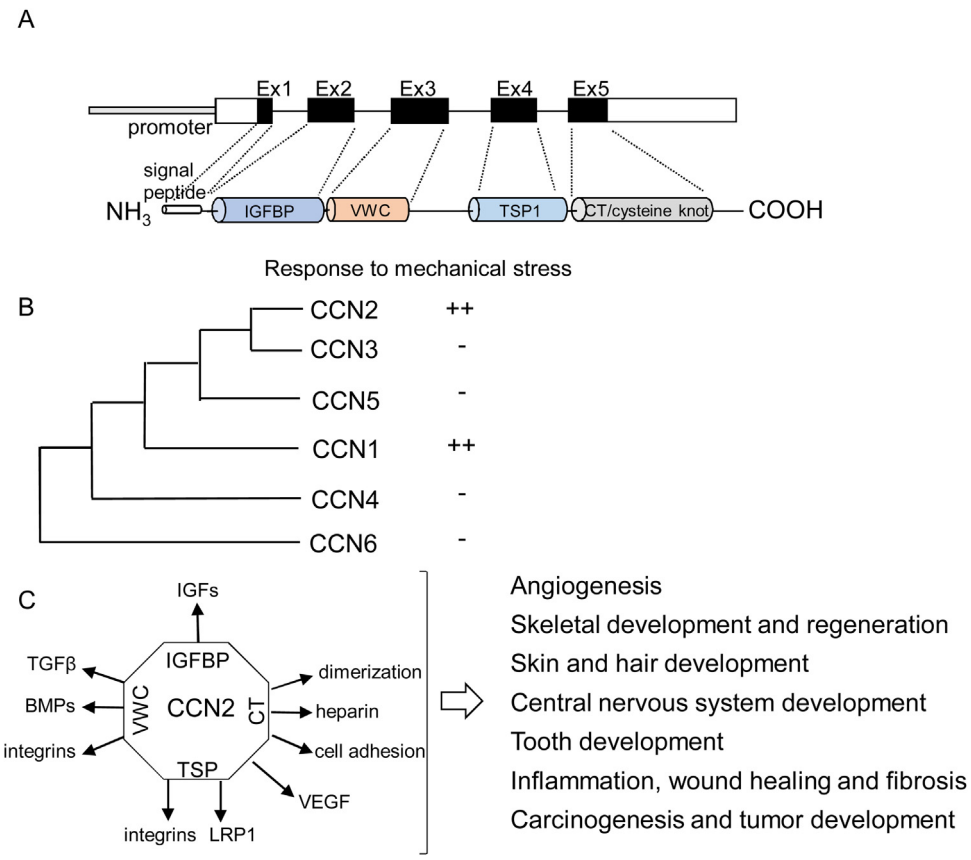

Fig. 1. (A) Illustration of the structures of the CCN2 gene and its product. The upper part of the figure illustrates the structure of the CCN2 gene. Solid boxes and solid lines in the CCN2 gene represent exons 1 to 5 (Ex1-Ex5) and introns, respectively. The lower part illustrates the structure of the CCN2 protein. IGFBP: IGF-binding protein-like module, VWC: von Willebrand factor type C module, TSP1: thrombospondin 1 type 1 repeat, CT: C-terminal module. (B) Dendrogram of CCN family members. ++ represents the mechano-sensing genes and – represents the genes insensitive to mechanical stress. (C) Interaction of each module of CCN2 with various factors and possible role of CCN2 under physiological and pathological conditions. Abbreviations in this figure are as follow: TGFβ, transforming growth factor β; BMP, bone morphogenetic protein; LRP1, low-density lipoprotein receptor-related protein 1; VEGF, vascular endothelial cell growth factor.

the cell type [3]. Our early reports demonstrated that CCN2 promotes the proliferation and differentiation of chondrocytes [17] and osteoblasts [18], as well as the differentiation of osteoclasts [19]. Regarding gene expression, the results of in vivo study revealed that CCN2 is strongly expressed in the pre-hypertrophic region of the growth plate [20] but that it is expressed to a lesser extent in articular cartilage tissues that are maintained without further growth [21]. These findings suggest that CCN2 works actively during development stages, whereas its physiological roles are unclear during adult stages. However, even at adult stages, CCN2 expression is increased when tissue modification, such as wound healing [22] and bone remodeling [23] occur. Mechanical loading is the one of the important factors that can provoke CCN2 production in adult tissues. As shown in Fig. 2, mechanoreceptors that sense mechanical stress include ion channels [24–26], integrin-focal adhesion complexes [24,27,28], reorganized actin [24,29], Rho guanosine triphosphatase (GTPase) family members [24,30], and G-protein-coupled receptors (GPCRs) [24,31]. In this review article, we summarize the characteristics of these mechanoreceptors. We also discuss the mechanism of CCN2 expression promoting chondrocyte differentiation via mechanoreceptors, as well as the interaction between CCN2 and the mechanoreceptors.

2. Mechanoreceptors that sense mechanical stress

Various cells including chondrocytes convert a mechanical stimulus into biochemical signals by means of special sensors that activate intracellular signaling pathways. These sensors that sense mechanical forces, such as stretch, compression, and hypo-osmotic stress, are called “mechanoreceptors;” and they include mechanosensitive ion channels [24–26], integrins [24,27,28], Rho GTPase family members [24,30], and several GPCRs [24,31], as stated above (Fig. 2). Mechanosensitive ion channels are transmembrane proteins that open or close according to alteration by physical forces. As a result, these mechanical forces are converted into electrochemical signals in the cells. Mechanosensitive ion channels are divided into 2 groups [32]. One is the non-selective depolarizing stretch-activated ion channel (SAC), which allows the influx of cations such as Ca^{2+}, Na^{+}, and K^{+} into the cell in response to mechanical or hypo-osmotic stress. SACs include the epithelial sodium channel (ENaC) family [33], the transient receptor potential (TRP) channel family [33–35], and the Piezo1 and Piezo2 channels [36]. The other one is the selective hyperpolarizing potassium channel, which includes the voltage-gated potassium channel ($VGCs_K$) and stretch-activated potassium channel ($SACs_K$). The pore in the $VGCs_K$ channel opens or closes depending upon the voltage in response to alteration of membrane potential due to the influx of a charged species, such as Ca^{2+}, but does not directly open or close when mechanical stress is applied [32,33]. $SACs_K$ channels are activated by mechanical stimuli and include TREK-1, TREK-2, and TRAAK, which have 4 transmembrane segments and 2 pore domains [32,35]. TREK and TRAAK stand for TWIK-related K^{+} (TREK) and TWIK-related arachidonic acid-activated K^{+} channels, respectively; and TWIK is the abbreviation for the tandem two-pore K^{+} domains in a weak inwardly rectifying K^{+} channel [32,35]. Additionally, Big K^{+} channels activated by Ca^{2+} (BK_{Ca} channels) are

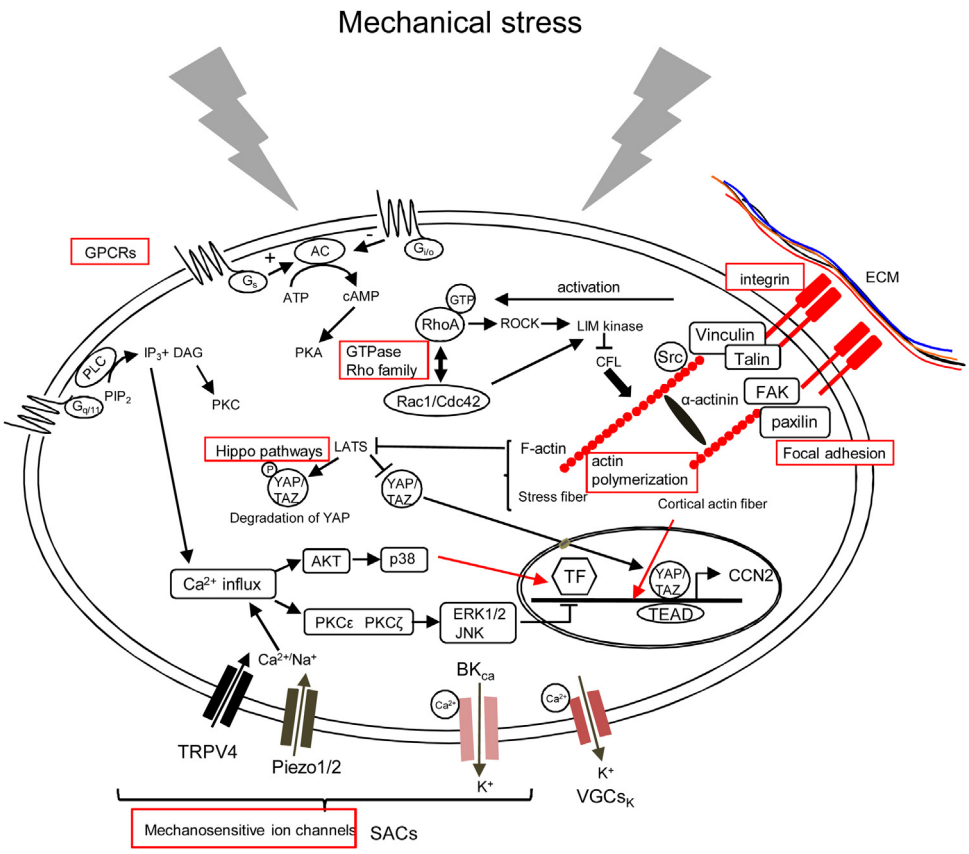

Fig. 2. Schematic diagram of possible mechanoreceptors regulating CCN2 expression. Various proteins that have been described in this article as potential mechanoreceptors in chondrocytes are displayed. These proteins have been shown to be activated by membrane stretch, by fluid shear stress or by LIPUS treatment, and to convert the mechanical signals into chemical ones. Finally, the gene expression of CCN2 is up-regulated via multiple intracellular chemical signals. Abbreviations in this figure are as follow: PLC, phospholipase C; AC, adenylyl cyclase; PIP2, phosphatidyl-inositol 4,5-biphosphate; IP3, inositol triphosphate; cAMP, adenosine 3',5'-cyclic monophosphate; PKA, protein kinase A; CFL, cofilin; TF, transcription factor.

another type of $SACs_K$; and their activation occurs by a mechanical stress-induced increase in the intracellular Ca^{2+} concentration [32,37].

Integrins are transmembrane cell-surface receptors that interact with components of the extracellular matrix (ECM), such as fibronectin, collagens, and laminin [27,28]. Integrins are heterodimers composed of α and β subunits and are expressed in several tissues and organs as a combination of at least 18 α subunits with 8 β subunits [27,28]. Between the cytoplasmic domain of the β subunit and cytoskeletal protein, actin, there are adaptor proteins including talin, vinculin, and paxillin. Focal adhesion kinase (Fak) and Src kinase family members, which are tyrosine kinases, are also located therein; and these molecules are involved in the formation of focal adhesions [38]. On the other hand, the actin cytoskeleton exists in 2 forms, monomeric globular actin (G-actin) and filamentous actin (F-actin); and it regulates cell shape by the conversion between G- and F-actin [29]. Mechanical forces control alterations of the actin cytoskeleton via a variety of regulatory proteins [29]. Among them, Rho GTPase family members, which include RhoA, Rac1, and Cdc42, are well-known as important regulators of actin dynamics [30,39]. RhoA exists in 2 forms, the guanosine diphosphate (GDP)-bound inactive and the guanosine triphosphate (GTP)-bound active form; and its active form activates Rho-associated kinase (ROCK), a downstream target of RhoA [30,39]. The activation of ROCK induces phosphorylation of cofilin via LIM kinase, which is an actin-binding kinase; and phosphorylated cofilin suppresses actin depolymerization, resulting in the formation of F-actin stress fibers [40]. When various cells are subjected to mechanical loading, adaptor proteins bind to the intracellular domain of integrins and form focal adhesions [39]. Mechanical stress-dependent conformational changes in Src and vinculin activate RhoA, and connect focal adhesions with F-actin stress fibers, activating signaling networks that regulate cell survival, proliferation, and differentiation [30,39,40]. On the other hand, Rac1 antagonizes RhoA activity; and Rac1 and Cdc42 promote the formation of F-actin networks leading to sheet-like lamellipodia and rod-like filopodia extensions, respectively [30]. These findings indicate that Rho GTPase family members are essential mediators connecting mechanical stimuli with the alteration of actin cytoskeletal organization. Next, changes in actin dynamics regulate gene expression promoting cell differentiation by the action of YAP (Yes-associated protein) and its paralog, TAZ (transcriptional coactivator with PDZ-binding motif), which are transcription coactivators regulated by the Hippo signaling pathway [41–45]. This Hippo pathway consists of a serine/threonine kinase cascade including LATS (large tumor suppressor) [41–45]; and it is activated by high cell density or decreased tension on actin stress fibers, resulting in the phosphorylation of YAP by LATS kinase [41–45]. Phospho-YAP is retained in the cytoplasm, where it is immediately degraded by proteasomes [41–45]. When the Hippo pathway is inactivated by low cell density or formation of actin stress fibers, YAP is not phosphorylated due to LATS inhibition. Consequently, YAP is translocated from the cytoplasm to the nucleus where it promotes gene expression activating multiple cellular events [41–45].

GPCRs are characterized by 7 membrane-spanning hydrophobic regions that couple with heterotrimeric G proteins composed of α-, β-, and γ-subunits [31]. When GPCRs interact with ligands, such as hormones, neurotransmitters, and small molecules, GPCRs

are subjected to conformational changes in their transmembrane domains; and G proteins are activated by the change from GDP to GTP [31]. Then, the Gα-GTP complex dissociates from GPCRs and Gβγ subunits, and interact with downstream effectors, resulting in the activation of signal transduction pathways [31]. The Gα-subunit family is divided into 4 groups based on functional effectors, $G_s\alpha$, $G_{i/o}\alpha$, $G_{q/11}\alpha$, and $G_{12/13}\alpha$ [46]. $G_s\alpha$ and $G_{i/o}\alpha$ subunits stimulate and suppress cAMP-dependent pathways by activating and inhibiting adenylyl cyclase, respectively. The $G_{q/11}\alpha$ subunit activates phospholipase C (PLC) and promotes calcium influx and phosphorylation of protein kinase C (PKC), whereas $G_{12/13}\alpha$ regulates actin cytoskeletal remodeling in cells by activating RhoA GTPase [30,46]. It is well-known fact that GPCRs translate physiological stimuli into biochemical signals, thereby contributing to cellular homeostasis. Interestingly, a recent study demonstrated that the $G_{q/11}\alpha$ protein-coupled angiotensin II type 1 receptor (AT_1R), which is one of the components of the renin-angiotensin system (RAS), is activated by mechanical loading in a ligand-independent fashion [47]. AT_1R is the first GPCR identified as a mechanosensitive receptor; and to date, several GPCRs including the endothelin type 1_a (ET_{1A}) receptor, histamine H_1 receptor, muscarinic M_5 receptor, and parathyroid hormone (PTH) receptor have been identified as mechanosensitive receptors similar to AT_1R [48–52].

3. Role of CCN2 as a pivotal regulator of mechanical stress response in cell differentiation

3.1. CCN2 is a mechano-sensing regulator in cartilage

As articular cartilage is an avascular and lymphatic-free tissue, nutrition is supplied to chondrocytes, which are the only cellular components, only from the streaming of synovial fluid made by joint movement [53]. Therefore, suitable mechanical loading under physiological conditions is essential to normal cartilage metabolism [53]; and their metabolic activity is influenced by several mechanical stress-induced critical factors regulating chondrocyte proliferation and differentiation [54]. Among them, CCN2 is a unique anabolic factor that is highly induced by mechanical loading and that promotes both the proliferation and differentiation of chondrocytes [5,17]. In our report published over a decade ago, we investigated the effect of the frequency of mechanical stress on CCN2 expression in cultured chondrocytes [5]. Our data demonstrated that CCN2 expression was further increased under continual mechanical loading for 0.5 h with a rest interval of 2.5 h than that under continuous mechanical loading for 3 h [5]. These findings indicated that the up-regulation of CCN2 is better promoted by continual short-term loading with a rest than that by continuous loading, suggesting that rest after mechanical loading plays an important role in the maintenance of chondrocyte viability. Furthermore, we showed that cyclic tension force at the magnitude of 15 kPa and frequency of 30 cycles/min, which is an excessive force for human chondrocytic HCS-2/8 cells [55], suppresses the gene expression of CCN2, but that mechanical stress of the same magnitude and frequency increases CCN2 expression in meniscus cells that have both fibroblastic and chondrocytic phenotypes [5]. These results indicate that sensitivity to mechanical loading is different depending on the cell type. Based on these data, we speculate that CCN2 expression may be useful as an indicator of chondrocyte viability and of the suitable magnitude of mechanical stress depending on the cell type.

Ultrasound is sound waves that are out of the range of audible sound, and it can affect cells and tissues by acting as a mechanical stress. Ultrasound therapy was introduced in the 1950s in the orthopedic field, and is currently an established therapy in the orthopedic clinic to promote the healing of bone fractures [56]. At present, ultrasound therapy is also one of the widely used physical treatments for knee osteoarthritis (OA). Ultrasound therapy is performed in 2 methods; i.e., one is continuous, and the other is pulsed [57–59]. Application of high-intensity continuous ultrasound (HICUS: intensity; 1–300 W/cm^2) generates a thermal effect and induces pain relief in the tissues [57–59]. On the other hand, application of low-intensity pulsed ultrasound (LIPUS: intensity; 1–100 mW/cm^2) changes cell membrane permeability and increases intracellular calcium levels; and, as a result, it promotes tissue regeneration with non-thermogenic and non-destructive actions [57–59]. Comparison between continuous and pulsed ultrasound for OA repair by using meta-analysis demonstrated that pulsed ultrasound is effective for both pain relief and functional improvement, whereas continuous ultrasound is effective only for pain relief, when compared with the non-treated group [60]. Therefore, we consider that LIPUS treatment is more useful than HICUS treatment for investigating the effect of ultrasound on chondrocyte differentiation. However, the mechanism by which this treatment affects chondrocyte differentiation and the related intracellular signaling pathways triggered by LIPUS are still unknown. Our previous study revealed that LIPUS increases the gene expression of aggrecan and type II collagen, which are 2 major representative factors for chondrocyte differentiation [8] and that LIPUS promotes tissue repair in an experimental model of rat meniscus injury [61]. Interestingly, we also have shown that the LIPUS-induced effects are abolished by *Ccn2*-deficiency [8]. Moreover, we found that the expression of genes of TRPV4 and BK_{ca} channels, which are mechanosensitive ion channels, is decreased in *Ccn2*-deficient chondrocytes and that the stability of both TRPV4 and BK_{ca} channel mRNAs is reduced by *Ccn2* knockdown [8]. From these findings, we consider that the insensitivity to LIPUS under *Ccn2* deficiency is due to the reduced expression of TRPV4 and BK_{ca} channels caused by depletion of CCN2. Taken together, these findings indicate that mechanical stress-induced CCN2 not only plays multiple roles in chondrocyte functions but that CCN2 also regulates the gene expression of mechanoreceptors, suggesting CCN2 to be a mechano-sensing regulator.

3.2. Regulatory mechanism of the gene expression and protein production of CCN2 by mechanical stress

Next, we now describe how mechanical stress translates into the gene expression of CCN2. Mechanical stress results in the activation of multiple signaling cascades, such as those involving cation influx by mechanosensitive ion channels [24–26], integrins linking extracellular matrix proteins to focal adhesions [24,27,28], Rho GTPase family members [24,30], and GPCRs [24,31]. In particular, RhoA GTPase links the formation of focal adhesions to actin rearrangement, and also functions as a transducer of intracellular signaling, leading to changes in gene expression and cell morphology [30,39]. Namely, RhoA GTPase plays a central role in mechano-transduction. Works from a number of laboratories have demonstrated the involvement of CCN2 expression in RhoA activation in several cell types. Interference of RhoA activation by botulinum ADP-ribosyltransferase (C3 transferase) and toxin B, which are inhibitors of RhoA signaling, inhibits the induction of CCN2 by lysophosphatidic acid (LPA), a known activator of RhoA in fibroblasts [62,63]. Additionally, inhibition of RhoA-associated kinase (ROCK), which is a downstream mediator of RhoA signaling, by a specific inhibitor of ROCK, also decreases the induction of CCN2 in vascular smooth muscle cells [64]. These findings indicate that activation of RhoA induces CCN2 expression in fibroblasts and vascular smooth muscle cells. Because it is well known that activated RhoA regulates actin polymerization [39], we next focus on the relationship between RhoA-activated actin stress fibers and CCN2 expression. Previous studies demonstrated that *Ccn2*

expression in fibroblasts and osteoblasts is respectively increased and decreased by treatment with jasplakinolide, which stabilizes actin stress fibers, and by that with latrunculin B, which disrupts F-actin and increase G-actin [6,62]. These data show that the induction of CCN2 expression is closely related to actin polymerization, suggesting that actin stress fibers have an important role in the stimulation of CCN2 expression. Then, how does actin polymerization regulate CCN2 expression? It is worthy of notice that YAP and its paralog, TAZ are transcriptional coactivator of the TEAD family regulating CCN2 expression as described in Section 2. As YAP/TAZ is translocated into the nucleus or stays in the cytoplasm according to the tension of the actin cytoskeleton, the expression of CCN2, which is one of the target genes, is increased by the formation of F-actin stress fibers when the cells are subjected to tension [42,65]. Therefore, the induction of CCN2 by activated RhoA is due to the retention of YAP in the nucleus by actin polymerization, at least in fibroblasts and mesenchymal stem cells. However, dedifferentiated chondrocytes that underwent several passages in culture show very prominent stress fibers, and these cells lose the round cell shape of mature chondrocytes and display a fibroblastic morphology [66]. Although these cells form F-actin stress fibers and RhoA is activated therein, their chondrocyte markers including type II collagen are lost [66–69]. Indeed, it has been reported that Sry-related high mobility group box 9 (SOX9), which is a master regulator of chondrocyte differentiation and maturation, is repressed via nuclear translocation of YAP/TAZ elicited by the formation of F-actin stress fibers [66–69]. Here, we should note that SOX9 directly increases CCN2 expression in chondrocytes at the transcriptional level [70]. Although nuclear translocation of YAP/TAZ may transiently induce CCN2 expression in chondrocytes, because of the decrease in SOX9, which increases and maintains CCN2 expression, the nuclear translocation of YAP/TAZ by RhoA activation eventually may have no effect of CCN2 expression in chondrocytes. As such, the fact that RhoA activation promotes and inhibits CCN2 expression in fibroblasts [62] and chondrocytes [66–70], respectively, suggests that CCN2 regulation via RhoA may depend on the cell-specific background of transcription factors.

It has been reported that Rac1 and Cdc42, which are other members of the Rho GTPase family, antagonize the effect of RhoA in various cells, as described in Section 2 [71]. Rac1 and Cdc42 have been shown to regulate cortical actin organization and to be involved in the formation of lamellipodia and filopodia, respectively [72,73]. Additionally, it has been demonstrated that Rac1 and Cdc42 positively regulate the expression of SOX9 and type II collagen in chondrocytes [74]. Moreover, pharmacological inhibition of Rac1 decreases CCN2 expression in chondrocytes, and inhibition of actin polymerization by cytochalasin D also results in a decrease in CCN2 mRNA and protein levels [75]. Our data also revealed that both actin polymerization with a cortical pattern and CCN2 expression are promoted in chondrocytes subjected to LIPUS treatment [8]. These findings suggest that cortical actin organization by Rac1 activation is involved in CCN2 expression induced by LIPUS treatment in chondrocytes. Collectively, cortical actin organization via Rac1 activation promotes the translocation of SOX9 into the nucleus; and as a result, CCN2 expression is up-regulated. On the other hand, although differentiating adipocytes undergo cortical actin organization as well, CCN2 expression is remarkably down-regulated in them [65]. Additionally, LIPUS treatment decreases cortical actin and increases CCN2 expression, repressing their adipogenic differentiation [65]. As it is reported that peroxisome proliferator-activated receptor (PPAR)γ, which is an essential transcription factor for adipogenesis, inhibits CCN2 expression [76], these findings suggest that the PPARγ decreased by LIPUS treatment increases CCN2 expression in adipocytes. However, additional studies are needed to determine the involvement of actin polymerization and LIPUS-induced CCN2 expression in those cells.

Integrins are heterodimeric cell-surface receptors consisting of non-covalently linked α and β subunits, and are one of the most important sensors of mechanical stress [24,27,28]. Integrins bind to extracellular matrix proteins, such as collagen, fibronectin, laminin, and so on, and activate intracellular signaling pathways that regulate cellular functions, including actin organization and morphological changes [24,27,28]. Also, integrins are immediate sensors of mechanical stress on cells, and interact with adaptor molecules such as focal adhesion kinase (FAK) via its intracellular domain, thereby connecting the actin cytoskeleton to various signaling molecules including extracellular signal-regulated kinase (Erk), phosphatidylinositol-3 kinase (PI3K), and Rho GTPase family [24,77]. These findings are observed without exception, and thus integrins regulate chondrocyte proliferation and differentiation via activation of many downstream pathways, including Ras/Raf/Erk, PI3K/Akt and Rho/Rac/Cdc42. Integrins $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$, and $\alpha_v\beta_5$ are present in cartilage [78]. Among them, at least integrins $\alpha_5\beta_1$, $\alpha_6\beta_1$, and $\alpha_v\beta_5$ have been shown to bind to CCN2 [14,15]. We clarified that *Ccn2*-deficient chondrocytes exhibit impaired proliferation and differentiation [15], suggesting that a part of this impairment is associated with abolished interaction between integrin α_5 and CCN2 [15]. In summary, integrins are the first receptors sensing mechanical stress and lead to enhanced expression of the CCN2 gene through their signal pathways. Also, as a ligand of integrins, CCN2 itself regulates the cellular actions induced by mechanical loading via the integrin pathways.

TRPV4, which is a cation-channel molecule, is activated by mechanical stress, and promotes the influx of Ca^{2+} into chondrocytes, resulting in increased steady-state levels of SOX9 mRNA and protein [79]. Our previous data demonstrated that Ca^{2+} influx into chondrocytes is increased by treatment with LIPUS and that phosphorylation of Erk and p38 is also enhanced [8]. Additionally, we reported that Ca^{2+} influx via GPCRs increase CCN2 production via the activation of Akt and p38, and decreases it via the activation of PKCε, PKCζ and Erk [80]. Furthermore, we confirmed that LIPUS increases the mRNA levels of Sox9, type II collagen, and aggrecan in human inner meniscus cells in culture [61]. As such, Ca^{2+} influx via TRPV4 activated by LIPUS regulates CCN2 and SOX9 expression through the activation of various signal pathways, resulting in the promotion of chondrocyte differentiation. Interestingly, we found that the gene expression of TRPV4 is reduced in *Ccn2*-deficient chondrocytes, compared with that in wild-type chondrocytes, and that *Ccn2* knockdown decreases the stability of TRPV4 mRNA in human chondrocytic HCS-2/8 cells [8]. Based on these findings, we speculate that CCN2 induced by mechanical stress contributes to the stability of the mechanoreceptor mRNA, forming a positive feed-back loop with the mechanoreceptor to enhance chondrocyte differentiation as a mechano-sensing regulator.

AT_1R belongs to the family of GPCRs, and it has been known that AT_1R interacts with angiotensin II (ANGII) and promotes myogenic vasoconstriction, cardiac hypertrophy, and kidney fibrosis [81]. However, a recent study found agonist-independent mechanical-induced AT_1R activation [30,47]. Several groups showed that the expression of AT_1R and AT_2R genes is detectable in chondrocytes [82,83], and we have shown that CCN2 is increased at mRNA and protein levels by treatment with ANGII (Nishida et al., unpublished data). Additionally, we have also shown that ANGII-induced phosphorylation of p38 is decreased by pretreatment with losartan, which is a specific blocker of AT_1R (Nishida et al., unpublished data). These findings indicate that ANGII promotes CCN2 expression through AT_1R activation and p38 phosphorylation. Because it has been reported that conformational changes in AT_1R elicited by mechanical stress are different from those caused by agonists [84], signaling pathways of CCN2 expression induced by mechanical

stress may differ from those triggered by ANGII. Hereafter, additional experiments are needed to clarify the signaling pathways of CCN2 regulation via agonist-independent mechanical AT_1R activation and the possible role of AT_2R as a mechano-sensing receptor in chondrocytes.

4. Conclusions

In this review article, we summarized the characteristics of the various mechanoreceptors, involved in chondrocyte differentiation (Fig. 2). We also discussed individual functions of these mechanoreceptors that are still in the process of investigation [24–31]. However, when cells, tissues, and organs are subjected to mechanical stress, not only individual mechanoreceptors would be triggered, but also cross-talk between mechanoreceptors including TRPV4-integrin, Rho-GPCRs, YAP/TAZ-GPCRs, and Rho-integrin would occur. Indeed, it has been reported that TRPV4 is directly associated with integrin $\alpha_2\beta_1$ and Src tyrosine kinase in a molecular complex in sensory neurons [85] and that GPCRs classified as $G_{i/o}\alpha$, $G_{q/11}\alpha$, and $G_{12/13}\alpha$ repress the activity of LATS, which is a kinase in Hippo pathways and induces YAP/TAZ activity [86]. Hereafter, in order to comprehensively understand mechano-sensing and/or mechano-transduction in mechanically challenged cells or tissues, further information on the various interactions among mechanoreceptors on those cells or tissues is needed. Also, we have discussed the mechanism of mechanical regulation of CCN2, as a mechano-sensing regulator, in this article. CCN2 is up-regulated by various mechanical stress, such as stretch, compression, fluid shear stress, and ultrasound in various types of the cells. In fact, tooth movement experiments using rodents in vivo reveals that CCN2 is up-regulated in osteocytes at both compression and tension sides [87]. The CCN2 produced by osteocytes promotes not only bone formation and resorption [23], but also apoptosis at the compression side [88]. As a result, CCN2 induced by mechanical stress enables tooth movement through the promotion of bone remodeling. In any case, optimal frequency and magnitude of the forces are dependent on the cell type, and CCN2 expression is increased by the mechanical stress optimal for the cells [55]. In contrast, if the cells are subjected to unsuitable mechanical stress, CCN2 expression is oppositely decreased [55]. These findings indicate that CCN2 can be a useful biomarker to sense optimal mechanical stress for cells including chondrocytes, meniscus cells, osteoblasts [6] and osteocytes. Mechanoreceptors and their mechanism of action of sensing and converting this optimal stress into chemical ones to induce CCN2 are still unknown and of particular interest.

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgments

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