Pharmacological Reports 71 (2019) 399–408

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Review article

# Discoidin domain receptor 2: An emerging pharmacological drug target for prospective therapy against osteoarthritis

Amresh Kumar[a], M. Dutta Choudhury[a,**], Parasar Ghosh[b], Partha Palit[c,*]

[a] Department of Life Science and Bioinformatics, Biotech Hub, Assam University, Silchar, Assam, India
[b] Department of Rheumatology, Institute of Post Graduate Medical Education and Research, Kolkata, India
[c] Department of Pharmaceutical Sciences, Drug Discovery Research Laboratorty, Assam University, Silchar, Assam, India

ARTICLE INFO

*Article history:*
Received 5 July 2018
Received in revised form 27 November 2018
Accepted 14 January 2019
Available online 15 January 2019

*Keywords:*
DDR2
RNAi
Osteoarthritis
TGF-β
Metalloproteinases

ABSTRACT

Discoidin domain receptor2 (DDR2), a cell membrane tyrosine kinase on chondrocytes surface plays main role in cell-ECM interaction during the progressive degeneration of articular cartilage in osteoarthritis. The degraded component of ECM, type II collagen upon DDR2 binding provokes synthesis of matrix metalloproteinases (MMPs), responsible for severe destruction of joint tissues. DDR2 knockout has been investigated to decline the expression of MMP-1 and 13. Previously, various molecules were effective in preclinical level against different targets in OA, but found to be collapsed in clinical trial due to insufficient target specificity and clinical toxicity. Review emphasizes the role of DDR2 in the degeneration of cartilage in osteoarthritis (OA) and its blocking by DDR2 antagonist attenuates the disease severity. DDR2 in chondrocytes contributes paramount role in degradation of cartilage at early stage of osteoarthritis *via* collagen 2 binding through the felicitation of TGF-β signaling molecule and other triggering factors. DDR2 involvement in regulation of matrix metalloproteinase (MMP), cross talking interaction in maintenance of ECM-chondrocytes, bone developments, interference RNA and designing the DDR2 antagonists have been critically investigated. The exploration may conclude that the DDR2 could be the novel pharmacological target to prevent the progression of osteoarthritis at early stage because of over expression of DDR2 and MMP which further promotes severe cartilage degeneration. Owing to pharmacological specificity of DDR2 in OA as drug target, it is to be hypothesized that development of safe molecules as DDR2 antagonist could be the good option in the treatment of OA with promising landmark.



**Contents**



* Principal corresponding author
** Joint corresponding author
*E-mail addresses:* drmdc@bioinfo.au.ac.in (M. Dutta Choudhury), itspartha_p@yahoo.com (P. Palit).

https://doi.org/10.1016/j.pharep.2019.01.007
1734-1140/

## Introduction

The Discoidin domain receptor was discovered by homology cloning based on catalytic kinase domain and considered as orphan receptor until 1997 when two independent groups discovered that several different collagens were ligand for DDR receptors [1,2]. A cell surface receptor tyrosine kinase, discoidin domain receptor 2 (DDR2) plays a key role in communication around the circumventing environments of extracellular matrix (ECM). The signaling role of DDR2 in the developments of bone and cartilage degradation due to deposition of ECM components has suggested the paramount contribution in patho-physiological scenario in cartilage [3]. The mechanical injuries, degradation of cartilage, inflammatory disorders and age-related changes cause accumulation of collagen at the injured site of joints. At the early stage of osteoarthritis, the expression level of DDR2 is elevated in chondrocytes because of collagen type II protein accumulation and proximity upon the degradation of cartilage. This leads to release of matrix metalloproteases (MMPs). The level of MMP-1 in human fibrosarcoma cell line HT1080 has found to be overexpressed through DDR2 activation, suggesting of regulatory role in cartilage degeneration [3]. The incremented expression of DDR2 enhances the binding of receptor to collagen type II, which in turn switch on the expression of MMP-13, causing a severe damage to extracellular matrix of cartilage, as well [4,5]. Therefore, DDR2 receptor in chondrocyte is sensed by the microenvironments created due to mechanical stress or biochemical insults. Collagen type II in cartilage could trigger more DDR2-dependent collagen signaling towards the pathological condition.

The DDR2 knockout mouse has been investigated to reduce expression of MMP-13 and MMP-1 activity and found to be declined the degradation of articular cartilage significantly in some studies [6,69]. Further, these facts were fortified by the deletion of DDR2 receptor in mice model of osteoarthritis (OA) and found to be reduced the extent of degradation of articular cartilage in knee joints [6]. The articular cartilage shows the mechanical integrity due to extracellular matrix. The ECM is composed of collagen type II protein in major amount, which forms 3D network and provides cartilage to resistant against tensile forces along and external injury [7].

At first, cartilage damage in OA reflects the loss of proteoglycan and collagen network that increases the swelling of tissues. The incremented expression of DDR2 receptor has also co-related with the depletion of cartilage in surgically induced OA model [8]. OA, a degenerative inflammatory joints disease is the results of imbalanced molecular homeostasis between catabolism and anabolism of cartilage. Cartilage degradation at the joint by various MMPs has been reported in the earlier studies but the exact molecular mechanism behind the developments of OA is still unclear for which it is very difficult to identify the suitable and safe therapeutic targets that effectively treat and cure the OA.

On viewing such critical scenario, we have tried to explore the prospect, future and significance of DDR2 and its activity towards the cartilage degeneration and understanding of probable mechanism of DDR2 antagonism for novel therapeutic target against OA in this study. We also try to recognize how upstream and downstream signaling of DDR2 expression activity in response to suitable ligand could control the OA pathogenesis in the present review.

Since, anti-MMP therapy [9], anti-iNOS therapy [10], TNF-α and IL-1β inhibitor (Diacerhein) [11] *etc*. are some of the disease modifying anti-osteoarthritis drugs have been used. But their action based on the evidence from the clinical trial and scientific literature suggesting the therapeutic efficacy in OA is fragile. Moreover, the current drugs including diseases modifying anti-arthritic drugs used for the treatment of OA provide only symptomatic relief to patients from pains and inflammation, but this pharmacological intervention does not attenuate the articular cartilage degeneration mediated OA disease syndrome. Therefore, there is a priority to switch the effective target to DDR2, which could suspend OA specific multi-direction signaling pathway to circumvent the degradation of cartilage.

## Genomic location, classification and regulation of DDR2

The gene encoding DDR2, also known as CD167b is located on the chromosome number 1 (1q23.3) and composed of 19 exon part, out of which 4–19 are coding exon [12]. In the early 90 s, several researchers have identified the protein with unusual N-terminal discoidin-I like domain and C-terminal kinases domain by the cloning of a novel class of transcripts and determined domain with 45% identical to Neurotrophin receptor, TrkA. Later on, these transcripts were given a unique name such as DDR, Trk E, NEP, CAK, RTK-6, PTK-3, MCK-10, CCK-2, TKT and Tyro-10. Furthermore, these proteins have been reported to have unusual receptor tyrosine kinase (RTK) structure [12–20]. DDR, Trk E, NEP, CAK, RTK-6, PTK-3, and MCK-10 are further classified and renamed as DDR1 while Tyro-10, CCK2 and TKT as DDR2 receptor based on the N-terminal homology [1,2]. DDR1 and DDR2 lies between the intersection of big receptor family. DDR family members (Fig. 1) are generally expressed in inflammation, fibrosis, cancer *etc*., out of which DDR2 only regulate the OA disease progression. In cancer, both DDR1 and DDR2 are expressed. In inflammatory osteoarthritis status are initiated by the aberrant activation of DDR with extracellular matrix collagen 2 as ligand in response to cartilage degeneration due to mechanical injury and ageing process in adult. Since, DDR2 is specifically expressed in joint cartilage and chondrocytes isolated from temporomandibular joint of DDR1-null mice by the collagen 2 ligand binding [21]. In order to activate DDR2 in erratic way, collagen 2 is released from the joint cartilage which further confirms the OA disease progression followed by the MMP-13 hyper expression [1]. The site of DDR1 expression is epithelial cells and immune cells including mononuclear cells whereas for DDR2 expression, in the mesenchymal cells including osteophytes and chondrocytes. However, DDR2 was also reported to express in neutrophils [1,22]. In case of normal cells, both DDR1 and DDR2 are not expressed whereas DDR1 but not DDR2 seems to get expressed in well differentiated epithelial cancer cells only. During the OA, both aggrecan and collagen are degenerated by enzyme metalloproteinase belonging to ADAMTS and metalloproteinase released from chondrocytes [23,22]. This cascade has been related to site where DDR2 is expressed may show strong evidence link to the human disease OA displaying age-dependent cartilage degeneration and mechanical injury.

The expression of DDR2 receptor has regulated by numerous types of factors depending upon the cell type. During the osteogenic differentiation, the transcription factorATF4 combined with CCAAT/enhancer binding site in the DDR2 promoter, which is responsible for induction of DDR2 transcription [24]. In case of hepatic cells, liver injury has been reported to be acted as a factor for the up-regulation of DDR2 mRNA expression [25]. As above said DDR2, a microenvironment sensor is tyrosine kinase receptor,

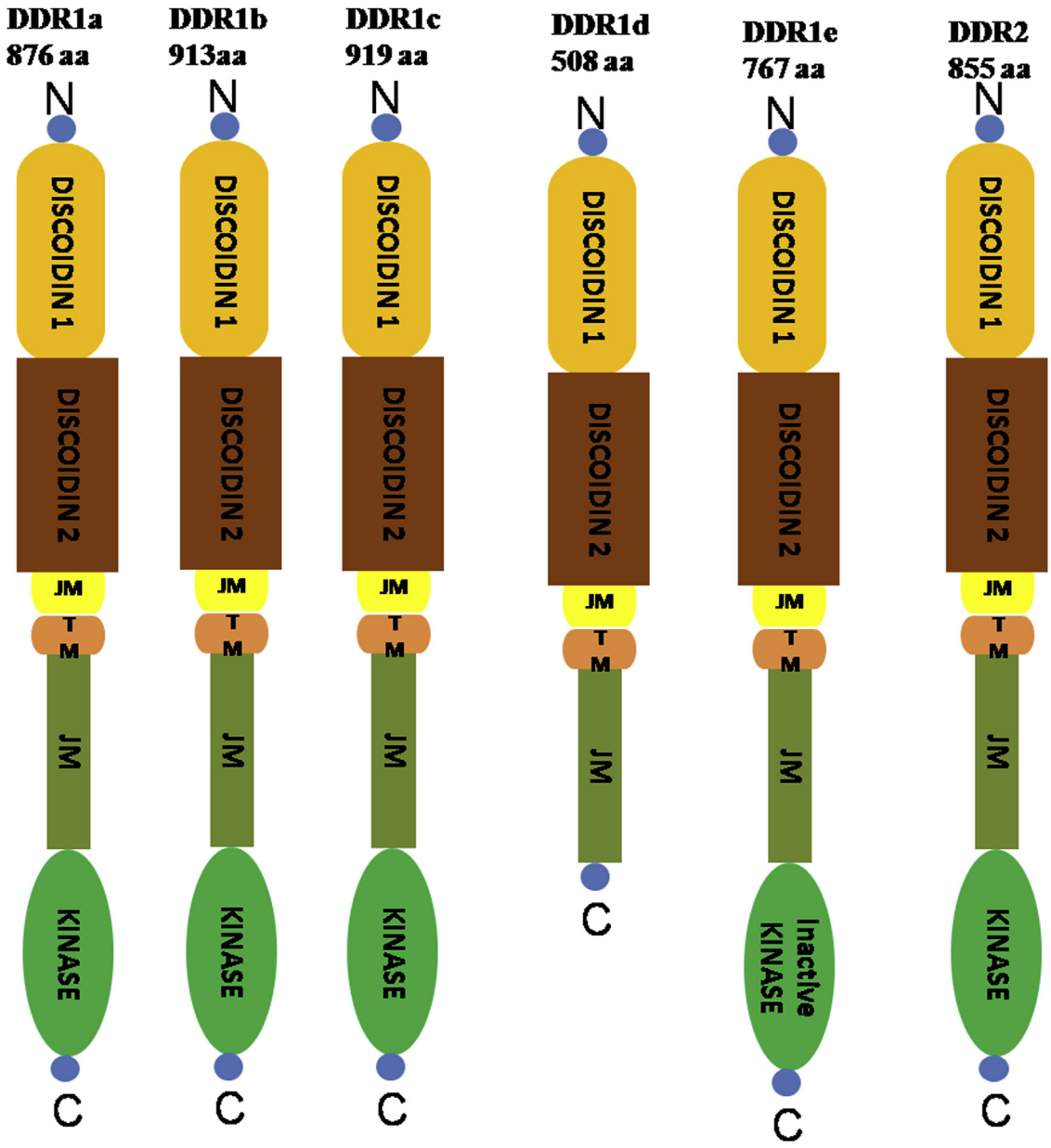


**Fig. 1.** Domain structure of DDR2 family.

which can be regulated by various factors depending upon the cell type, in which DDR2 is being expressed.

**How does DDR2 receptor activated to induce OA**

Alternation of joints by mechanical stress produces microcracks and deterioration of natural pores in subchondral bone provides channels for cross talk through diffusion of small molecules [26,27]. At early stage of OA, chondrocytes in the injured cartilage releases transforming growth factor beta (TGF-β) *via* SMAD 2/3 signaling network, which contributes in maintenance of quiescent phase of chondrocytes, aggrecan and collagen II. Yes, it is true that TGF-β is released from the damaged cartilage in the initial stage of OA. The chondrocytes in cartilage are the responsible for maintaining the integrity of tissue. The chondrocytes respond the damaged collagen network at injury site by incrementing the level of transforming growth factor-β (TGF-β), integrin and collagen synthesis. It may facilitate the attempt of being repaired towards the damaged cartilage. If it fails to repairs the tissue, damaged components such as collagen type II may integrate with DDR2 receptor in chondrocytes to activate it by binding of collagen 2 with the DDR2 active site and could secrete MMP-13, leading to severe cartilage damage (OA) in response to TGF-β and collagen networking signaling pathway in negative mode of role. In addition to protective role of smad 2/3 signaling, it may also promote TGF-β formation of chondrophytes and osteophytes [28]. Formation of osteophytes at the margin of cartilage is characteristic properties of OA. Collagen II ligand binding in DDR2 provokes the receptor activation that further induces negative impacts on ECM and chondrocytes. The up-regulation of DDR2 expression level in presence of collagen II on chondrocytes is the keys event in pathogenesis of OA as reported earlier [29]. Further studies again fortify that collagen II may exacerbate OA by enhancing the induction and increasing the level of MMP-13 following the aberrant activation and binding with DDR2, which degrade the collagen matrix and making a step for the positive feedback loop [30–32]. Early depletion of cartilage in OA causes the degradation of proteoglycan led to exposure of collagen II, which may further

interact with chondrocytes upon binding to DDR2 [33]. Thus, the extrinsic activation of DDR2 upon integration with collagen II may induce the overexpression of hypertrophic markers such as MMP-13 following the upregulation of TNF-α [30] and thus TGF-β acts an initiator of OA in the subchondral bone joints [34]. TGF-β1, may eventually lead to activation of DDR2 and consider their importance in the development of OA. Earlier sign in articular degeneration is high production of proteoglycan in mouse model of OA [35]. Over expression of TGF-β1level in human OA tissue is also outlined when compared to healthy articular cartilage [36], which also supports the involvement of TGF-β1 in early onset of OA. Those condensed progenitor cells inside the osteophytes are supposed to differentiates in to chondrocytes, which undergoes chondrogenesis to produces collagen II and Aggrecans. TGF-β induced serine proteases (HTRA1) disrupts the pericellular matrix may be one of the earliest events occur in the activation of chondrocyte cell surface receptor [37]. Therefore, TGF-β may indirectly involve in activation of DDR2 receptor in chondrocytes as it allows the aggregation of collagen II in pericellular matrix. At early stage of cartilage damage, the depletion of proteoglycans occurs resulting in exposure of collagen II with chondrocytes.

During the entire life of individual, articular chondrocyte as well as subchondral bone receives acute or chronic stress. Joints homeostasis is maintained by biomechanical stress, as rapid loss of proteoglycan in joints occurs due to immobilization or disuse. These osteophytes are fibrocartilage capped with bony out growth at margin of joints. The experiment on Murine OA model clarified that the osteophytes derived from MSC-periosteal lining cells at junction of bone-cartilage but not at synovial lining [38,39].

The activation of DDR2 receptor upon binding of type II collagen is dependent upon the structural motif present on the receptors. The DDR2 receptor preferentially binds to the collagen II but this interaction is also prevented by intact/uninjured proteoglycan network of pericellular matrix.

NMR was employed for the determination of un-ligated domain 1 structure of DDR2 receptor. The collagen-binding site on the same was identified by cross saturation experiment and mutagenesis [40]. Birgit leitinger identified the binding site of collagen with DDR2 receptor, which was three spatially adjacent surface loops within the DS domain of DDR2, contains the collagen binding sites that involves in DDR2 signaling [41]. The DDR2 receptor recognizes the specific amino acid sequence of collagen. Initially, the recombinant triple helical variants of collagen type II were used for mapping the DDR2 binding sites that mapped the specific DDR2 binding sites to the second quarter of its collagenous domains [42]. The activation of DDR2 receptor displays the collagen specificity as it binds with collagen type II [41] and type X [42] favorably. GVMGFO (O = hydroxyproline) motif in the triple helix peptide of the ligand was identified to enable the crystal structure determination of the complex of DDR2 discoidin 1 domain [43]. This is a polar structure with GVMGFO motif being accommodated in an amphipathic binding pocket.

During OA, the early cartilage damage causes the depletion of proteoglycan, lead to exposure of collagen type II protein, which further interact with chondrocytes *via* DDR2 [1,33]. Therefore, the cytoplasmic domain of DDR2 receptor is phosphorylated at tyrosine residue upon the binding of type II collagen, which commences the down-stream signaling pathway, inducing the expression of IL-6, IL-1β and MMP-13 mRNA in the deep layer of cartilage and resulting in cartilage destruction in joints of patients with osteoarthritis [44,45]. Besides these, mechanical injury followed by the excess cartilage overloading may destroy the integrity of chondrocyte and osteophyte cells, in turn may cause osteoarthritis in patients without known earlier bone joint abnormalities. The cartilage degeneration and subsequent cell death of chondrocyte cells is occurred in response to mechanical injury due to release of reactive oxygen species. It is most interesting thing that mechanical injury mediated induction of OA disease progression has been initiated by the DDR2 expression and augmented activity as reported by the earlier reports [21,46,47].

## Role of DDR2 receptor in the development of bone in human

The significance of DDR2 receptor in the skeleton growth of human being could not be ignored, which has revealed by analysis of rare genetic disease, chondrodysplasia termed as spondylo-meta-epiphyseal dysplasia (SMED-SL) with short limbs and abnormal calcifications. This Autosomal disease has been characterized by presence of short stature, short limbs, broad fingers, bone abnormalities and premature calcifications. These autosomal diseases caused by the occurrence of three missense mutations in DDR2 and one spliced site mutation were identified in eight patients from seven different consanguineous families [48]. The mutation caused in DDR2 receptor leading to SMED-SL was analyzed by carrying out experiment in the human cell line to realize the cellular and biochemical mechanism. The three-missense mutation in DDR2 resulting in trafficking defect making the DDR2 protein retained in endoplasmic reticulum whereas forth mutation resulted in DDR2, which is perfectly trafficked to the cell surface but failed to interact with collagen due to mutation in the key residue of collagen binding sites [41]. Thus, the Autosomal disease SMED-SL is caused by at least two mutation leading to loss of function of DDR2 receptor that lack the ability to interact with collagen protein, resulting it in to severe skeleton abnormalities. This fact supports the essential contribution of DDR2 in human bone growth and developments. A current finding of DDR2 receptor contribution in the development of Murine Temporomandibular Joints (MTJ) has revealed by analyzing the primary culture of TMJ articular chondrocytes from wild type and DDR2slie/slie mice that showed abnormalities in chondrocytes maturity and mineralization in the absence of DDR2 [49]. This study also signifies the DDR2 is necessary for the normal development of TMJ condyle and maintain homeostasis of the extracellular matrix of joins. At the early stage of human developments, DDR2 receptor do contribute in bone growth and mineralization but mutation in this gene causes rare Autosomal diseases (SMED-SL) in a few cases where irregular mineralization is reported. It may be due to irregular/lacks of regulatory mechanism involved in aberrant DDR2 expression and at late stage of life individual are prone to OA like condition. The mechanical stress, surgical damage, *etc.* at joint tissue causes loss of extracellular matrix. The collagen that further binds DDR2 receptor in chondrocytes causes severe damage to cartilage by metalloproteinase's but the intact pericellular matrix in turn protect DDR2 receptor being activated. Although DDR2 involve in bone development at early stage of life as per scientific report but due to changes in the pathophysiological condition of joints where DDR2 level gets incremented. Therefore, DDR2 seems to be dual mode of expression profile depending upon physiological condition and stages of life. It is also responsible for high expression at early stage of OA developments.

## DDR2 receptor and MMP-13 expression

Type II collagen protein in articular cartilage is highly stable structure as its half-life is 117 years [43]. The chondrocytes have limited ability to synthesis type II collagen protein in the matrix of mature articular cartilage once the collagen gets disrupted. Collagenase-3 (MMP13) plays important role in the progression of OA as it has ability to cleave triple helix structure of collagen type II protein efficiently than that of MMP-1 [42].

Altered mechanical damage, aging, obesity, *etc*. in OA are driving force toward degradation of pericellular matrix of chondrocytes. It enhances the exposure of chondrocytes towards type 2 collagen in matrix. The interaction of chondrocyte with Type 2 collagen results in upregulated expression of DDR2 receptor and MMP-13, subsequently degradation of type II collagen and aggrecan occur [42,50]. Deposited fragments of aggrecan and type II collagen may further increase the synthesis of MMP-13 upon integration with DDR2 receptor [30] or through binding to another receptor [51]. DDR2 receptor in chondrocytes may enhance its activation with TGF-β being up-regulated in destructed ECM. In turn it may acts as positive feedback loop for expression of MMP-13 causing severe damage to cartilage through IL-1β and TNF-α induction. In report mention on heterozygote DDR2 null mice revealed the partial protection against the experimental OA mice model and also facilitated evidence of diminished MMP13 expression level [6], fortifying that DDR2-MMP13 loop involve in onset of OA. The attenuation of DDR2 receptor activation in chondrocytes by the antagonist may block the both loop of DDR2 activation *via* TGF-β and type II collagen, leading to down-regulation of MMP13 through IL-1β and TNF-α. Therefore, we may conclude that blockage of DDR2 receptor through ligand antagonism could play a protective role in chondrocytes formation through inhibiting the destruction of cartilage by MMP-13 by suppressing the TNF-α, IL-1β.

MMP, an effector and indicator molecule in pathological state that provides cartilage health under both physiological circumstances and osteoarthritic state, which have already been studied earlier [52]. Collagen type II molecules is released upon joint injury that could act on DDR2 receptor through RAS/RAF/MEK/ERK and p38 signaling pathways that further activate MMP-13 to damage extracellular matrix [4,6]. Some studies have also been provided evidence that collagen-2 involves in expression of IL-1, IL-6, IL-8, IL-1β as well as MMP-1, 2, 13 *via* mitogen activated protein kinase p38 (MAPKp38) and Nuclear factor kappa B (NF-kβ) signaling pathway [53]. Based on the above findings, it may be concluded that RAS/RAF/MEK/ERK and p38 could be the intermediate molecules through which MMP-13 expression may be incremented upon the binding of collagen type II to DDR2 receptor in chondrocytes. This may provide important clue where MMP breakdown the extracellular cartilage matrix exposing chondrocytes to collagen type II, which induces the extra release of degradative enzyme, causing further degradation of cartilage. Since, the MMP-13 expression in human cartilage with osteoarthritis has been previously reported to be upregulated compared to that of normal [43,52,54,55]. A similar observation has also been reported in knee joint cartilage of mouse model showing the osteoarthritis like changes due to constitutive expression of MMP-13 [56]. In support of this, a recent study in mice deleted with MMP-13 enzyme demonstrated delay the progression of articular tissue [23]. However, the up regulation of DDR2 receptor implicates MMP-13 induction in chondrocyte [29,57]. The earlier report indicates that MMP-13 is major effectors in mechanically induced joint destruction in case of osteoarthritis. However, MMP-13 may be over expressed followed by their up regulation in case of other type of arthritis such as rheumatoid arthritis, but there is no chance of possible involvement of DDR2 activated IL-1β, IL-6 upregulation followed by the MMP-13 hyperexpression. However, few reports suggest the involvement of DDR2 receptor in fibroblast cells in rheumatoid arthritis but that is not sufficient evidences to establish the direct connection of DDR2 and way of its activation along with MMP13 expression are key regulator for initiation of RA disease progression. Moreover, RA is basically an autoimmune disorder where, pro-inflammatory cytokines like IL-1β and TNF-α are upregulated at initial stage followed by the activation of joint matrix metalloproteinase expression. Moreover, the activation of the DDR2 happens only in the downstream cascade of disease progressive signaling pathway at later-stage. So, therapy with DDR2 antagonist might not be fruitful in case of RA. Conversely, the important biomarker associated with degradation of articular cartilage at early phase of OA may comprises collagen ligand mediated DDR2 activation followed by a number of matrix-digesting enzymes such as metalloproteinase family, the disintegrin and metalloproteinases with thrombospondin type 1 motif (ADAMTS) family, MMP-9, MMP-13, aggrecanase *etc*. [58]. Out of which, attention may be focused on MMP-13 as it is significantly over-expressed in osteoarthritic joints and cartilage in the later stage following DDR2 receptor activation, which is hardly recognized in normal cartilage and RA disease initiation.

Other types of arthritis, MMP-13 may be activated by other signaling pathway by passing the DDR2 signaling. So DDR2 activation mediated MMP-13 expression could be the specific and crucial signaling route for development of OA disease specific progression.

## Signaling of DDR2 receptor and its regulation

Being the tyrosine kinase receptor, downstream signaling of DDR2 receptor is initiated by phosphorylation of cytoplasmic tyrosine residue upon ligand binding (Type II collagen) but the authentic facts or detail information of tyrosine phosphorylation upon the binding of collagen protein is not still clear to the researcher. A recent study on the phosphoproteomic showed that the collagen provoked DDR2 auto-phosphorylation were identified at two sites of the kinase domain (tyr684 and tyr813) in the DDR2 signaling pathway [59]. Additionally, an intriguing fact came in light about the site of JM domains (tyr481) that was found to be phosphorylated constitutively [60] but phosphorylation of tyr471, which was reported to be the docking site for adaptor shcA. Further Tyr471was not recognized utilizing anti-phosphotyrosine immune-precipitation and peptide identification following the protein digestion [60]. The expression of DDR2 receptor in various systems is different and implicates the involvements with several transcription factor/complexes. The ATF4-C/Ebb transcription factor is responsible for DDR2 expression in osteogenic differentiation system [24]. In case of rat VSM cells, hypoxia or hyperbaric oxygen responsible for the enhanced My-Max binding activity of DDR2 promoter region lead to the increased expression of DDR2 receptor [54]. However, the signaling and regulatory mechanism of DDR2 receptor is quite different depending upon the site of expression and types of molecules being sensed. In case of osteoarthritis, cartilage produces type II collagen in major amount which binds to DDR2 receptor provoking the activation of MMPs for degradation of cartilage and DDR2 itself being activated in this cascade. Therefore, either inactivation or proper antagonist binding into DDR2 active site in OA induced mice model may stop the extent of disintegration of cartilage and the severity of diseases.

## DDR2 receptor in cross talking mechanism at joints

Cross talking pathway provides us a better understanding of the chondrocytes behavior as chondrocytes expressed several receptors that get activated by components of an extracellular matrix upon damage in articular cartilage. DDR2 receptor in OA chondrocytes has been highly expressed representing the key events in the progression of OA [29]. The binding of type II collagen protein of cartilage preferentially induces DDR2 expression in chondrocytes but this interaction is also prevented by intact/uninjured proteoglycan of extracellular matrix (ECM). The cross taking pathway in intact ECM supports receptor to enable the cell polarity, differentiation and survival which are important for the

normal cell functioning. The ECM of articular cartilage is disrupted in OA resulting in the production of collagen type II protein, aggrecan *etc*. which allows it to interact with the DDR2 receptor or any other unknown receptor. DDR2 receptor in chondrocytes further induces the expression of MMP-13 *via* up-regulation of TNF-α and IL-1β that contributes to the degeneration of articular cartilage pre-dominantly [4,33] as depicted in Figs. 2 and 3. This is considered to be one of the key mechanisms in chondrocytes at the early stage of progression of osteoarthritis. Other findings again have strengthened the contribution of DDR2 receptor activation in the progression of OA as DDR2 appears to mediate collagen type II dependent release of catabolic cytokine IL-6 in primary human chondrocytes [4,24]. In integration to it, *in vitro* studies have demonstrated that the DDR2 receptor activation is responsible for osteoblasts differentiation and chondrocytes maturation *via* RUNX2 (Runt-related transcription factor-2) activation pathway [61]. This all information fortifies the paramount role of chondrocytes extracellular matrix in maintaining the cartilage integrity and highlights the conception of early structural alteration of ECM by the involution of DDR2 receptor in persuading the mechanism of OA progression.

## The contribution of interference RNA and epigenetic in OA attenuation

Interference RNA, a cellular mechanism for post-transcriptional gene silencing that is associated with mRNA. The potential of RNAi has successfully silenced the wide range of protein coding genes. Its high specificity sanctions the attenuation of diseases cognate alleles. Gene silencing performed by RNAi against DDR2 receptor demonstrated the reduction in expression level of IL-6 in chondrocytes, which inferred the DDR2 receptor contribute the significant role in collagen mediated release of IL-6 [53].

Recent studies corroborated the regulation of gene expression at post-transcription level through microRNA mediated RNA interference is the novel mechanism. The expression of microRNA profile in human OA chondrocytes has demonstrated to be correlated with expression of MMP-13 with specific microRNA [62–64]. In the previous studies it has been established that DDR2 mediated MMP-13 expression in chondrocytes in OA is carried out. Another study showed the overexpression of mir-27b suppresses the activity of reporter construct with 3′-UTR of human MMP-13 mRNA leading to the inhibition of IL-1β mediated MMP-13 expression in chondrocytes [65]. Collagen II mediated DDR2 activation can show the variable behavior depending upon the cell type. siRNA mediated DDR2 silencing in lung micro fibroblast cell showed prominent reduction in the DDR2 expression followed by the blockage of ECM fragments accumulation such as fibronectin and collagen [22]. Therefore, information derived from the work done on the role of RNAi, epigenetic factor, siRNA, *etc*. have strongly suggested that inhibition or suppression of DDR2 activation in chondrocytes or fibroblast cultures *in vitro* diminishes the expression level of MMP-13, IL-1β and IL-6 mediated MMP-13. This transpired due to blockage of phosphorylation and the expression of DDR2, which completely check the down regulatory signaling pathway pertaining to degradation of cartilage tissues. Advantage associated with therapeutic application of RNAi, siRNA against DDR2 receptor in cartilage may provide efficacy and specificity of the treatment and reducing the toxicity simultaneously. A few researches in the context of DDR2 silencing using RNAi therapy has been carried out to establish the role of DDR2 in improvement in OA cartilage. However, work on siRNA mediated DDR2 silencing in lung micro fibroblast cell showed prominent reduction in the DDR2 expression followed by the blockage of ECM fragments accumulation such as fibronectin and collagen [22], which fortify the potential role in blocking the damage of ECM. In

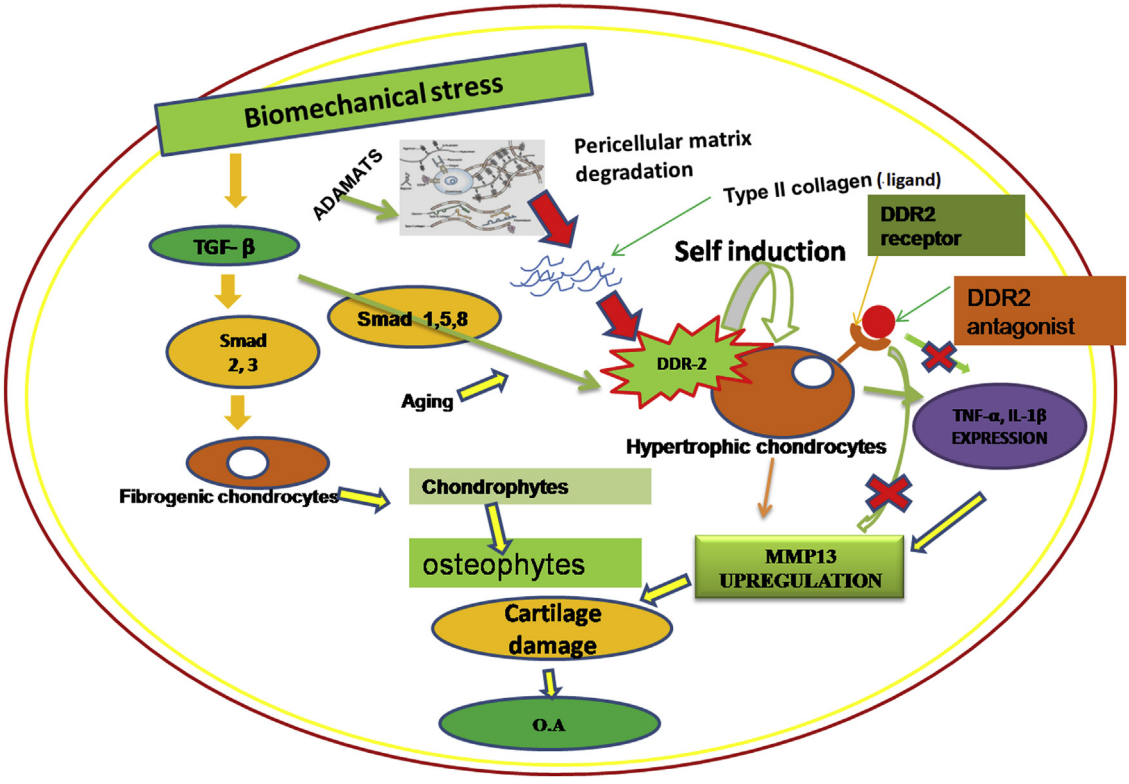


**Fig. 2.** Overview of cross talking pathway of osteoarthritis disease progression and its probable management by DDR2 antagonist.

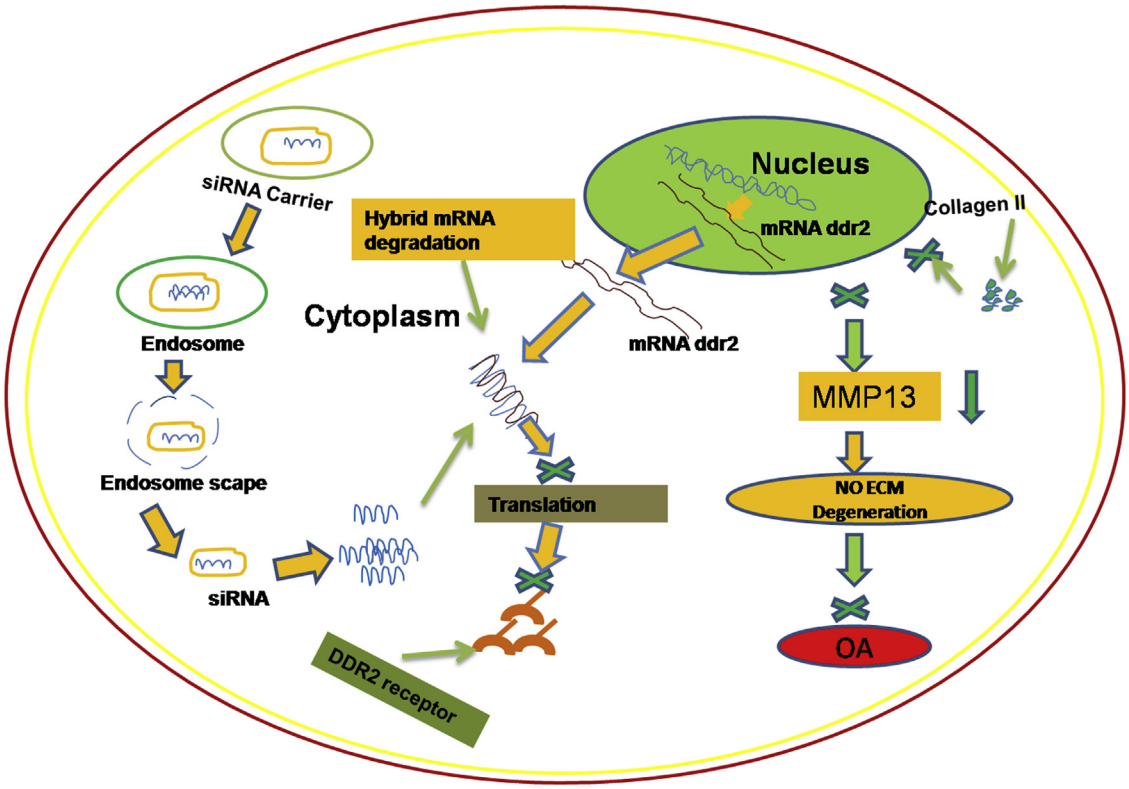


**Fig. 3.** Role of siRNA in silencing of DDR2 gene in attenuation of OA.

order to strengthen the RNAi role in suppression of DDR2 expression, much more investigative research in human OA cartilage need to be executed to verify the robustness of RNAi technology in OA treatments. Therefore, DDR2 receptor targeting via interference RNA technology or pharmacological development of diseases modifying drugs could be the prospective treatment option. It may bring possible remedy against Osteoarthritis.

## DDR2 receptor antagonism and OA

DDR2 is a special kind of receptor that distinguished from other RTKs by discoid (DS) domain structurally of 162 amino-acid long motif in extracellular part. The reduction in the level of DDR2 expression in mouse model is concerned with the delayed progression of condylar cartilage degeneration, induced with either type XI collagen or partially discectomy [66]. Actinomycin D, an antibiotic has been used clinically for the treatment of cancer. A new biological function of Actinomycin D has been reported to be intervened the activation of DDR2 expression in the insect cell-based method [67]. Other small DDR antagonists have also been reported but it is not concrete to DDR2. Moreover, increased expression of the collagen receptor discoid in domain receptor 2 in articular cartilage has a prime important and regulatory event in the pathogenesis of OA [4,6,29]. Therefore, DDR2 antagonist could be the new insight into the drug discovery and development of diseases modifying drug against the OA in the upcoming future. DDR2 expression may be the primary stage cascade for the initiation of OA disease. Consequently, designing, development of suitable and concrete DDR2 antagonist molecule could be the impressive therapeutic approach towards the curing of OA in near future. If some lead macro or micro molecules would be designed specifically against DDR2 as antagonist, then they could prevent the collagen 2 proximity and subsequent binding towards DDR2 for initiating the OA diseases explained in the Fig. 4.

## Prospect of DDR2 antagonist

DDR2, a receptor of tyrosine kinase has been found now to be reported to play a significant role in onset of OA at the early stage of diseases progression. The DDR2 are the receptor for extracellular collagen and activated upon the binding of collagen resulting in complex signaling networks expounded by above mentioned findings. An *in silico* molecular docking study of various natural and synthetic molecules against the DDR2 receptor is one of the ways to design and develop the DDR2 antagonist as anti-OA drug through lead optimization followed by the assaying of those compounds through the receptor marker in chondrocyte and osteophyte cell culture and *in vivo* assay to validate its efficacy. We are endeavoring to develop potent and selective inhibitor against DDR2, which further could be implicated pharmacologically to demonstrate the inhibitory impact on kinase activity of DDR2. Others have reported some DDR2 inhibitors [68] but the validation neither of these compounds at cellular model level nor the degree of selectivity for DDR2 has explored. The inhibition of DDR2 receptor expression with the antagonist in the patients with OA could completely block the down streaming signaling network, provoking the chondrocytes not to release MMP-13, IL-6, TNF-α, *etc.* in ECM and may check the cartilage degradation initiated by the OA [69]. However anti- MMP-13, IL-6 and TNF-α agents could manage the OA at the last stage of disease pathway, but they could not heal the OA specifically, as MMP-13, IL-6 and TNF-α up-regulation may occur in several inflammatory disorders including rheumatoid arthritis, psoriasis and other auto-immune disorders. Designing of selective DDR2 antagonist which may strongly bind with the active site of tyrosine kinase receptor of DDR2 may inhibit the adhesion of collagen 2 ligand with the receptor in the osteophytes cell surface. This phenomenon may lead to arrest the further progression of OA disease markedly and ameliorate the disease successfully.

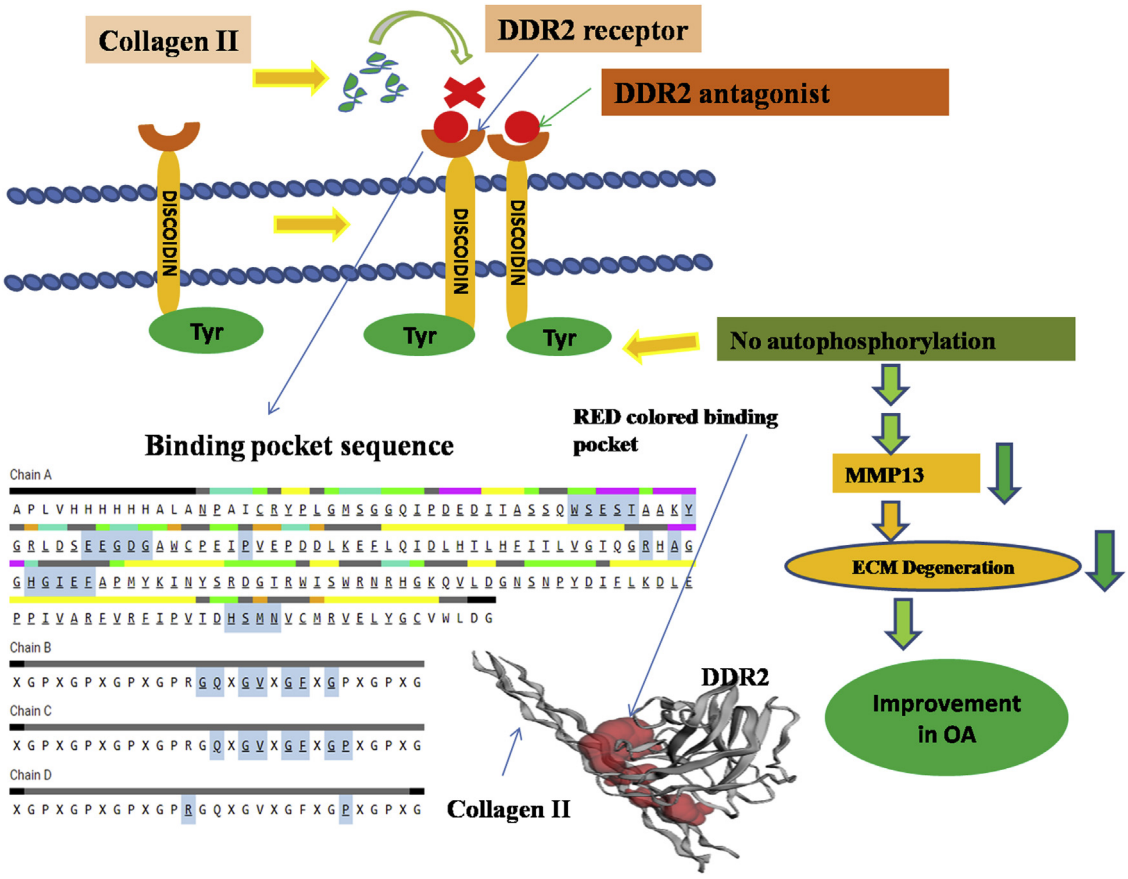


**Fig. 4.** DDR2 receptor activation and possible antagonism by the ligand binding followed by the downstream signaling pathway in order to understand the attenuation of OA pathogenicity. DDR2 active site sequence and structure model conjugated with collagen 2 agonist ligand binding has been presented for better understanding disease progression.

## Discussion

OA, one of the disease conditions for which treatment available is symptomatic relieve from pains but unable to control the diseases progression and permanent cure. Since, osteoarthritis progression cannot be halted if we don't prevent the early events of onset of OA and specific triggering factors responsible for disease initiation. With above-mentioned review of the studies suggest that DDR2 receptor could be the promising drug target [69] against the OA for noteworthy therapeutic management. The increment of expression level of MMP-13, a key regulator of matrix destruction associated with the early pathogenesis of OA is up regulated byDDR2 receptor activation in chondrocytes in mouse model and human patients' sample [24]. The over-expression of DDR2 in many models of OA is one of the key factors responsible for early stage of osteoarthritis progression because of the decrease in the expression of DDR2 receptor in chondrocytes of mouse model attenuates the cartilage degeneration [24].

Current challenge is to use our understanding for early mechanism of OA, which regulating the chondrocytes response to develop emerging treatment strategies. Extensive research on DDR2 response to ECM environments in OA is required so the effective strategy for new therapies could be developed for eradicating the disease severity. Some intermediate molecule in signaling network of DDR2 and MMP13 could be identified to understand the down-regulatory mechanism of degradation of cartilage but up-streaming factors of DDR2 expression needs to be investigated properly to design potential drug against OA. What are the prime and specific biochemical causative agents for OA disease progression through DDR2 hyper-expression mediated activation that needs to be re-investigated for developing potential drug molecules against OA?

## Conclusion

The involvement of DDR2 receptor activation in pathogenesis of OA seems to be active players as it contributes in severe destruction of cartilage *via* metalloproteinase expression. The information presented in this review illustrates that the DDR2 activation may involve in the OA at the early stage. Identification of molecules as antagonist to DDR2 receptor could be the promising therapeutic targets against the OA through lead optimization. It may intervene the down regulatory pathway, in which metal-loproteinases are involved in being activated. As downstream signaling mediators such as MMP expression, IL-6, IL-12, IL-1β and TNF-α up-regulation are the common features of all auto-immune disorders including rheumatic arthritis, celiac disease, diabetes mellitus type 1, Graves' disease, inflammatory bowel disease, multiple sclerosis, psoriasis etc. But DDR2 expression is not actively related with those auto-immune disorders significantly at preliminary stages. However, DDR2-agonistic action mediated MMP expression, IL-6, IL-12, IL-1β, TNF-α up-regulation through TGF-β and collagen signaling are solely related with the onset progression of OA disease. Therefore, designing of DDR2 receptor antagonist for blocking the collagen type 2 ligand protein binding through TGF-β signaling rather than the developing inhibitor of MMP-13, MMP-1, MMP-9, IL-6, IL-12, IL-1β and TNF-α could be the rational, effective, specific and safe drug discovery approach

towards the OA treatment and possible cure. Research in the epigenetic and RNAi analysis against DDR2 in OA is at plinth but the available information may suggest the various mechanisms involve in pathophysiology of OA. Pharmacological inhibitors against the DDR2 receptor need to be identified through *in silico* modeling and subsequent preclinical and clinical studies for the OA patients, which could have highest therapeutic value with lowest side effects for anti-osteoarthritis blockbuster drug development. Although still extensive research in respect to DDR2 association in osteoarthritis needs to be undertaken in order to reach in the successful development of treatment regimen for the permanent cure of this devastating disease. Apart from *in vitro* screening of DDR2 antagonist molecule, *in vivo* experiment should be carried out in near future to develop an established and potential disease modifying anti-osteoarthritis drug molecule. In order to establish such model, the expression level of DDR2 from fibroblast cells in chondrocytes should be determined in the mechanical injury/oxalo-acetate induced osteoarthritis mice model. The DDR2 expression level of must be measured and compared with the uninjured/oxalo-acetate untreated mice to validate the functional role of DDR2 in the osteoarthritis initiation. This experimental model could play a major role for successful *in vivo* screening of the anti-osteoarthritis drug through DDR2 signaling in near future.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgment

This work was supported by DBT JRF fellowship ref. no. DBT/JRF/BET-16/I/2016/AL/90-466 and F.30-136/2015(BSR).

## References

[1] Vogel W, Gish GD, Alves F, Pawson T. The discoidin domain receptor tyrosine kinases are activated by collagen. Mol Cell 1997;1:13–23.
[2] Shrivastava A, Radziejewski C, Campbell E, Kovac L, McGlynn M, Ryan TE, et al. An orphan receptor tyrosine kinase family whose members serve as nonintegrin collagen receptors. Mol Cell 1997;1:25–34.
[3] Labrador JP, Aconitia V, Tuckerman J, Lin C, Oleos E, Manes S, et al. The collagen receptor ddr2 regulates proliferation and its elimination leads to dwarfism. EMBO Rep 2001;2:446–52.
[4] Xu L, Peng H, Wu D, Hu K, Goldring MB, Olsen BR, et al. Activation of the discoidin domain receptor 2 induces expression of matrix metalloproteinase 13 associated with osteoarthritis in mice. J Biol Chem 2005;280:548–55.
[5] Us J, Yu J, Ren T, Zhang W, Zhang Y, Liu X, et al. Discoidin domain receptor 2 is associated with the increased expression of matrix metalloproteinase-13 in synovial fibroblasts of rheumatoid arthritis. Mol Cell Biochem 2009;330(1–2):141–52.
[6] Xu L, Servais J, Polar I, Kim D, Lee PL, Chung K, et al. Attenuation of osteoarthritis progression by reduction of discoid in domain receptor 2 in mice. Arthritis Rheum 2010;62:2736–44.
[7] Gentile C, Concede R. Cartilage and bone extracellular matrix. Cur Pharm Des 2009;15(12):1334–48.
[8] Siam S, Irina K, Mare L, Hannes T, Marina A, Andres A, et al. Evaluation of correlation of articular cartilage staining for ddr2 and proteoglycans with histological tissue damage and the results of radiographic assessment in patients with early stages of knee osteoarthritis. Int J Clin Exp Pathol 2015;8:5658–65.
[9] Clutterbuck AL, Aspin KE, Harris P, Alloway D, Mubashir A. Targeting matrix metalloproteinases in inflammatory conditions. Curr Drug Targets 2009;10 (12):1245–54.
[10] Miyasaka N, Hirata Y. Nitric oxide and inflammatory arthritis's. Life Sci 1997;61 (21):2073–81.
[11] De Isla NG, Mainard D, Muller S, Stoltz JF. In vitro effects of diacerein on NO production by chondrocytes in response to proinflammatory mediators. Biomed Mater Eng 2008;18(1 Suppl):S99–S104.
[12] Kern T, Helmrich U, Gruninger A, Bohme B, Wolf G, Rübsamen-Waigmann H, et al. Structure, expression and chromosomal mapping of tkt from man and mouse: a new subclass of receptor tyrosine kinases with a factor viii-like domain. Oncogene 1993;8:3433–40.
[13] Johnson JD, Edman JC, Rutter WJ. A receptor tyrosine kinase found in breast carcinoma cells has an extracellular discoidin-like domain. Proc Natl Acad Sci U S A 1993;90:5677–81.
[14] Marco ED, Cutuli N, Guerra L, Cancedda R, Luca MD. Molecular cloning of trke, a novel trk-related putative tyrosine kinase receptor isolated from normal human keratinocytes and widely expressed by normal human tissues. J Biol Chem 1993;268:24290–6.
[15] Zerlin M, Julius MA, Goldfarb M. Nep A novel receptor-like tyrosine kinase expressed in proliferating neuroepithelia. Oncogene 1993;8:2731–9.
[16] Perez JL, Shen X, Finkernagel S, Sciorra L, Jenkins NA, Gilbert DJ. Identification and chromosomal mapping of a receptor tyrosine kinase with a putative phospholipid binding sequence in its ectodomain. Oncogene 1994;9:211–9.
[17] Perez JL, Jing SQ, Wong TW. Identification of two isoform of cak receptor kinase that are expressed in breast tumor cell line. Oncogene 1996;12:1469–77.
[18] Lai C, Lemke G. Structure and expression of the tyro 10 receptor tyrosine kinase. Oncogene 1994;9:877–83.
[19] Sanchez MP, Tapley P, Saini SS, He B, Pulido D, Barbacid M. Multiple tyrosine protein kinases in rat hippocampal neurons: isolation of ptk-3, a receptor expressed in proliferative zones of the developing brain. Proc Natl Acad Sci U S A 1994;91:1819–23.
[20] Alves F, Vogel W, Mossie K, Millauer B, Höfler H, Ullrich A. Distinct structural characteristics of discoidin subfamily receptor tyrosine kinases and complementary expression in human cancer. Oncogene 1995;10:609–18.
[21] Borza CM, Pozzi A. Discoidin domain receptors in disease. Matrix Biol 2014;34:185–92.
[22] Zhao H, Bian H, Bu X, Zhang S, Zhang P, Yu J, et al. Targeting of discoidin domain receptor 2 (DDR2) prevents myofibroblast activation and neovessel formation during pulmonary fibrosis. Mol Ther 2016;24:1734–44.
[23] Little CB, Braai A, Burkhardt D, Smith SM, Fusing AJ, Web Z, et al. Matrix metalloproteinase 13-deficient mice are resistant to osteoarthritic cartilage erosion but not chondrocyte hypertrophy or osteophyte development. Arthritis Rheum 2009;60:3723–33.
[24] Lin KL, Chou CH, Hsieh SC, Hwa SY, Lee MT, Wang FF. Transcriptional upregulation of ddr2 by atf4 facilitates osteoblastic differentiation through p38 mapk-mediated runx2 activation. J Bone Miner Res 2010;25:2489–503.
[25] Elvira O, Kazuo I, Francis JE, Lieming X, Li-Hsien W, Hsin CL, et al. Ddr2 receptor promotes mmp-2–mediated proliferation and invasion by hepatic stellate cells. J Clin Invest 2001;108:1369–78.
[26] Botter SM, van Osch GJ, Clockaerts S, Waarsing JH, Weinman's H, van Leeuwen JP. Osteoarthritis induction leads to early and temporal subchondral plate porosity in the tibial plateau of mice: an in vivo microfocal computed tomography study. Arthritis Rheum 2011;63:2690–9.
[27] Gu XI, Palacio-Mancheno PE, Leong DJ, Borisov YA, Williams E, Maldonado N, et al. High-resolution micro arthrography of hard and soft tissues in a murine model. Osteoarthr Cartil 2012;20:1011–9.
[28] Yang X, Chen L, Xu X, Li C, Huang C, Deng C. TGF-beta/Smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage. J Cell Biol 2001;153:35–46.
[29] Xu L, Peng H, Glasson S, Lee PL, Hu K, Ijiri K, et al. Increased expression of the collagen receptor discoidin domain receptor 2 in articular cartilage as a key event in the pathogenesis of osteoarthritis. Arthritis Rheum 2007;56:2663–73.
[30] Tchetina EV, Kobayashi M, Yasuda T, Meijers T, Pidoux I, Poole AR. Chondrocyte hypertrophy can be induced by a cryptic sequence of type II collagen and is accompanied by the induction of MMP-13 and collagenase activity: implications for development and arthritis. Matrix Biol 2007;26:247–58.
[31] Yasuda T, Tchetina E, Ohsawa K, Roughley PJ, Wu W, Mousa A, et al. Peptides of type II collagen can induce the cleavage of type II collagen and aggrecan in articular cartilage. Matrix Biol 2006;25:419–29.
[32] Jennings L, Wu L, King KB, Hämmerle H, Cs-Szabo G, Mollenhauer J. The effects of collagen fragments on the extracellular matrix metabolism of bovine and human chondrocytes. Connect Tissue Res 2001;42:71–86.
[33] Xu L, Polur I, Servais JM, Hsieh S, Lee PL, Goldring MB, et al. Intact pericellular matrix of articular cartilage is required for unactivated discoidin domain receptor 2 in the mouse model. Am J Pathol 2011;179:1338–46.
[34] Zhen G, Wen C, Jia X, Li Y, Crane JL, Mears SC, et al. Inhibition of TGF-β signaling in mesenchymal stem cells of subchondral bone attenuates osteoarthritis. Nat Med 2013;19(6):704–12.
[35] Xu L, Polur I, Lim C, Servais JM, Dobeck J, Li Y, et al. Early-onset osteoarthritis of mouse temporomandibular joint induced by partial discectomy. Osteoarthr Cartil 2009;17(7):917–22.
[36] Kawamura I, Maeda S, Imamura K, Setoguchi T, Yokouchi M, Ishidou Y, et al. SnoN suppresses maturation of chondrocytes by mediating signal cross-talk between transforming growth factor-β and bone morphogenetic protein pathways. J Biol Chem 2012;287(34):29101–13.
[37] Xu L, Golshirazian I, Asbury BJ, Li Y. Induction of high temperature requirement A1, a serine protease, by TGF-b1 in articular chondrocytes of mouse models of OA. Histol Histopathol 2014;29:609–18.
[38] Blaney Davidson EN, Vitters EL, van Beuningen HM, van de Loo FA, van den Berg WB, van der Kraan PM. Resemblance of osteophytes in experimental osteoarthritis to transforming growth factor beta-induced osteophytes: limited role of bone morphogenetic protein in early osteoarthritic osteophyte formation. Arthritis Rheum 2007;56:4065–73.
[39] Bakker AC, van de Loo FA, van Beuningen HM, Sime P, van Lent PL, van der Kraan PM, et al. Overexpression of active TGF-beta-1 in the murine knee joint: evidence for synovial-layerdependent chondro-osteophyte formation. Osteoarthr Cartil 2001;9:128–36.
[40] Osamu I, Masanori O, Noritaka N, Naoki G, Nobuo N, Ichio S. Structural basis of the collagen-binding mode of discoidin domain receptor 2. EMBO J 2007;26:4168–76.

[41] Ali BR, Xu H, Akawi NA, John A, Karuvantevida NS, Langer R, et al. Trafficking defects and loss of ligand binding are the underlying causes of all reported ddr2 missense mutations found in smed-sl patients. Hum Mol Genet 2010;19:2239–50.
[42] Mitchell PG, Magna HA, Reeves LM, Lopresti-Morrow LL, Yocum SA, Rosner PJ, et al. Cloning, expression, and type II collagenolytic activity of matrix metalloproteinase-13 from human osteoarthritic cartilage. J Clin Invest 1996;97:761–8.
[43] Shlopov BV, Lie WR, Mainardi CL, Cole AA, Chubinskaya S, Hasty KA. Osteoarthritic lesion: involvement of three different collagenases. Arthritis Rheum 1997;40:2062–74.
[44] Pulsatelli L, Addimanda O, Brusi V, Pavloska B, Meliconi R. New findings inosteoarthritis pathogenesis: therapeutic implication. Ther Adv Chronic Dis 2013;4(1):23–43.
[45] McDonald LT, Johnson SD, Russell DL, Young MR, LaRue AC. Role of a novel immune modulating DDR2-expressing population in silica-induced pulmonary fibrosis. PLoS One 2017;12(7) e0180724.
[46] Buckwalter JA, Anderson DD, Brown TD, Tochigi Y, Martin JA. The roles of mechanical stresses in the pathogenesis of osteoarthritis: implications for treatment of joint injuries. Cartilage 2013;4(4):286–94.
[47] Leitinger B. Discoidin domain receptor functions in physiological and pathological conditions. Int Rev Cell Mol Biol 2014;310:39–87.
[48] Bargal R, Cormier-Daire V, Ben-Neriah Z, Merrer ML, Sosna J, Melki J, et al. Mutations in ddr2 gene cause smed with short limbs and abnormal calcifications. Am J Hum Genet 2009;84:80–4.
[49] Ge C, Mohamed F, Binrayes A, Kapila S, Franceschi RT. Selective role of discoidin domain receptor 2 in murine temporomandibular joint development and aging. J Dent Res 2018;97(3):321–8.
[50] Matyas J, Atley L, Ionescu M, Eyre D, Poole A. Analysis of cartilage biomarkers in the early phases of canine experimental osteoarthritis. Arthritis Rheum 2004;50:543–52.
[51] Leitinger B, Steplewski A, Fertala A. The D2 period of collagen II contains a specific binding site for the human discoidin domain receptor DDR2. J Mol Biol 2004;344(4):993–1003.
[52] Reboul P, Pelletier JP, Tardif G, Cloutier JM. The new collagenase, collagenase-3, is expressed and synthesized by human chondrocytes but not by synoviocytes. A role in osteoarthritis. J Clin Invest 1996;97:2011–9.
[53] Klatt AR, Zech D, Kühn G, Paul-Klausch B, Klinger G, Renno JH, et al. Discoidin domain receptor 2 mediates the collagen II dependent release of interleukin-6 in primary human chondrocytes. J Pathol 2009;218:241–7.
[54] Bau B, Gebhard PM, Haag J, Knorr T, Bartnik E, Aigner T. Relative messenger rna expression profiling of collagenases and aggrecanases in human articular chondrocytes in vivo and in vitro. Arthritis Rheum 2002;46:2648–57.
[55] Tetlow LC, Adlam DJ, Woolley DE. Matrix metalloproteinase and proinflammatory cytokine production by chondrocytes of human osteoarthritic cartilage: associations with degenerative changes. Arthritis Rheum 2001;44:585–94.
[56] Neuhold LA, Killar L, Zhao W, Sung ML, Warner L, Kulik J, et al. Postnatal expression in hyaline cartilage of constitutively active human collagenase-3 (mmp-13) induces osteoarthritis in mice. J Clin Invest 2001;107:35–44.
[57] Sunk IG, Bobacz K, Hofstaetter JG, Amoyo L, Soleiman A, Smolen J, et al. Increased expression of discoidin domain receptor 2 is linked to the degree of cartilage damage in human knee joints: a potential role in osteoarthritis pathogenesis. Arthritis Rheum 2007;56:3685–92.
[58] Matyas J, Astley L, Ionescu M, Eyre D, Poole A. Analysis of cartilage biomarkers in the early phases of canine experimental osteoarthritis. Arthritis Rheum 2004;50:543–52.
[59] Iwai LK, Payne LS, Luczynski MT, Chang F, Xu H, Clinton RW, et al. Phosphoproteomics of collagen receptor networks reveals shp-2 phosphorylation downstream of wild-type ddr2 and its lung cancer mutants. Biochem J 2013;454:501–13.
[60] Ikeda K, Wang LH, Torres R, Zhao H, Olaso E, Eng FJ, et al. Discoidin domain receptor 2 interacts with src and shc following its activation by type I collagen. J Biol Chem 2002;277:19206–12.
[61] Zhang Y, Su J, Yu J, Bu X, Ren T, Liu X, et al. An essential role of discoidin domain receptor 2 (ddr2) in osteoblast differentiation and chondrocyte maturation via modulation of runx2 activation. J Bone Miner Res 2011;26:604–17.
[62] Yamasaki K, Nakasa T, Miyaki S, Ishikawa M, Deie M, Adachi N, et al. Expression of microrna-146a in osteoarthritis cartilage. Arthritis Rheum 2009;60:1035–41.
[63] Jones SW, Watkins G, Le Good N, Roberts S, Murphy CL, Brockbank SM, et al. The identification of differentially expressed microRNA in osteoarthritic tissue that modulate the production of TNF-alpha and MMP13. Osteoarthr Cartil 2009;17:464–72.
[64] Iliopoulos D, Malizos KN, Oikonomou P, Tsezou A. Integrative microrna and proteomic approaches identify novel osteoarthritis genes and their collaborative metabolic and inflammatory networks. PLoS One 2008;3:e3740.
[65] Akhtar N, Rasheed Z, Ramamurthy S, Anbazhagan AN, Voss FR, Haqqi TM. microRNA-27b regulates the expression of matrix metalloproteinase 13 in human osteoarthritis chondrocytes. Arthritis Rheum 2010;62:1361–71.
[66] Salazar A, Polur I, Servais JM, Li Y, Xu L. Delayed progression of condylar cartilage degeneration, by reduction of the discoidin domain receptor 2, in the temporomandibular joints of osteoarthritic mouse models. J Oral Pathol Med 2014;43:317–21.
[67] Siddiqui K, Kim GW, Lee DH, Shin HR, Yang EG, Lee NT, et al. Actinomycin D identified as an inhibitor of discoidin domain receptor 2 interactions with collagen through an insect cell based screening of a drug compound library. Biol Pharm Bull 2009;32(1):136–41.
[68] Richters A, Nguyen HD, Phan T, Simard JR, Grütter C, Engel J, et al. Identification of type II and III ddr2 inhibitors. J Med Chem 2014;57:4252–62.
[69] Manning LB, Li Y, Chickamauga NS, Li X, Xu L. Discoidin domain receptor 2 as a potential therapeutic target for development of disease-modifying osteoarthritis drugs. Am J Pathol 2016;186(11):3000–10.