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Review Article

Long noncoding RNAs: a new regulatory code in osteoarthritis

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Abstract: It is reported that long noncoding RNAs (lncRNAs) were expressed aberrantly in cartilage of osteoarthritis (OA). Current evidence indicates that lncRNAs not only serve as positive or negative regulators of OA, but also crosstalk with multiple potential targets to impact on the critical events in OA process. This review summarized the lncRNAs identified in OA to date, discussed their influence on the survival of chondrocytes and synoviocytes, arthritis-associated factors, and angiogenesis, and indicated the potential in diagnosis, therapy, and prognosis.

Keywords: Long noncoding RNA, osteoarthritis, cartilage

Introduction

Articular cartilage homeostasis is tightly orchestrated and maintained in a balance between anabolic and catabolic processes by the activity of the chondrocytes. When joints are subjected to altered loading caused by malalignment or trauma [1], catabolism often predominates and causes joint abnormalities, including synovitis, cartilage degradation, subchondral bone sclerosis, and osteophyte formation, which can be defined as osteoarthritis (OA) collectively [2]. In 1990, OA was estimated as the eighth leading non-fatal burden of life all over the world, whereas in 2000 it became the sixth [3, 4]. The pathogenesis of OA is complex and involves interplay of multiple factors such as genetic predisposition, altered mechanical loading, and the imbalance between anabolic and catabolic factors. It is thought that the imbalance plays a major role in cartilage degradation in OA [5].

Given that articular cartilage is normally avascular and has little intrinsic regenerative capacity, OA is a challenging disease to treat. There have been no effective therapies discovered to ameliorate or stop OA progression. Recent studies have shed light on the connection between noncoding RNAs (ncRNAs) and OA development [6]. The ncRNAs can be broadly divided into small ncRNAs and long ncRNAs (lncRNAs) [7]. It is known that lncRNAs, which are mRNA-like and more than 200 nucleotides in length, are transcribed in mammalian genomes pervasively [8]. In the past decade, the lncRNAs emerge as novel regulators of numerous biological processes where they serve as guides, signals, decoys, and scaffolds [9, 10], and have effects on a broad spectrum of development and diseases [11-17].

LncRNAs have been reported to play critical roles in the development of bone and cartilage tissue [18, 19]. It aroused interest in aberrant expression of lncRNAs in OA cartilage which might influence the balance between anabolic and catabolic phase of joint cartilage. It is suggested that lncRNAs could be applied for diagnosis and prognosis, and could serve as a personalized therapeutic biomarker to impede, stop, and even reverse OA progression [20-22]. In the current review, we mainly summarized and emphasized the roles of lncRNAs in the OA progression, and harnessed them for the treatment of OA.

Table 1. Selected lncRNAs identified to date in the regulation of the chondrocytes and synoviocytes survival

LncRNA	Proposed mechanism of action	Reference
GAS5	Suppress miR-21 expression to inhibit the autophagic response and stimulate apoptosis	[30]
PCGEM1	Sponge for miR-770 to regulate synoviocytes proliferation, apoptosis, and autophagy	[40]
UFC1	Interact with miR-34a to promote chondrocytes proliferation and inhibit apoptosis	[37]
uc.343	Cis-regulate HOXC8 to impact chondrocytes cycle	[22]

Abbreviations: GAS5, Growth arrest-specific 5; PCGEM1, prostate cancer gene expression marker 1.

LncRNAs regulate the fate of cells

Table 1 summarized several lncRNAs that played demonstrated roles in the fate of chondrocytes and synoviocytes. These representative lncRNAs were selected to illustrate the diverse targets and mechanisms of lncRNAs in the regulation of chondrocytes and synoviocytes survival (**Table 1**, **Figure 1**).

Chondrocytes

Recently, many studies reported both apoptotic and non-apoptotic cell death in OA chondrocytes [23]. It is demonstrated that chondrocyte death is responsible for the severity of cartilage degradation [24-27], suggesting that apoptosis could be of diagnostic valve and be a potent option for the new therapeutic target.

It is known that Growth Arrest-Specific 5 (GAS5) is expressed in various tissues with multiple splice isoforms differentially and widely [28], and the inhibition of GAS5 will suppress cell apoptosis [29]. Several studies revealed that the expression level of GAS5 was significantly upregulated in OA chondrocytes, and RNA FISH analysis showed that GAS5 was positioned at nucleus and cytoplasm in OA chondrocytes but merely at nucleus in healthy chondrocytes [30].

MiR-21 is one kind of well-known onco-microRNAs. It has been shown experimentally that miR-21 targeted numerous genes involved in tumor growth and metastasis, for example, inhibiting tumor suppressors, such as PTEN and PDCD4 in gastric cancer [31], Tipe2 in immune diseases [32], and methionine adenosyltransferase in hepatoma cells [33]. It is demonstrated that miR-21 was suppressed in OA patients and the modulation of miR-21 influenced apoptosis and autophagy of OA chondrocytes [30].

It is observed that miR-21 was upregulated and GAS5 was downregulated in breast tumor tissues, indicating a negative correlation between GAS5 and miR-21 in several breast cancer cell lines [34]. Song et al observed a reciprocal repression of GAS5 and miR-21 during OA pathogenesis. The upregulation of GAS5 decreased the level of miR-21 expression significantly and regulated cartilage degradation [30]. It is supposed that GAS5 regulated cell survival by acting as the sponge of miR-21 and thereby contributed to the pathogenesis of OA. However, the possible inter-regulatory network between miR-21 and GAS5 has not been well studied.

Previous studies have shown that lncRNA UFC1 regulated cell survival positively and was expressed aberrantly in colorectal cancer and liver cancer [35, 36]. Recently, functional studies demonstrated UFC1 functioned as the promotor of proliferation and the inhibitor of apoptosis of chondrocytes, and it is reported that the expression of UFC1 was downregulated in OA cartilage [37]. It is known that UFC1 could interact with miR-34a in OA chondrocytes. MiR-34a could stimulate apoptosis of OA chondrocytes, while silencing miR-34a could reduce chondrocytes apoptosis effectively [38]. This observation suggests that the interplay between UFC1 and miR-34a could regulate the survival of chondrocytes, and restoring the expression of UFC1 has the potential to relieve or stop cartilage degradation.

It is shown that Homeobox gene C8 (Hoxc8) knock-down chondrocytes appeared to be with prolonged duration and delayed exit from M-phase [39], which implicated that Hoxc8 could control cell cycles to affect the proliferation of chondrocytes and cartilage development at this critical time point. LncRNA uc.343 was reported to reside upstream of Hoxc8 and cis-regulate Hoxc8. LncRNA uc.343 was upregulated in OA cartilage and was correlated with Hoxc8 positively in SW1353 cells treated with IL-1β [22]. These results provide evidence that

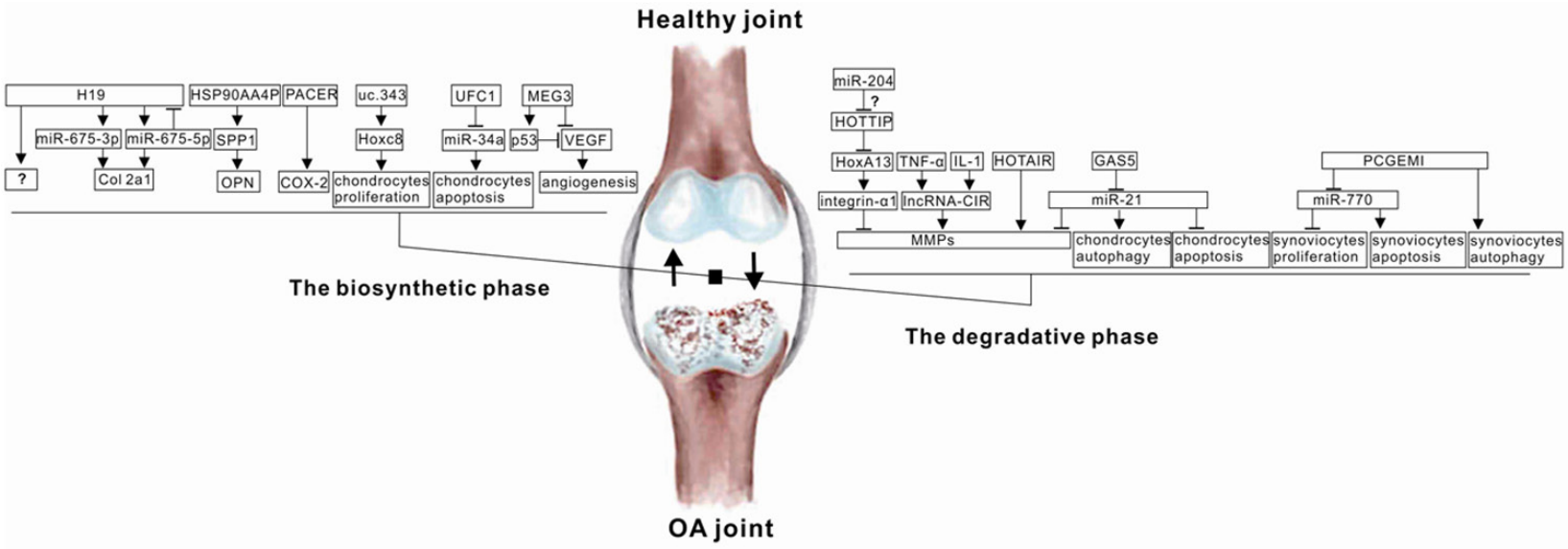

Figure 1. Schematic mode of lncRNAs and signaling pathways involved in the OA process. LncRNA stimulated or inhibited diverse targets to impact on the balance between the biosynthetic phase and the degradative phase of joint cartilage.

Table 2. Selected lncRNAs identified to date in the regulation of arthritis-associated factors

LncRNA	Proposed mechanism of action	Reference
HOTAIR	Upregulate MMPs expression	[6]
HOTTIP	Inhibit HoxA13/integrin-α1 signaling pathway to promote cartilage degradation	[7]
GAS5	Suppress miR-21 expression to upregulate MMPs expression	[30]
lncRNA-CIR	Upregulate MMP-13 expression	[21]
HSP90AA4P	Regulate SPP1/OPN pathway	[22]
PACER	Positively regulate COX-2 production	[60]
H19	Encode miR-675-3p and miR-675-5p; Negatively regulated by miR-675-5p; Modulate Col2a1 expression	[20, 63]

Abbreviations: HOTAIR, HOX transcript antisense RNA; MMPs, Matrix metalloproteinase; HOTTIP, HoxA distal transcript antisense RNA; lncRNA-CIR, Cartilage Injury Related lncRNA; SPP1, Secreted phosphoprotein 1; OPN, osteopontin; PACER, p50-associated cyclooxygenase 2-extragenic RNA; COX-2, Cyclooxygenase 2.

uc.343 might target Hoxc8 to regulate chondrocytes cycle progression.

Synoviocytes

Hyperplasia of synoviocytes is a hallmark of OA and the fibroblast-like synoviocytes can secrete proinflammatory cytokines to degrade cartilage [40]. It is well-known that prostate cancer gene expression marker 1 (PCGEM1) was overexpressed in prostate cancer. Overexpression of PCGEM1 decreased doxorubicin-induced expression of p53 and p21Waf1/Cip1, and suppressed apoptosis in LNCaP cells [41]. LncRNA PCGEM1 was also overexpressed in OA synoviocytes. Overexpression of PCGEM1 boosted proliferation of synoviocytes, activated beclin-1, and depressed PARP and caspase-9 [40]. MiR-770 is reported to suppress synoviocytes proliferation and stimulate synoviocytes apoptosis significantly, and the level of miR-770 decreased in OA synoviocytes. It is demonstrated that PCGEM1 suppressed miR-770 by direct binding in synoviocytes, and led to the hyperplasia of synoviocytes [40].

LncRNAs regulate arthritis-associated factors

Table 2 summarized several lncRNAs that targeted the arthritis-associated factors. These representative lncRNAs were selected to illustrate the diverse signaling pathways and mechanisms of lncRNAs in the regulation of arthritis-associated factors (**Table 2**, **Figure 1**).

MMPs

Matrix metalloproteinases (MMPs) are well-known factors responsible for cartilage degradation. Hox transcript antisense intergenic RNA (HOTAIR) was upregulated in knee OA cartilage as well as the synovial fluid of temporomandibular joint (TMJ) OA cartilage, according to microarray analysis [6]. IL-1β treatment of TMJ condylar cartilage enhanced the expression of MMP-1, MMP-3, and MMP-9 dramatically, whereas the effects were reversed by HOTAIR knockdown [42], indicating that HOTAIR functioned as a regulator of MMPs.

HoxA distal transcript antisense RNA (HOTTIP), locating in 5' end of the HoxA cluster, encodes the lncRNA which could suppress HoxA-13 [43]. In OA chondrocytes, HOTTIP was upregulated significantly, while HoxA-13 was downregulated. In addition, it is reported that HoxA-13 could regulate integrin-α1 positively [7]. Overexpression of integrin-α1 subunit could promote chondrogenesis, whereas integrin-α1 knockdown could increase MMP-2 synthesis and contribute to cartilage degradation at younger mice [44]. Therefore, HOTTIP may serve as the promotor of cartilage degradation via inhibiting the HoxA-13/integrin-α1 signaling pathway. It is shown that miR-204 suppressed HOTTIP expression in hepatocellular carcinoma [45], and it arouses the interest whether miR-204 targets HOTTIP and negatively regulates HOTTIP in cartilage, which remains further studied.

It is reported that miR-21 interacted with MMPs by indirect targeting. In laryngeal squamous cell carcinoma, miR-21 was relevant to cell migration and tumorigenicity via regulation of MMP-2 expression [46]. The expression levels of miR-21 in cerebral ischemia and renal fibrosis were associated with the regulation of MMP-9 [47, 48]. RECK and TIMP3, the major

inhibitors of MMPs, have been known as targets of miR-21 in glioma cells [49]. The downregulation of miR-21 increased the expression level of MMP-13 significantly in OA chondrocytes [30]. The overexpression of GAS5 in chondrocytes in vitro led to the increment of MMPs, and GAS5 acted as a negative regulator of miR-21, indicating that GAS might serve as a sponge of miR-21 to regulate cartilage degradation. However, the specific inter-regulatory network between miR-21 and GAS5 remains to be further elucidated.

Cartilage Injury Related lncRNA (lncRNA-CIR), a vimentin pseudogene, was upregulated in OA cartilage and could induce degradation of cartilage extracellular matrix in vitro. It is reported that knockdown of lncRNA-CIR increased expression of cartilage associated genes (collagens I/II and aggrecan), while its overexpression caused the increment of MMP-13. TNF-α and IL-1, two critical mediators of OA, could stimulate the expression of lncRNA-CIR [21]. Overall, lncRNA-CIR plays a key role in the pathogenesis of OA, but the precise molecular mechanisms need to be deciphered.

OPN

Secreted phosphoprotein 1 (SPP1) deficient mice were apt to developing OA [50]. SPP1 encoded osteopontin (OPN). OPN is a well-characterized regulator of cartilage mineralization [51]. It is found that OPN was distributed at pericelluar sites in cartilage [52], while OPN was upregulated in osteoarthritic cartilage [53] and promoted pathologic mineralization [54]. SPP1 resided at upstream of HSP90AA4P and served as the cis-regulated target of HSP90AA4P. It is reported that HSP90AA4P was downregulated in OA cartilage [22]. These evidences lead to speculation that HSP90AA4P might function as a protector of cartilage by SPP1/OPN pathway.

COX-2

Cyclooxygenase 2 (COX-2) plays a crucial role in regulating the arachidonic acid pathway and prostaglandin E2 production [55], which is presumed to stimulate inflammation and pain in OA cartilage [56, 57]. The expression of COX-2 was significantly lower in late OA than that in early OA [58], indicating it may play different roles in different stages of OA. It is shown that inhibitors of COX-2 delayed the resolution of inflammation [59]. LncRNA p50-associated COX-2 extragenic RNA (PACER) is positioned adjacent to COX-2 and is reported to positively regulate COX-2 production [60]. It has been shown that PACER was induced in OA chondrocytes by various proinflammatory cytokines [61], indicating that PACER was a key regulator in the inflammatory response of joint cartilage. However, lncRNA expression of chondrocytes responding to proinflammatory stimuli was rapid and transient. In knee and hip OA cartilage, PACER was reported to be downregulated [61], suggesting a pathologic reduction in the ability of the cartilage tissue to resolve aberrant inflammation.

Col II

The lncRNA H19 expressed abundantly in embryonic tissue of endodermal and mesodermal origin and diverse tumors [62, 63]. H19 generated miR-675-5p and miR-675-3p, whereas miR-675-5p suppressed the expression of H19 [64], which made a self-regulatory feedback. It is shown that inhibition of H19 downregulated COL2a1, and overexpression of H19 upregulated COL2a1, while overexpression of miR-675 could rescue COL2a1 in H19-depleted chondrocytes [65], indicating H19 regulated COL2a1 which was mediated by miR-675. However, less is known about the direct target of miR-675 for repressing COL2a1.

The expression of H19 and miR-675 were upregulated under anabolic conditions and downregulated under catabolic conditions [20, 65], suggesting that H19 and/or miR-675 might be of diagnostic value as metabolic indicators of OA. However, H19 and miR-675 were expressed higher in cartilage tissue of knee OA according to cDNA array analysis [20]. It raises a question of whether the upregulation of H19 and/or miR-675 in OA chondrocytes functions as a compensatory effort in extracellular matrix synthesis and matrix destruction antagonism during OA development. Interestingly, the variation of miR-675 regulation was more than fourfold below that of H19, inferring that only a fraction of H19 was degraded to provide miR-675.

LncRNAs regulate angiogenesis

Cartilage is normally avascular, and the invasion of blood vessels is an essential step in

Table 3. Selected lncRNAs upregulated or downregulated in the OA cartilage

Upregulated in OA cartilage			Downregulated in OA cartilage	
H19	GAS5	BX096395	MEG3	UFC1
HOTAIR	HOTTIP	BI015463	PACER	AK289744
RP11-445H22.4	CTD2574D22.4	uc.277	HSP90AA4P	AL137603
PMS2L2	PCGEM1	RP11-195E11.3	RP11-396J17.1	AP003175.1
lncRNA-CIR	uc.343	BC044611	BQ045000	RP11-102H24.1
AK054860	AL359062	AL832767	RP11-46402.5	

ossifications. OA is a disease closely associated with angiogenesis. Many studies highlighted the importance of angiogenesis in OA as well as its contribution to progressive joint damage [66]. Vascular endothelial growth factor (VEGF) is a crucial mediator of angiogenesis. It has been shown that VEGF could regulate hypertrophic cartilage remodeling and vascular invasion into growth plate cartilage [67], and the vasculature offers a conduit to recruit cells that involved in cartilage resorption and bone deposition [68]. Therefore, the inhibition of angiogenesis presents a novel therapeutic approach to reduce inflammation and pain in OA.

Maternally expressed gene 3 (MEG3) is a maternally expressed lncRNA and a tumor suppressor gene [69]. It is suggested that MEG3 may inhibit tumor progression through inhibiting angiogenesis [70]. Recently, a study reported that the expression of lncRNA MEG3 was decreased in OA and that its expression levels were reversely associated with VEGF levels [71]. It has been indicated that MEG3 stimulated p53-mediated transcriptional activation [72, 73]. P53 could reversely regulate VEGFA transcription by binding to the transcription factor Sp1 sites on the VEGFA promoter [74]. These results above indicate that MEG3 inhibited angiogenesis by means of p53 pathways (**Figure 1**). However, the detailed mechanisms by which MEG3 inhibits angiogenesis remain to be elucidated.

Summary

LncRNA regulates the OA progression through sophisticated and multi-layered influences on the balance between the biosynthetic phase and the degradative phase (**Figure 1**). In this review, we summarized the roles of lncRNAs played in the survival of chondrocytes and synoviocytes, arthritis-associated factors, and angiogenesis. These evidences points out lncRNAs as new regulators in OA, which are likely to be the diagnostic, therapeutic, and prognostic biomarkers.

However, enormous challenges need to be overcome before the clinical use. Most studies presented the differences of expression of lncRNAs between OA cartilage and normal cartilage [6], and several lncRNAs were tested and verified by Polymerase chain reaction (PCR) (summarized in **Table 3**), while there were a tiny fraction of evidence confirming the mechanisms. It is highlighted that one lncRNA has multiple potential targets, which might coordinate or antagonize each other's functions. The crosstalk between lncRNAs and targets might depend on the tissue source and the stage of OA process, which increases the difficulties in predicting the prognosis and the side effects of lncRNA-based therapies. In spite of these difficulties, the development of lncRNA-based diagnosis, therapy, and prognosis, after being validated the efficacy and safety in animals, will be seen in next few years, and could be applied to OA clinically and other conditions associated with chronic inflammation.

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Disclosure of conflict of interest

None.

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