REVIEW

Wnt and Rho GTPase signaling in osteoarthritis development and intervention: implications for diagnosis and therapy

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Abstract

Wnt and Rho GTPase signaling play critical roles in governing numerous aspects of cell physiology, and have been shown to be involved in endochondral ossification and osteoarthritis (OA) development. In this review, current studies of canonical Wnt signaling in OA development, together with the differential roles of Rho GTPases in chondrocyte maturation and OA pathology are critically summarized. Based on the current scientific literature together with our preliminary results, the strategy of targeting Wnt and Rho GTPase for OA prognosis and therapy is suggested, which is instructive for clinical treatment of the disease.

Introduction

The disability burden and prevalence of osteoarthritis (OA) in both developed and developing countries is increasing due to an aging population. OA is a degenerative joint disease that is characterized by cartilage degradation and osteophyte formation. It involves multiple components of the joint, including the synovial joint lining, peri-articular bone and adjacent supporting connective tissue elements [1]. Current OA treatment modalities mainly function as intermittent symptom relief without long-term improvement in disease prognosis due to our current limited understanding of OA pathophysiology. Better understanding of the underlying mechanisms of OA initiation and progression might therefore facilitate identification of appropriate therapeutic targets for OA treatment [2].

The mechanism of OA is currently not well defined, as multiple factors can in more than one way lead to

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articular cartilage destruction and loss of joint function. Recently, increasing numbers of studies have implicated chondrocyte terminal differentiation (hypertrophy-like changes) in the pathogenesis of OA. This is similar to the chondrocyte differentiation process during endochondral ossification (EO). The close resemblance between terminal differentiation in OA cartilage and EO suggests that new OA therapeutic targets can potentially be identified from EO biology. Normal articular chondrocytes located at the ends of long bones do not develop into the hypertrophic state, thus avoiding terminal differentiation. However, OA chondrocytes lose their stable phenotype and undergo hypertrophy, which is characterized by cell enlargement as well as expression of various chondrocyte maturation and osteogenesis markers such as COLX [3], matrix metalloproteinase (MMP)13 (also known as collagenase 3) [3-5], a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-5 [6-8], osteopontin, osteocalcin, Indian Hedgehog [9], Runx2 [10], vascular endothelial growth factor (VEGF) [11], and transglutaminase-2 (TG-2) [12].

The developmental biology of EO is of key importance in understanding the process of OA, and there is much scientific evidence indicating that signaling pathways modulating joint formation and homeostasis are of central importance in the pathogenesis of OA. The Wnt signaling pathway is well established to be a key regulator in EO [13,14], a process through which bone and articular cartilage are formed. At the same time, most studies support the notion that activation of Wnt/β-catenin signaling is associated with articular chondrocyte matrix catabolism and stable phenotype loss [15]. Recent years have also seen a number of studies indicating that Rho GTPases play central roles in both chondrocyte differentiation and articular chondrocyte physiology, which will be discussed below.

Wnt and Rho GTPase signaling and their interaction

In the canonical Wnt signaling pathway, most β-catenin in the cytoplasm is sequestered within an oligomeric

complex of casein kinase, axin, the adenomatous poly-posis coli tumor suppressor protein (APC) and glycogen synthase kinase 3β (GSK3β) [16]. However, when Wnt ligands bind to cell membrane receptors, signaling through the frizzled receptors inhibits this degradation process, thereby increasing the levels of free cytoplasmic β-catenin. Accumulation of cytoplasmic β-catenin results in its translocation to the nucleus, where it binds to transcription factors such as lymphoid enhancing factor (LEF)/T cell factor (TCF) to generate a transcriptionally active complex that targets genes such as those encoding MYC, cyclin D1, MMP3 and CD44 [17]. In addition, there are some natural extracellular inhibitory factors that regulate canonical wnt signaling, including members of the secreted frizzled receptor protein (sFRP) family, Dickkopf (Dkk) proteins [18], Wnt inhibitory factor [19], cerberus [20] and sclerostin [21] (Figure 1).

The Rho family of GTPases includes 20 members, which are 'Ras-like' proteins. Amongst these, Cdc42, Rac1, and RhoA have been intensively studied. Guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs) and guanine nucleotide dissociation inhibitors (GDIs) are all regulators of the switch between the active and inactive forms of Rho-GTP. Rho GTPases have also been referred to as 'molecular switches' for transducing signals from the chondrocyte extracellular matrix to affect cytoskeletal actin dynamics and cellular morphology, which in turn regulate cell proliferation, apoptosis and gene expression [22].

Meanwhile, a new study indicates that Rho GTPases play a role in nuclear transportation of cytoplasmic β-catenin. Constitutive activation of Rac1 in colon cancer cells significantly enhances TOPFlash promoter activity and nuclear β-catenin accumulation. This effect is inhibited by dominant-negative Rac1 [23]. Mutation of RacGap50C, a negative regulator of Rac1, in *Drosophila* embryos stimulated canonical Wnt signaling [24]. Similarly, Rac1-specific activator Tiam1 was demonstrated to transcriptionally activate β-catenin/TCF complexes in response to Wnt3a [25]. In another study, Wu and colleagues [26] reported that Rac1 acted cooperatively with JNK2 activation during β-catenin phosphorylation and nuclear localization. This was further supported by phenotype similarity between Rac1 and β-catenin ablation in mouse limb bud ectoderm. Although neither stabilization nor nuclear localization of β-catenin re-quires RhoA activation, Wnt3a induction of osteogenic differentiation of stem cells requires both RhoA and β-catenin activation [27] (Figure 1).

By contrast, much less attention has been paid to non-canonical Wnt signaling, which is characterized as being β-catenin/TCF independent. One example of non-canonical Wnt signaling is the planar cell polarity pathway, which promotes cell organization in a particular
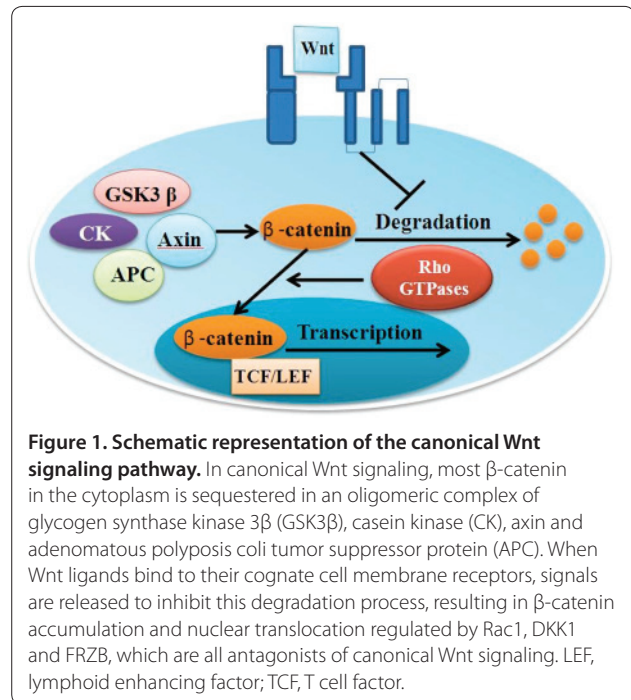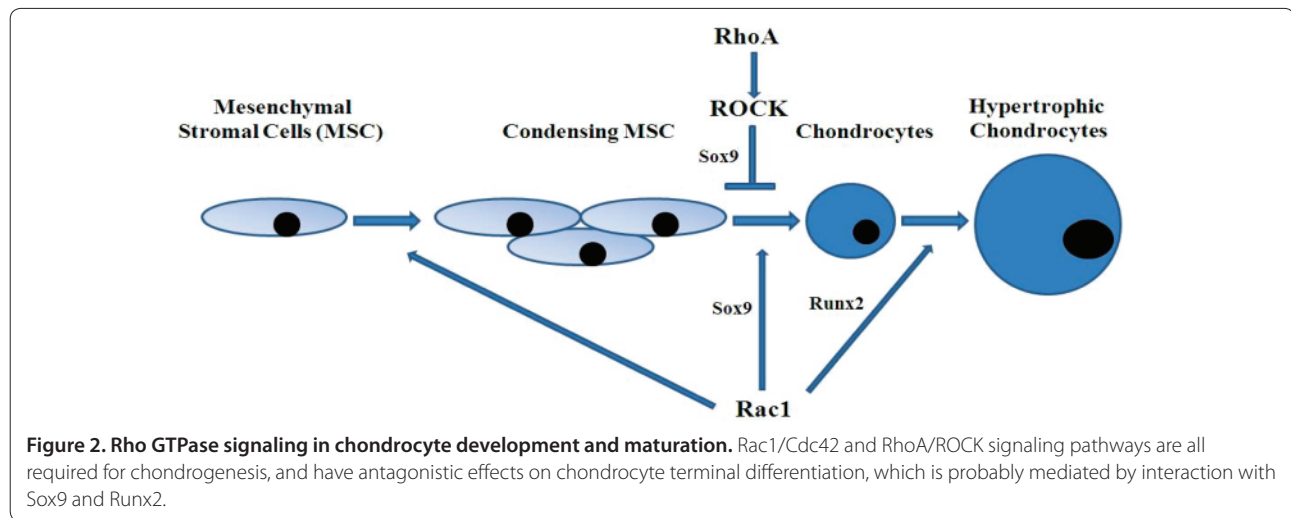

Figure 1. Schematic representation of the canonical Wnt signaling pathway. In canonical Wnt signaling, most β-catenin in the cytoplasm is sequestered in an oligomeric complex of glycogen synthase kinase 3β (GSK3β), casein kinase (CK), axin and adenomatous polyposis coli tumor suppressor protein (APC). When Wnt ligands bind to their cognate cell membrane receptors, signals are released to inhibit this degradation process, resulting in β-catenin accumulation and nuclear translocation regulated by Rac1, DKK1 and FRZB, which are all antagonists of canonical Wnt signaling. LEF, lymphoid enhancing factor; TCF, T cell factor.

orientation [28,29], through the action of Rho GTPases on assembly of the actin cytoskeleton [30,31].

Roles of Wnt and Rho GTPases in regulating chondrocyte hypertrophy and maturation

Canonical Wnt signaling is known to induce chondrocyte hypertrophy and final maturation. During skeletal development and growth, chondrocyte hypertrophy, calcification, and expression of MMPs, ADAMTS and VEGF in limb buds or growth plates require activation of canonical Wnt signaling [32,33]. Forced expression of the constitutively active form of LEF in chick chondrocytes stimulates ectopic EO [34]. Additionally, mis-expression of Frzd-1, a Wnt antagonist, led to delayed chondrocyte maturation, metalloprotease expression and marrow/bone formation [35], thus suggesting a positive role of Wnt signaling in promoting chondrocyte maturation. These data confirmed the pivotal role of Wnt-β-catenin in chondrocyte maturation and hypertrophy during EO.

Recent studies also suggest that GTPases play a significant role in both chondrocyte development and maturation. Rac1 and Cdc42 are co-expressed in both articular and growth plate chondrocytes, and they func-tion to accelerate the rate of chondrocyte differentiation by increasing COLX promoter activity [36]. Kerr and colleagues [37] found that levels of active Rho GTPases increased with chick chondrocyte maturation. The acti-vated Rac1 expression induced chondrocyte enlargement and MMP13 upregulation, suggesting a positive role of Rac1 in chondrocyte maturation. Additionally, Rac1 and

Figure 2. Rho GTPase signaling in chondrocyte development and maturation. Rac1/Cdc42 and RhoA/ROCK signaling pathways are all required for chondrogenesis, and have antagonistic effects on chondrocyte terminal differentiation, which is probably mediated by interaction with Sox9 and Runx2.

Cdc42 are required for chondrocyte condensation mediated by N-cadherin and act as positive regulators of chondrogenesis [38]. The regulatory effect on chondrocyte differentiation was verified by gene mutation studies in mice. *In vivo*, Rac1-deficient growth plates displayed delayed ossification, reduced chondrocyte proliferation and increased apoptosis [39], partly due to reduced mitogenic activity through Rac1-inducible nitric oxide synthase-nitric oxide signaling in EO [40]. Similar results were observed in limb bud development. One study reported that the specific deletion of Rac1 (Msx-2 cre) caused severe truncations of limb buds due to impaired nuclear transport of β-catenin [26]. Studies by Kamijo and colleagues reported that both Rac1 [41] and Cdc42 [42] are essential for interdigital programmed cell death through regulation of Bmp, Msx1, and Msx2 gene expression.

A study by Beier and colleagues [43] demonstrated an antagonistic effect of RhoA/ROCK signaling on chondrocyte differentiation, in contrast to Rac1/Cdc42 signaling [44]. Over-expression of RhoA in ATDC5 cells resulted in delayed hypertrophic differentiation with reduced COLX and MMP13 expression. However, pharmacological inhibition of RhoA/ROCK by Y27632 increases *Sox9*, *COLII* and aggrecan mRNA levels during chondrogenesis in monolayer culture systems. The observed effects of RhoA/ROCK signaling appeared to be antagonistic in a three-dimensional micromass culture system [45]. Similarly, the study by Lassar and colleagues also reported that RhoA/ROCK signaling regulated Sox9 transcriptional activity through actin polymerization mediated by protein kinase A phosphorylation of Sox9 [46]. By contrast, studies of D'Lima and colleagues [47] demonstrated that ROCK, a downstream effector of RhoA, directly phosphorylates Sox9, which in turn regulates chondrogenesis. This suggests that RhoA functions

through signaling pathways other than ROCK in modulating chondrogenesis [48]. Recently, Sox9 has been demonstrated to correlate with Mef2c in modulating chondrocyte terminal differentiation [49], suggesting that Rho GTPases may function upstream of Sox9 during chondrocyte differentiation.

In summary, Rac1/Cdc42 and RhoA/ROCK signaling pathways are all expressed during chondrogenesis and have adverse effects on chondrocyte terminal differentiation (hypertrophy-like change). The Rac1/Cdc42 signaling pathway accelerates chondrocyte hypertrophy while the RhoA/ROCK signaling pathway delays chondrocyte maturation through regulation of Sox9, as illustrated in Figure 2, but the underlying mechanisms are still poorly understood.

Canonical Wnt signaling and pathological changes in osteoarthritis

Wnt-β-catenin signaling is activated in both human and mice OA cartilage. In fact, many animal model studies utilizing a genetic approach have strengthened this view. Mechanical injury, a major cause of OA, leads to down-regulation of Wnt antagonist FRZB and up-regulation of ligand Wnt16 and target genes encoding β-catenin, Axin-2, C-JUN and LEF-1 [50]. Furthermore, transcriptome analysis demonstrated that expression of Wnt1-inducible signaling protein 1 (WISP-1) is increased two-fold in cartilage lesions compared to healthy intact cartilage [51]. These findings indicate that Wnt signaling may function as an OA initiation factor upon mechanical injury. Corr and colleagues [52,53] first reported that Arg200Trp and Arg324Gly Frzb variants, encoding sFRP3, an extracellular inhibitor of Wnt-β-catenin signaling, contributed to genetic susceptibility of women to hip OA. However, the same conclusions were not reached by another two groups that investigated other populations

[54,55]. Although Min and colleagues [56] thought that these two variants are also associated with other generalized OA at multiple sites, there is still no direct evidence implicating Frzb variants in knee OA. Frzb knockout mice display increased cartilage damage and thicker cortical bone formation [57]. Given the close relationship between bone shape and OA development, Baker-Lepain and colleagues [58] believed that SNPs in Frzb are associated with the shape of proximal femur and further contribute to hip OA development. However, some pertinent questions remain: do these two variants increase wnt ligand binding with the Frizzled protein to activate Wnt-β-catenin signaling; and does mis-function of Frzb in chondrocytes directly lead to OA or Frzb modulation of bone shape, disrupting mechanical loading on cartilage and consequently leading to OA? The inhibition of Dickkopf-1 (Dkk1), a negative regulator of Wnt-β-catenin signaling, has been reported to be able to reverse the bone-destructive characteristics of rheumatoid arthritis to the bone-forming characteristics of OA [59]. Another study on the mouse OA model also demonstrated that control of Dkk1 expression prevents joint cartilage deterioration in osteoarthritic knees through attenuating the apoptosis regulator Bax, MMP3 and RANKL (receptor activator of nuclear factor kappa-B ligand) [60]. Additionally, Blom and colleagues [61] showed that stimulation of Wnt-induced signaling protein 1 (WISP1) in chondrocytes resulted in IL1-dependent induction of MMPs and aggrecanase, suggesting induction of chondrocyte maturation. *LRP5* is located in chromosome 11q12-13, which is thought to be an OA susceptibility locus [62]. *Lrp5-/-* mice displayed increased cartilage degradation, probably due to low bone mass density [63]. These studies thus provide indirect evidence for Wnt-β-catenin participation in OA progression. Zhu and colleagues [64] provided direct evidence for the first time that β-catenin is implicated in the development of OA. The conditional activation of β-catenin in articular chondrocytes of adult mice caused OA-like cartilage degradation and osteophyte formation, and this was associated with accelerated chondrocyte maturation and MMP13 expression. Later, the authors reported a somewhat contradictory finding that selective suppression of β-catenin signaling in articular chondrocytes also causes OA-like cartilage degradation in Col2a1-ICAT (inhibitor of β-catenin and TCF) transgenic mice [65]. This led Kawaguchi [66] to hypothesize that β-catenin induces chondrocyte maturation similarly to Runx2, whereas it suppresses chondrocyte apoptosis similarly to osteoprotegerin (Table 1).

Although most current studies in the scientific literature demonstrate the involvement of canonical Wnt-β-catenin signaling in OA development, the role of this signaling pathway in OA pathophysiology is actually dependent on patient characteristics. For instance, two SNPs in FRZB were initially thought to be associated with an increased risk of primary hip OA among female patients [52,53]. However, conflicting data were reported by different studies [54,55]. The relationship between FRZB SNPs and human OA development may be dependent on the characteristics of the patient population, that is, sex and age-related differences. Excessive or insufficient β-catenin signaling in mice chondrocytes has been shown to increase susceptibility to OA phenotype [64,65], thus suggesting that balanced β-catenin levels are essential for maintaining homeostasis of articular chondrocytes. Factors impairing this balance could lead to pathological changes in chondrocytes by promoting either terminal differentiation or apoptosis.

Moreover, because OA is a systemic joint disease affecting overall joint tissues, including cartilage, subchondral bone and synovium, imbalance of β-catenin signaling in tissues other than cartilage could also initiate or promote OA development. For example, because canonical Wnt signaling has direct roles in osteogenesis, excessive Wnt signaling can also lead to increased bone formation, which might be associated with osteophyte formation. Two Wnt antagonists, sFRP1, which binds to RANKL [67], and DKK1, which promotes osteoprotegerin secretion [58], can alter the balance between osteoclast and osteoblast development. Additionally, upregulated DKK1 levels in synovial fibroblasts contribute to synovial hypervascularity in OA [68], which would imply that modulating DKK1 expression in synovial fibroblasts may be a potential therapeutic strategy for OA-induced synovitis and joint degradation.

Rho GTPases and pathological changes in osteoarthritis chondrocytes

With increasing recognition of the role of Rho GTPase activities in chondrocyte hypertrophy-like changes, their effects on OA have attracted much attention and have been investigated using both human genetic studies and animal models. Epidemiological studies from different groups reported a relationship between SNPs in *RhoB* and OA susceptibility in some populations [69,70]. Meanwhile, rodent OA models treated with the Rho kinase inhibitor AS1892802 displayed alleviation of cartilage damage [71]. RhoB is downregulated in OA articular chondrocytes and is thought to be responsible for significant DNA damage observed in the pre-apoptotic phenotype of OA chondrocytes [72]. RhoA-ROCK signaling is thought to be involved in early phase response to abnormal mechanical stimuli, which is accepted as a contributory factor to OA initiation and progression [73]. In addition, RhoA-ROCK signaling has also been demonstrated to interact with other pathological factors associated with OA such as transforming growth

Table1. Overview of the roles of various elements of the Wnt signaling pathway in osteoarthritis development, as demonstrated by human genetic studies or animal models
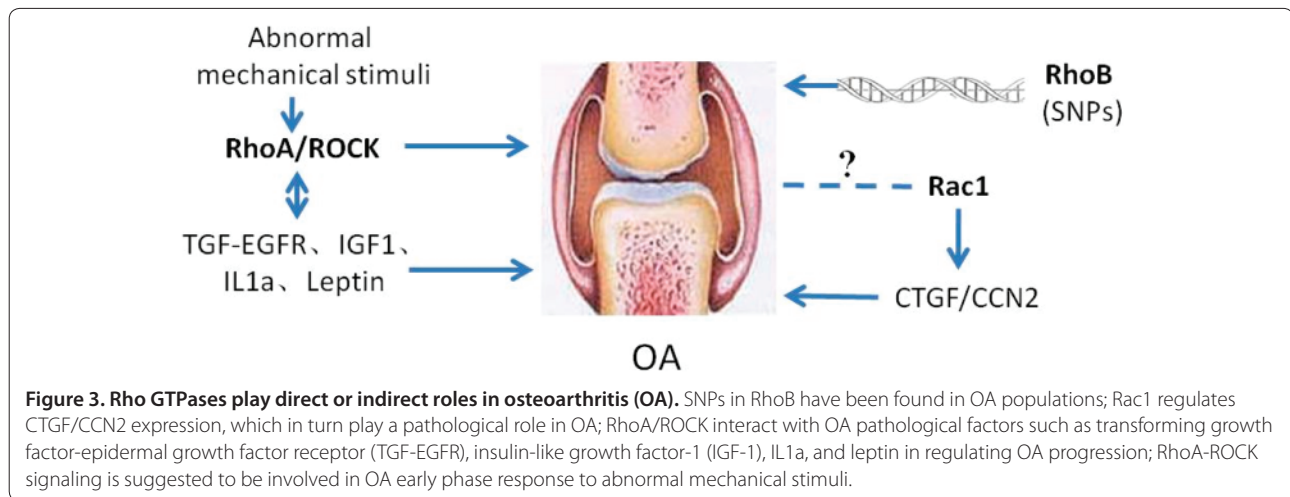
	Element treatment/SNPs	Effect on Wnt signaling	Results	Conclusions
Receptor				
LRP5	Haplotype (C-G-C-C-A) in LRP5	Inhibition	This haplotype predisposes to increased risk of OA	LRP5 variant may predispose patients to OA [56]
	Lrp5 knockout in mice	Inhibition	Increased cartilage degradation, decreased bone mineral density	Loss of function of Lrp5 leads to OA [57]
Wnt ligands				
Wnts (up-regulated in OA)	Mechanical injury	Activation	Up-regulation of Wnt16/WISP-1, down-regulation of FRZB, up-regulation of β-catenin, axin-2, C-JUN and LEF-1	Mechanical injury activates Wnt signaling [43]
Wnt antagonists				
Frzb (up-regulated in OA)	Arg200Trp/Arg324Gly Frzb variants	Activation	These two variants are associated with female hip OA from an epidemiological viewpoint	These two variants confer genetic susceptibility to female hip OA [46,47]
	Arg200Trp/Arg324Gly Frzb variants	Activation	These two variants are associated with other generalized OA by epidemiological analysis	These two variants contribute to female hip OA [50]
	Frzb knockout mice	Activation	Increased cartilage damage, thicker cortical bone formation	Loss of function of Frzb contributes to the development of OA [51]
DKK1 (up-regulated in OA)	Inhibition of DKK1 by antibody	Activation	Blocks bone erosion, promotes bone formation, reverses RA to OA	Wnt signaling is a key regulator of joint remodeling [53]
	OA rat knees were treated with end-capped phosphorothioate Dkk-1 antisense oligonucleotide (Dkk-1-AS)	Inhibition	Alleviated Mankin score, cartilage fibrillation, and serum cartilage degradation markers	Dkk1 expression prevents OA cartilage destruction and subchondral bone damage [54]
Transcription factor				
β-Catenin (up-regulated in OA)	Activation of β-catenin in articular chondrocytes	Activation	OA-like cartilage degradation, osteophyte formation, accelerated chondrocyte maturation and MMP13 expression	Wnt/β-catenin activation promotes OA development by accelerating chondrocyte maturation [58]
	Suppression of β-catenin in articular chondrocytes	Inhibition	OA-like cartilage degradation, increased chondrocyte apoptosis	Wnt/β-catenin inhibition promotes OA development by increasing chondrocyte apoptosis [59]

LEF, lymphoid enhancing factor; MMP, matrix metalloproteinase; OA, osteoarthritis; RA, rheumatoid arthritis.

factor-epidermal growth factor receptor signaling factors [74], IL1a, insulin-like growth factor-1 (IGF-1) [75] and leptin [76], suggesting a global role of RhoA-ROCK in OA progression. With regards to the Rac1/Cdc42 signaling pathway in OA progression, Cdc42-GTP content decreases [77] while Rac1-GTP increases with chondrocyte aging. This provides new insights into age-related OA development. Additionally, Rac1 regulates CTGF/CCN2 gene expression [78], which is upregulated in OA, and has been shown to be beneficial for articular cartilage regeneration in a mono-iodoacetate (MIA)-induced OA model and articular cartilage defect model [79]. A recent study by Long and colleagues [80] showed

that Rac1 is involved in Fnf-induced MMP13 production, thus suggesting a metabolic role of Rac1 activation in cartilage (Figure 3).

The role of Rho GTPases in OA progression may not only be limited to cartilage, but may also involve synovium and osteochondral bone. Rac and its regulators - GEFs and GAPs - have been proven to play vital roles in STAT signaling transduction [81-85], which is essential for the inflammatory response, thus suggesting the important role of Rac GTPases in OA joint inflammation [86]. Our preliminary results also showed that intra-articular administration of the Rac1 inhibitor NSC2376 efficaciously decreases mRNA transcript levels of

Figure 3. Rho GTPases play direct or indirect roles in osteoarthritis (OA). SNPs in RhoB have been found in OA populations; Rac1 regulates CTGF/CCN2 expression, which in turn play a pathological role in OA; RhoA/ROCK interact with OA pathological factors such as transforming growth factor-epidermal growth factor receptor (TGF-EGFR), insulin-like growth factor-1 (IGF-1), IL1a, and leptin in regulating OA progression; RhoA-ROCK signaling is suggested to be involved in OA early phase response to abnormal mechanical stimuli.

pro-inflammatory factors in joint tissue (unpublished data). Moreover, Rho GTPases also have important roles in mature osteoclasts by regulating the formation of actin rings and resorption lacunae [87] and are required for osteoclast differentiation [88]. The definitive role of Rho GTPase expression in osteochondral bone that contributes to OA progression needs to be further studied.

Our preliminary study investigating human OA cartilage shows that Rac1 is activated in OA chondrocytes and the level of Rac1-GTP is greatly upregulated by IL1b in a chondrocyte monolayer culture system (unpublished data), suggesting the important role of Rac1 in pro-inflammatory factor-induced OA progression. Furthermore, primary chondrocytes from OA calcified cartilage (one phenotype of OA) is significantly inhibited by the Rac1 specific inhibitor NSC23766, as demonstrated by Alizarin Red staining (unpublished data). Constitutive over-expression of Rac1 resulted in up-regulation of COLX, Runx2 and ADAMTS-5 and intra-articular injection of NSC23766 delayed mice OA development (unpublished data). Due to the high level of expression of Rac1 in human and mouse articular chondrocytes (Figure 4), further studies are focusing on the role of Rac1 in OA development *in vivo*, and its underlying mechanism. Additionally, the defined role of Rho GTPase in OA progression should be further investigated with animal models utilizing both genetic and pharmacological tools.

As mentioned earlier, Wnt/β-catenin signaling activation leads to elevated articular chondrocyte catabolism, hypertrophy-like changes and cartilage degradation, which are all key features of OA [66]. Rho GTPases have recently been discovered to function as key mediators of β-catenin nuclear translocation and the available data demonstrated significant roles of GTPases in chondrocyte hypertrophy, maturation and OA development [69-80]. Interaction between canonical Wnt signaling

and GTPases independent of actin cytoskeletal changes in OA development has not yet been addressed. The preliminary results from our study indicate that Rho GTPase modulation of OA may partially function through control of β-catenin nuclear translocation in canonical Wnt signaling.

Wnt signaling and Rho GTPases as targets for OA treatment

Current treatment modalities of OA, including pharmacological and surgical procedures, are mainly focused on promoting partial regeneration and relieving pain. For example, acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDS) and cyclooxygense 2 (COX-2) [89] are all utilized to relieve arthritic pain and can achieve good short-term results. Surgical treatment, including lavage, abrasion arthroplasty and microfracture, has long been considered as a palliative therapy for pain, possibly due to removal of inflammatory factors and bone marrow mesenchymal stem cell-mediated fibrous cartilage regeneration on the subchondral bone [90]. Concerns about later re-emergence of pain and durability of the newly formed fibrous cartilage by micro-fracture makes it imperative to develop more effective OA treatment modalities.

Recently, tissue engineering for cartilage regeneration has achieved much progress. Autologous chondrocyte implantation has often been used to treat simple cartilage defects [91,92]. However, chondrocytes in the newly formed cartilage by these procedures are likely to undergo calcification and hypertrophy-like changes, thereby affecting cartilage function [93]. Therefore, to improve therapeutic efficacy and maintain the functional status of regenerated cartilage, OA treatment should be focused on removing the causes or risk factors of OA. Small molecules targeted to OA-specific molecular pathophysiology may be a good strategy.
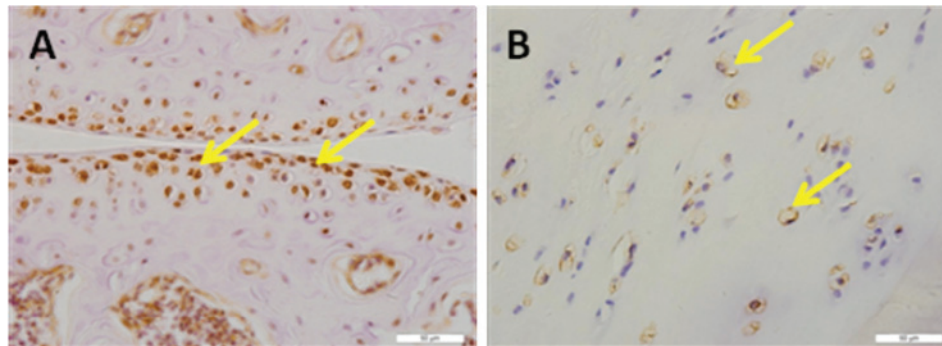
Figure 4. Rac1 is expressed in both mouse and human cartilage. (A) Robust expression of Rac1 was detected at the surface and middle zones of mouse cartilage but chondrocytes in calcified zone displayed reduced expression. **(B)** Rac1 is distributed ubiquitously in human articular cartilage. Arrows in both panels indicate representative Rac1-positive chondrocytes. Scale bars = 50 μm.

Available evidence suggests a critical role of Wnt signaling in EO as well as OA development. Excessive levels of some Wnt ligands and β-catenin have been observed in degenerating cartilage. However, this seems to be a paradox because several Wnt signaling antagonists, including DKK1, FRP1, FRP2, and FRP4, are strongly expresssed in OA synovium and cartilage. This may possibly be explained by the conjecture that both gain or loss of function of Wnt/β-catenin signaling would disrupt cartilage homeostasis and lead to pathological changes associated with OA. Aberrant expression of Wnt ligands and Wnt antagonists in synovium may function as an early signal to initiate OA, which in turn can be utilized as an easily accessible OA prognostic marker.

Both genetic and experimental studies have highlighted the great potential of locally modulating the Wnt signaling pathway to alter OA prognosis. Rho GTPases have been recently discovered to modulate β-catenin nuclear translocation and control β-catenin/TCF transcription activity. An altered level of Rho GTPases in articular chondrocytes might therefore be recognized as a new marker for OA development. Hence, Rho GTPases may be good targeting candidates to develop small molecule drugs for OA therapy. In fact, many ROCK inhibitors have recently emerged and have been reported in the patent literature. Some of these are utilized for inflammatory disorders such as multiple sclerosis and asthma. In particular, fasudil hydrochloride, a potent ROCK inhibitor, has been clinically used to treat cerebral vasospasm [94] and pulmonary hypertension [95].

Although blocking the activity of some members of the Rho GTPases family is able to prevent chondrocytes from undergoing hypertrophy and ossification, there are several pertinent problems to be solved before this strategy can be utilized as a means of OA therapy. Theoretically, Rho GTPases interact with the Sox9 and Runx2 pathways in maintaining a fine balance between chondrogenesis and chondrocyte terminal differentiation.

The underlying mechanism needs further investigation to identify more specific intervening signal molecules implicated in chondrocyte hypertrophy-like changes. Alternatively, Rho GTPase effectors could be more promising drug targets, because each of these effectors mediates specific roles of Rho GTPases. To date, modulating Rho GTPases to prevent chondrocytes from undergoing hypertrophy-like change has been evaluated in several animal studies and have demonstrated significant efficacy in OA therapy [71]. However, many scientific questions about the application of Rho GTPases for OA treatment still remain to be answered.

Last but not least, since Wnt and Rho GTPases have important signaling roles in numerous cell types, systemic administration of modulators of these pathways could be dangerous. Localized drug delivery may be a solution. Some biomaterials, such as chitosan and alginate microspheres, may serve as delivery vehicles for controlled drug release in designated tissues. Because Wnt and Rho GTPase signaling pathways modulate both early chondrogenesis (which should be promoted for cartilage repair) and hypertrophic differentiation (which should be suppressed), there should ideally be programmed drug administration for initial activation of these signaling pathways to promote chondrogenesis, followed by inhibition at a later time point to prevent chondrocyte terminal differentiation. Unpublished results from our lab showed that mesenchymal stem cells seeded on biomaterials incorporated with cytokines promoted cartilage repair. Thereafter, intra-articular injection of Rho GTPase inhibitors at a later time point could block terminal differentiation of the newly formed chondrocytes.

Conclusion
OA articular chondrocytes undergo hypertrophy-like changes, which is a similar process to EO. Wnt/β-catenin and Rho GTPases, mainly RhoA, Rac1 and Cdc42, are

well recognized as crucial regulators or mediators of chondrocyte development and chondrocyte hypertrophy during EO. It is now well established that Wnt/β-catenin and Rho GTPases have similar roles in OA progression and local modulation of the Wnt signaling pathway delays OA development. Preliminary studies have illustrated that Rac1 inhibition suppressed OA articular chondrocytes from undergoing hypertrophy-like changes both *in vivo* and *in vitro*. Moreover, Rac1 inhibitors may also be promising drugs for preventing chondrocyte ossification in cartilage tissue engineering. Other members of the Rho GTPase family may also possess similar potential as molecular targets for OA therapy. It was only in the last few years that the roles of Rho GTPases in modulating chondrocyte development and OA were intensively studied. Their regulatory effects on chondrocyte hypertrophy-like change warrants the use of Rho GTPase activators or inhibitors for OA prevention and cartilage tissue engineering. However, several concerns need to be addressed before Rho GTPase modulation is utilized as a means of OA therapy: the dosage and timing of intervention should be carefully investigated; appropriate controlled release systems may potentiate sustained function of Rho GTPases in OA joints; and drugs targeting specific effectors of Rho GTPases should be further developed to avoid side effects.

Abbreviations
ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; EO, endochondral ossification; FRP, frizzled receptor protein; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; IL, interleukin; LEF, lymphoid enhancing factor; MMP, matrix metalloproteinase; OA, osteoarthritis; RANKL, receptor activator of nuclear factor kappa-B ligand; sFRP, secreted frizzled receptor protein; SNP, single-nucleotide polymorphism; TCF, T cell factor; VEGF, vascular endothelial growth factor.

Competing interests
The authors declare that they have no competing interests.

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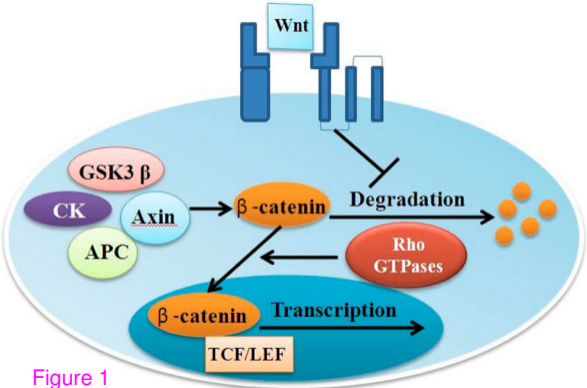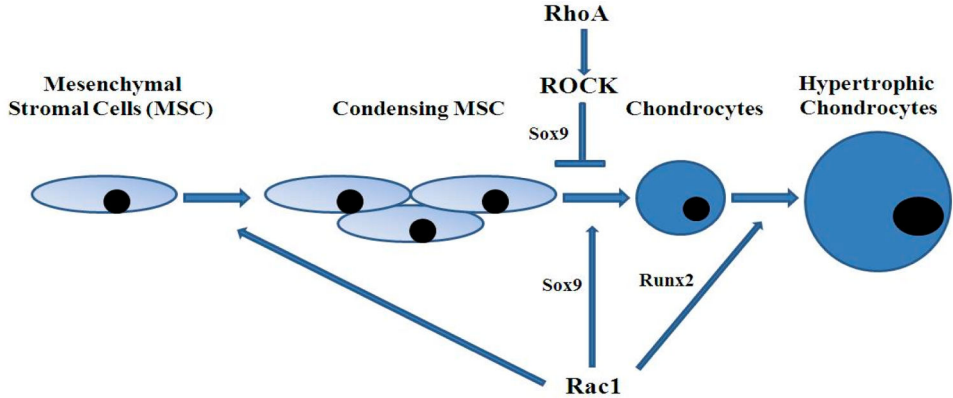95. Doggrell SA: **Rho-kinase inhibitors show promise in pulmonary hypertension.** *Expert Opin Investig Drugs* 2005, **14:**1157-1159.

Figure 1

Figure 2

Figure 4