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Review

Tyrosine kinases regulate chondrocyte hypertrophy: promising drug targets for Osteoarthritis

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SUMMARY

Osteoarthritis (OA) is a major health problem worldwide that affects the joints and causes severe disability. It is characterized by pain and low-grade inflammation. However, the exact pathogenesis remains unknown and the therapeutic options are limited. In OA articular chondrocytes undergo a phenotypic transition becoming hypertrophic, which leads to cartilage damage, aggravating the disease. Therefore, a therapeutic agent inhibiting hypertrophy would be a promising disease-modifying drug. The therapeutic use of tyrosine kinase inhibitors has been mainly focused on oncology, but the Food and Drug Administration (FDA) approval of the Janus kinase inhibitor Tofacitinib in Rheumatoid Arthritis has broadened the applicability of these compounds to other diseases. Interestingly, tyrosine kinases have been associated with chondrocyte hypertrophy. In this review, we discuss the experimental evidence that implicates specific tyrosine kinases in signaling pathways promoting chondrocyte hypertrophy, highlighting their potential as therapeutic targets for OA.

Introduction

OA is a highly prevalent degenerative joint disorder worldwide[1]. OA is characterized by a slow-progressive degeneration of the articular cartilage, dysregulation of subchondral bone remodeling and synovial inflammation which ultimately lead to loss of joint function and chronic pain[2]. Despite the main risk factors for OA have been identified[3], there is still no effective disease-modifying treatment available.

Accumulating data suggest that articular chondrocytes undergo a phenotypic shift and become hypertrophic in OA[4]. This phenotypic change resembles the process of endochondral ossification, which naturally occurs in the growth plate for the elongation of long bones and lasts from early ante natal period until puberty. Within this process, the temporary cartilage found at the early embryonic stages of endochondral bones differentiates into hypertrophic cartilage and eventually enters apoptosis or differentiates to osteoblasts[5], leading to replacement by bone. However, this process should not occur in articular cartilage. Recent studies have confirmed by single cell RNA-seq that hypertrophic differentiation takes place in osteoarthritic articular cartilage[6,7].

One of the main characteristics of hypertrophic chondrocytes is the upregulation of proteolytic enzymes, which drive the degradation of extracellular matrix (ECM) components such as type II Collagen and Aggrecan. These enzymes are mainly Matrix Metallopeptidase (MMP) 13 and a desintegrin and Metallopeptidase with Thrombospondin Motif (ADAMTS) 4 and 5. In addition, hypertrophic chondrocytes produce Alkaline Phosphatase (*ALPL*) that enhances the calcification of the matrix and increase the expression of hypertrophic-related genes, such as type 10 Collagen (*COL10A1*) and Runt-related transcription factor 2 (*RUNX2*)[2]. Whereas, a healthy and proliferative chondrocyte is characterized by the expression of SRY-box transcription factor (*SOX9*) and type II collagen as well as Bagpipe homeobox homolog (*BAPX1*) (Fig. 1). Therefore, understanding the mechanisms that drive the terminal differentiation of hypertrophic chondrocyte and control the levels of ECM-degrading enzymes is important for developing effective therapies for OA.

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Interestingly, recent insights into the field of OA pinpoint that Tyrosine kinases (TKs) may play an important role in regulating chondrocyte hypertrophy. These enzymes are highly involved in cellular differentiation and are the main regulators in several pathways[3]. However, there is no study that provides an overview of the TKs associated with chondrocyte hypertrophy and the pathways that are involved in this phenotypic transition. To this end, this review aims to evaluate which TKs have been related to chondrocyte hypertrophy during OA pathogenesis and provide a clear overview of the signaling pathways that they activate.

Main pathways driving chondrocyte phenotype

Various pathways have been associated with chondrocyte hypertrophy that takes place during bone development (Fig. 2). Indian hedgehog (IHH) and parathyroid hormone–related protein (PTHrP) are known to generate a negative feedback loop, which allows to maintain chondrocyte homeostasis. Namely, IHH promotes hypertrophy while PTHrP blocks it[8]. Moreover, TGF-β has been shown to prevent hypertrophy via Smad2/3 signaling, through modulating the levels of main chondrocyte phenotype-determining transcriptional regulators such as SOX9 and RUNX2. Conversely, TGF-β might promote hypertrophy when it activates Smad1/5/9 phosphorylation[9]. Another pathway that contributes to chondrocyte hypertrophy is wingless-type (Wnt) signaling. Wnt induces the stabilization of β-catenin in the cytoplasm, allowing its translocation to the nucleus and this eventually enables the transcription of *RUNX2*[10]. Similarly, pro-inflammatory cytokines such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α, have also shown to upregulate the expression of hypertrophic markers and downregulate *SOX9* by stabilizing nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κβ) and hypoxia inducible factor 2α (Hif-2α)[3,8,99]. Other pathways including mitogen-activated protein kinase (MAPK) signaling, phosphoinositide 3-kinase (PI3K)- protein kinase B (AKT) and janus kinase (JAK) 2- (signal transducer and activator of transcription factor (STAT), however, may lead to opposite responses depending on the ligand-receptor by which the transduction is initiated[2].

The involvement of tyrosine kinase members in driving chondrocyte hypertrophy

TKs are a large subclass of protein kinases, which play a major role in cellular signaling. Through their ability to phosphorylate other proteins at their tyrosine residues, TKs are able to control most fundamental cellular processes such as cell proliferation and differentiation, cell metabolism as well as cellular survival[11]. Recent studies have entailed TKs as the most pursued targets in current pharmacological research[3,12] and importantly, there are already several TK inhibitors that are FDA approved[13].

Current advances in the field of OA have suggested that both, receptor and non-receptor TKs, are associated to articular chondrocyte catabolism (Table I). Some TKs have been associated to the synthesis of inflammatory mediators, such as anexelekto (AXL) and

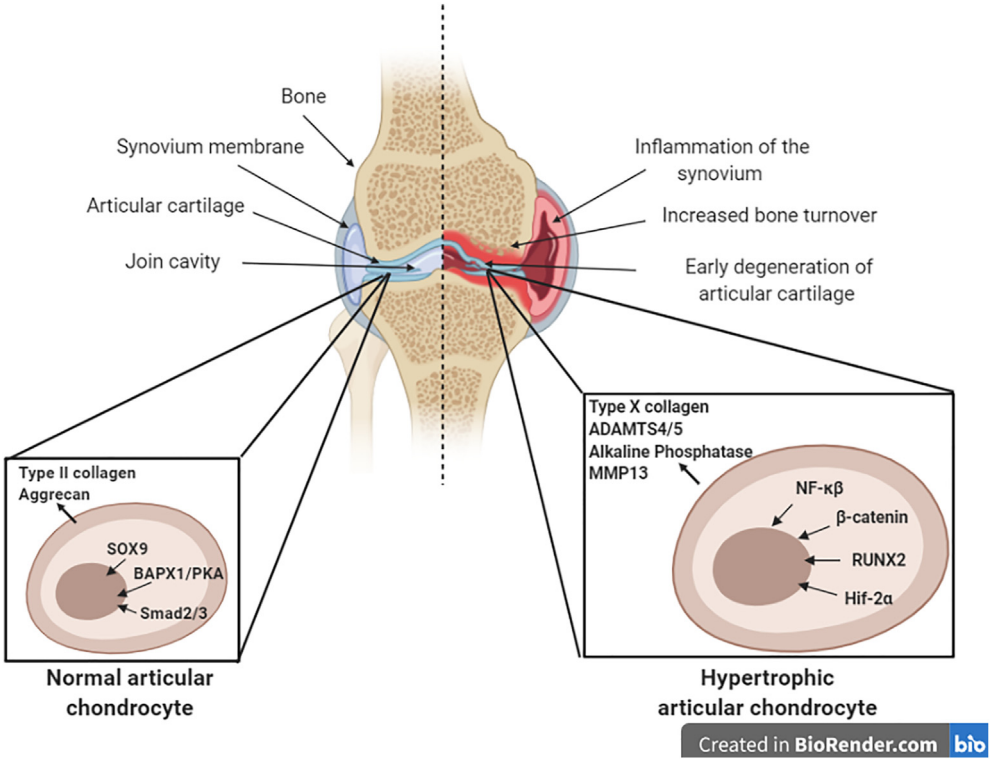

Fig. 1

Schematic representation of main characteristics of normal articular cartilage compared to an osteoarthritic cartilage. On the left, a normal articular chondrocyte is depicted together with the positive stimuli that induce chondrocyte homeostasis. Conversely, on the right a hypertrophic chondrocyte is shown, including the signaling and responses that occur during OA.

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tyrosine-protein kinase receptor (TYRO)-3[14], others have been shown to be increased in OA chondrocytes, such as human epidermal growth factor receptor 4 (ERBB4), insulin receptor (IR), c- mesenchymal ephithelial transition factor (MET) and vascular endothelial growth factor (VEGFR)-3[15–18], to inhibit catabolic factors such as ephrin type B receptor(EPHB) 4[19], or to modulate cartilage degradation such as lck yes novel tyrosine kinase (LYN), protein tyrosine kinase 5 (FRK), hematopoietic cell kinase (HCK) and spleen tyrosine kinase (SYK)[20,21]. In spite of this information, the specific role of these TKs in the mechanisms underlying chondrocyte hypertrophy remains to be elucidated.

Receptor tyrosine kinases

Receptor tyrosine kinases (RTK) are the major subgroup of the tyrosine kinase family and are essential components of signal transduction pathways that participate in intercellular communication. Out of 58 types of RTKs that are found in the human genome[46], ten have been associated to chondrocyte hypertrophy and are described below.

Fibroblast growth factor receptor 1 and 3

Fibroblast growth factor receptors (FGFRs) are ubiquitous regulators of development involved in wound healing, angiogenesis and cartilage homeostasis[1,47]. Mutations of FGFR genes are the basis of a broad range of skeletal developmental disorders such as craniosynostoses and chondrodysplasia[29]. FGFRs are activated through binding of fibroblast growth factors (FGFs) ligand and co receptor heparan sulfate to the extracellular domain of the receptor, which induces FGFR dimerization and transphosphorylation of tyrosine residues. Stimulation of the tyrosine kinase domain leads to activation of various downstream signaling proteins either from MAP kinases or PI3K/AKT pathway[47]. So far, four signaling members of FGFRs have been reported FGFR1, FGFR2, FGFR3 and FGFR4[48]. FGFR-1 and FGFR-3 thoroughly investigated in clinical trials as drug targets for various cancers and skeletal dysplasia[49,50], have shown to be the most predominant receptors expressed in human cartilage[28]. Thus, they are suggested to play essential roles in cartilage upon interaction with the FGF-2 and fibroblast growth factor 18 (FGF-18) ligands[1,2,30,51]. It has been consistently shown that the ratio of FGFR-3 to FGFR-1 is significantly reduced in OA, and that this is mediated by the upregulation of FGF-2[1,2]. Hence, suggesting

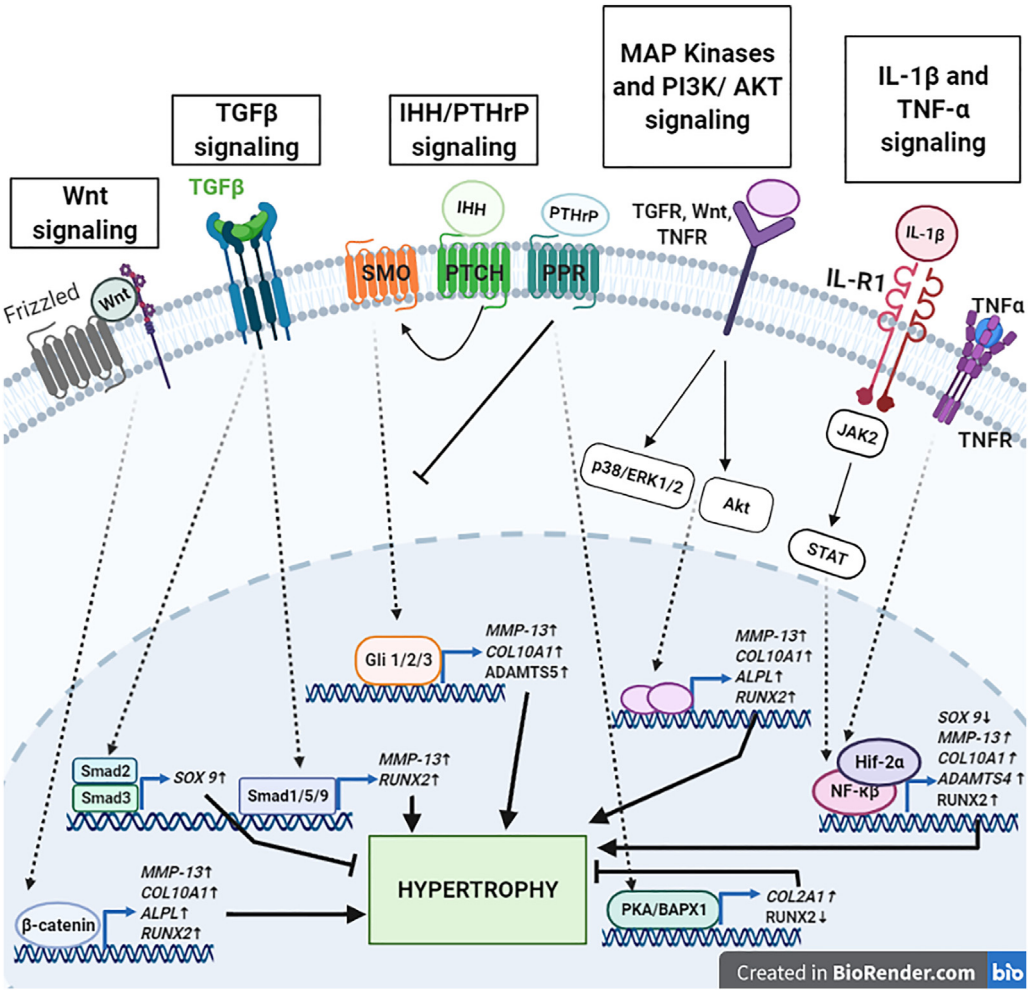

Fig. 2 **Overview of the signaling pathways involved in the regulation of chondrocyte homeostasis and their relation to chondrocyte hypertrophy.** The arrows and T-shaped lines signify positive and negative actions, respectively.

	Tyrosine Kinase	TK Family	Reference
Receptor	AXL	AXL	14
	TYRO-3		14
	DDR1	DDR	22,23
	DDR2		24–26
	EGFR	EFGR	27
	ERBB4		18
	EPHB 4	EPH	19
	FGFR-1	FGFR	1,2,14,28
	FGFR-3		29–31
	IGF1-R	INSR	32,33
	IR		34
	c-MET	MET	17
	ROR2	ROR	35
	TRK-A	TRK	8,36,37
	VEGFR-1	VEGFR	38,39
	VEGFR-2		16,38,39
	VEGFR-3		8,16,36,37,40
Non-receptor	FAK	FAK	41,42
	JAK2	JAK	43–45
	FRK	FRK	20
	FYN	SRC	3,14
	HCK		20
	LYN		20
	SYK	SYK	21

Table I Overview of the receptor and non-receptor tyrosine kinases that have been associated to chondrocyte catabolism

these FGFRs mediate opposing effects in human chondrocytes[28]. Suppression of FGFR-1 by G141, a non-ATP-competitive FGFR-1 inhibitor, impaired the expression of *MMP13, ADAMTS5* and *COL10A1* as well as reduced the loss of aggrecan in FGF-2 treated human chondrocytes. Additionally, G141 could delay the progression of cartilage degradation in a mouse model of OA[1]. Similarly, inhibition of components of the FGFR-1 signaling pathway, specifically FGFR-1 and ERK, abolished FGF-2 driven catabolic events in chondrocytes *in vitro*[2]. Similar effects were observed using Fgfr-1 conditional knock out mice[28]. Another morphogen that is overexpressed in OA-chondrocytes, FGF-23, can also activate FGFR-1. The addition of FGF-23 to healthy articular chondrocytes led to increased *RUNX2* expression and downregulation of *SOX9*, which is indicative of a hypertrophic phenotype[51].

On the other hand, FGFR-3 is essential for cartilage protection and inhibition of hypertrophic phenotype. In fact, Fgfr-3 (−/−) mice develop spontaneous OA, evidenced by an accelerated degradation of cartilage in association with an upregulation of *Mmp13* and *Col10a1*[1,30]. Further evidence demonstrated that *Fgfr-3* levels were reduced in *mtorc1* knockout mice that displayed cartilage degradation and chondrocyte hypertrophy[52]. Interestingly, exogenous stimulation of FGFR-3 by FGF-18 was able to ameliorate OA[31]. Of note, in a randomized phase II clinical trial the intra-articular injection of FGF-18 increased cartilage thickness, and substantially reduced cartilage loss[53]. Taken together, recent advances have shown that FGFR-1 induces chondrocyte hypertrophy while FGFR-3 prevents it.

Discoidin domain receptor 1 and 2

The discoidin domain receptors (DDRs) family comprises two members, DDR1 and DDR2, and are the only type I transmembrane receptor TKs that specifically bind to and are activated by collagen[54]. DDRs mediate cell–collagen interactions under both, normal and pathological conditions. Of note, DDRs regulate cell proliferation, migration, adhesion and tumor metastasis[55]. DDRs can be activated by different types of collagen and each receptor has different affinities for each one. Namely, only DDR1 binds to collagen IV[54,56], whereas DDR2 preferentially binds collagen II[57] and X[58]. Both DDRs activate signaling pathways including PI3K/AKT and rat Sarcoma virus (RAS)/extracellular signal regulated (ERK)/p38[59–63].

Provided the affinity of DDR2 for collagen type II and X, it was previously believed that this was the only DDR receptor involved in OA pathogenesis[64]. Several *in vitro* studies investigating the role of DDR2 in OA suggested that cumulative injuries in joint tissues lead to increased collagen II cleavage that results in DDR2 activation by tyrosine autophosphorylation[24–26]. DDR2 signaling induce overexpression of hypertrophic markers such as *Mmp13*, *Alpl* and *Col10a1 in vivo*[65]. OA severity was attenuated in *Ddr2*-deficient mutant mice[66]. A recent publication has highlighted that inhibition of DDR1 in OA-mice models decreased expression of *Mmp13* and *Col10a1*[22]. In the light of these findings, it can be elucidated that both DDRs may be involved in promoting chondrocyte hypertrophy.

Insulin-like growth factor 1 receptor

Insulin-like growth factor receptor 1 (IGF1-R), along with insulin receptor (IR) and IGF2-R, is a member of the IGF signaling pathway that is essential for cell growth and tissue differentiation[67]. Interestingly, a number of clinical trials involving IGF1-R inhibition in breast cancer patients have been published[68–70]. This receptor mainly mediates the effect of insulin-like growth factor 1 (IGF1) and IGF2. Ligand-activated IGF1-R subsequently recruits and activates signaling intermediates, that include members of the insulin-receptor substrate (IRS) family and Shc. Activation of IRs induces activation of the PI3K/AKT pathway. Conversely, Shc leads to activation of the MAPK/ERK pathway[71]. Besides tumorigenesis, IGF1-R is thought to be involved in the regulation of articular cartilage metabolism via IGF-1 binding[33]. Namely, stimulation of IGF-1R by IGF-1 impaired interleukin (IL)-1β induced NF-κβ activation in human chondrocytes. Consequently, hypertrophic differentiation was prevented[32]. Together these findings suggest a protective role of IGF1-R in OA progression.

Epidermal growth factor receptor

The epidermal growth factor receptor (EGFR, ErbB1) is a member of the ErbB family of receptors, which also includes ErbB2, ErbB3 and ErbB4. EGFR is crucial for the development of several organs during embryonic and postnatal stages[72]. Through its interaction with its growth factor ligands, transforming growth factor (TGF)-α and epidermal growth factor (EGF), EGFR undergoes a transition from monomeric to an active homodimer. Subsequent dimerization leads to protein autophosphorylation and downstream activation of MAPK, AKT and c-jun N-terminal kinase (JNK) pathways. Importantly, EGFR has shown to promote inflammatory processes upon TGF-α binding[73]. Overexpression of EGFR has been so far related to cancer, fibrosis and inflammatory diseases. Current literature on the field of OA, suggests that TGF-α and EGFR are involved in the molecular events that influence joint cartilage degeneration, since overexpression of both proteins was observed in degenerating cartilage in experimental knee OA[73]. Moreover, levels of anabolic genes such as *Acan* and *Col2a1* were reduced, whereas *Mmp13* levels were increased in articular chondrocytes in response to TGF-α[73]. Particularly, EGFR signaling is thought to drive ECM degradation through the activation of MAPK pathways, which involves the phosphorylation of p38 and ERK kinases. This latter event is essential for the translocation of β-catenin into the nucleus

and the expression of catabolic genes. Moreover, downregulation of EGFR prevents thrombin-induced *Mmp-13* expression in human chondrocytes via PI3K/AKT[27]. Clinical studies specifically targeting EGFR in OA have not hitherto been reported, albeit it could be of interest provided the participation of EGFR in the regulation of hypertrophic markers.

Vascular endothelial growth factor receptor 1 and 2

Vascular endothelial growth factor receptors (VEGFRs) are responsible for binding with their ligands VEGFs to promote angiogenesis and vasculogenesis during development, wound healing and endochondral ossification. Pathophysiological effects of VEGF/VEGFR signaling have been described in diseases such as cancer and rheumatoid arthritis[74,75]. In fact, VEGFR inhibitors have been used in clinical trials and were approved by FDA as drug targets for the treatment of cancer, pulmonary fibrosis and macular degeneration[76,77]. There are three subtypes of VEGF receptors, including VEGFR-1, VEGFR-2 and VEGFR-3, which can be activated by diverse structurally related VEGFs. Binding of VEGF to its receptor leads to protein homo- or heterodimerization, consequently inducing autophosphorylation of the tyrosine residues of the receptor and phosphorylation of downstream signal mediators that mainly participate in MAPK or PI3K/AKT pathways[78]. Besides their involvement in cancer progression, all VEGFRs have also appeared to be highly expressed in OA chondrocytes[16,38]. However, only the role of VEGF-A and its receptors VEGFR-1/-2 have been extensively studied in the pathophysiology of OA. Both receptor TKs are thought to drive vascular invasion of articular cartilage upon secretion of VEGF-A, a phenomena that is more pronounced in sever OA cartilage[39,79]. Both, *in vitro* and *in vivo* studies, showed that shRNA -mediated knockdown of VEGF-A was able to ameliorate OA. Namely, VEGF-A inhibition protected cartilage from hypertrophy by decreasing *Mmp13*, *Runx2* and *Col10a1*[39]. Inhibition of VEGF-A in an OA animal model reduced articular cartilage degradation. In contrast to healthy chondrocytes, VEGF-A downregulation increased expression of *Acan* and *Col2a1* and reduced *Mmp-13 and Adamts5* levels. Since VEGF-A can bind to both VEGFR-1 and -2, further studies investigating the specific roles of these receptors and their targeted inhibition in OA progression would be of interest.

Tropomyosin receptor kinase A

Tropomyosin receptor kinase A (TrkA), also recognized as high affinity nerve growth factor (NGF) receptor, is a member of the neurotrophic tyrosine kinase receptor (NTKR) family. Two other members constitute this family and are designated TrkB and TrkC. As a kinase, TrkA is responsible for functional NGF signal transduction to promote neuronal differentiation and proliferation, nociceptor response and avoidance of apoptosis. Precisely, the NGF-activated TrkA undergoes dimerization and autophosphorylation at multiple tyrosine residues, leading to the activation of diverse intracellular pathways such as PI3K/AKT and MAPK signaling. In many occasions, the NGF-TrkA complex is internalized via endocytosis and subsequently activates several signaling pathways[80]. NGF-TrkA signaling play a role in the pathophysiology of cancer and OA. Indeed, NGF and TrkA were found to be expressed in human articular chondrocytes and they were upregulated in osteoarthritic chondrocytes in accordance with the degree of tissue injury[81]. Besides the involvement of NGF-TrkA signaling in neuropathic pain in OA, this pathway was suggested to participate in chondrocyte homeostasis and hypertrophy[82]. Namely, it was found that the expression of an agonist of NGF and TrkA in human articular chondrocytes promoted chondrocyte hypertrophy. Conversely, inhibition of NGF receptor could also downregulate *SOX-9* and increase the expression of hypertrophy-related genes, including *ALPL* and *RUNX2*. The fact that both, over-activation and inactivation, of NGF-TrkA pathway give similar outcomes might be explained by the disruption of the feedback loop between IHH and PTHrP, two factors involved in regulating the homeostasis of articular chondrocytes[8]. Since 2015, a number of randomized clinical trials have been performed to evaluate the efficacy of TrkA inhibitors GZ389988, ASP7962 and ONO-4474 as treatment for OA knee pain and physical function. Whereas ASP7962 was unable to ameliorate pain and physical function, GZ389988 and ONO-4474 were able to safely reduce pain and gain physical function[36,37,40]. However, no structural changes were measured that could be associated with hypertrophy. Further research is required to decipher whether TrkA inhibition is a disease-modifying OA drug.

Receptor tyrosine kinase-like orphan receptor 2

Receptor tyrosine kinase-like orphan receptor (ROR) family consists of two members, ROR1 and ROR2, which are essential for regulating skeletal and neuronal development, cell polarity and migration[83]. The RORs are single-pass transmembrane receptors, with the unique feature that their cysteine-like rich domain resembles that of the Frizzled receptors and thus, non-canonical WNT ligands can bind to the receptor. Interaction of a non-canonical WNT ligand (WNT5A) with ROR triggers the association of ROR with other WNT co-receptors and subsequent RTK activation occurs. The signal is then transduced to downstream pathways such as WNT, PI3K/AKT, MAPK/ERK and Rho/yes-associated protein (YAP), which leads to the expression of genes related with cell proliferation and survival[84]. Since RORs are involved in cell proliferation and they are rarely expressed in adult tissues, they have become an attractive target for cancer therapy[85,86]. Nonetheless, a recent study showed that specifically ROR2 signaling was upregulated in OA cartilage and impaired chondrocyte differentiation via Rho/YAP pathway. ROR2 inhibition in primary human chondrocytes suppressed expression of *ADAMTS4/5* and increased *SOX9*. Furthermore, blocking ROR2 in a mouse OA model reduce pain and cartilage damage[35]. This study suggests that ROR2 is associated with WNT5A and hypertrophy and thus, ROR2 could also be a potential target for OA.

Cytoplasmic non-receptor tyrosine kinases

Non-receptor tyrosine kinases (NRTKs) are enzymes that are involved in the transduction of signals initiating from extracellular clues and which often interact with transmembrane receptors[87]. Three members (JAKs, FAK, Fyn) have been described to play a role in chondrocyte hypertrophy.

Janus kinase 2

Janus kinases (JAKs) family is constituted by four members, including JAK1, JAK2, JAK3 and Tyrosine kinase 2 (Tyk2), which are crucial components of various signal transduction pathways that control cellular survival and differentiation, among others. JAKs can be activated by either cytokines/interferons or growth factors. Upon the activation of JAKs by ligand-induced receptor oligomerization, these enzymes then phosphorylate the receptor that initiated the signal transduction and create docking sites for signaling molecules, especially members of the signal transducer and activator of transcription (STAT) family. Subsequently, the phosphorylated STATs form homo- or heterodimers that translocate into the nucleus to induce transcription of specific gene targets[88]. One of the members of the JAK family that is well-known to promote cell proliferation, JAK2, has recently been found to be activated in OA chondrocytes[89]. IL-1β highly increases JAK2/STAT3 phosphorylation in rat chondrocytes *in vitro*. Namely, the inhibition of JAK2/STAT3 pathway by JAK2 inhibitors such us AG490, prevented *Mmp-13* overexpression and the degradation of type II collagen[45]. Conversely, other studies

have found JAK2 to present a protective role in OA. In fact, JAK2/STAT3 inhibition by miR-375 resulted in upregulation of *Adamts5* and *Mmp13* and downregulation of *Col2a1* and *Acan* in mouse chondrocytes[44]. Moreover, JAK2/STAT1 signaling could lead to the inhibition of hypertrophy in rat chondrocytes via parathyroid hormone (PTH)/PTHrP receptor down-regulation upon FGFR3 activation[43]. In summary, JAK2 has a pivotal role in chondrocyte hypertrophy.

Focal adhesion kinase

Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that belongs to the FAK family, which also includes proline-rich tyrosine kinase 2 (PYK2). FAK has been suggested to play an essential role in integrin-mediated signal transductions and is important for early stages of cell migration and angiogenesis[90]. Activation of FAK occurs by disruption of an auto-inhibitory molecular interaction between the amino terminal four-point-one ezrin radixin moesin (FERM) domain and the central kinase domain. Upon activation, FAK associates with Src family kinases to initiate several signaling pathways including PI3K/AKT and MAPK. A number of studies *in vitro* suggested a critical role of FAK in angiogenesis during cancer progression[91]. On the other hand, FAK was also found to be involved in the pathophysiology of OA. Specifically, FAK could induce chondrocyte hypertrophic differentiation in response to factor XIIIa (FXIIIa) and transglutaminase 2 (TG2). Both transglutaminases are known to undergo physiologic upregulation in OA cartilage. In particular, TG2 mobilization to the cell surface is mediated by FXIIIa, upon its interaction with α1β1 integrin and thus, results in the phosphorylation of FAK and p38 MAP kinase. The activation of these protein kinases then led to increased levels of *COL10A1* in bovine articular chondrocytes[42]. FAK may act as a positive regulator of chondrocyte hypertrophy since

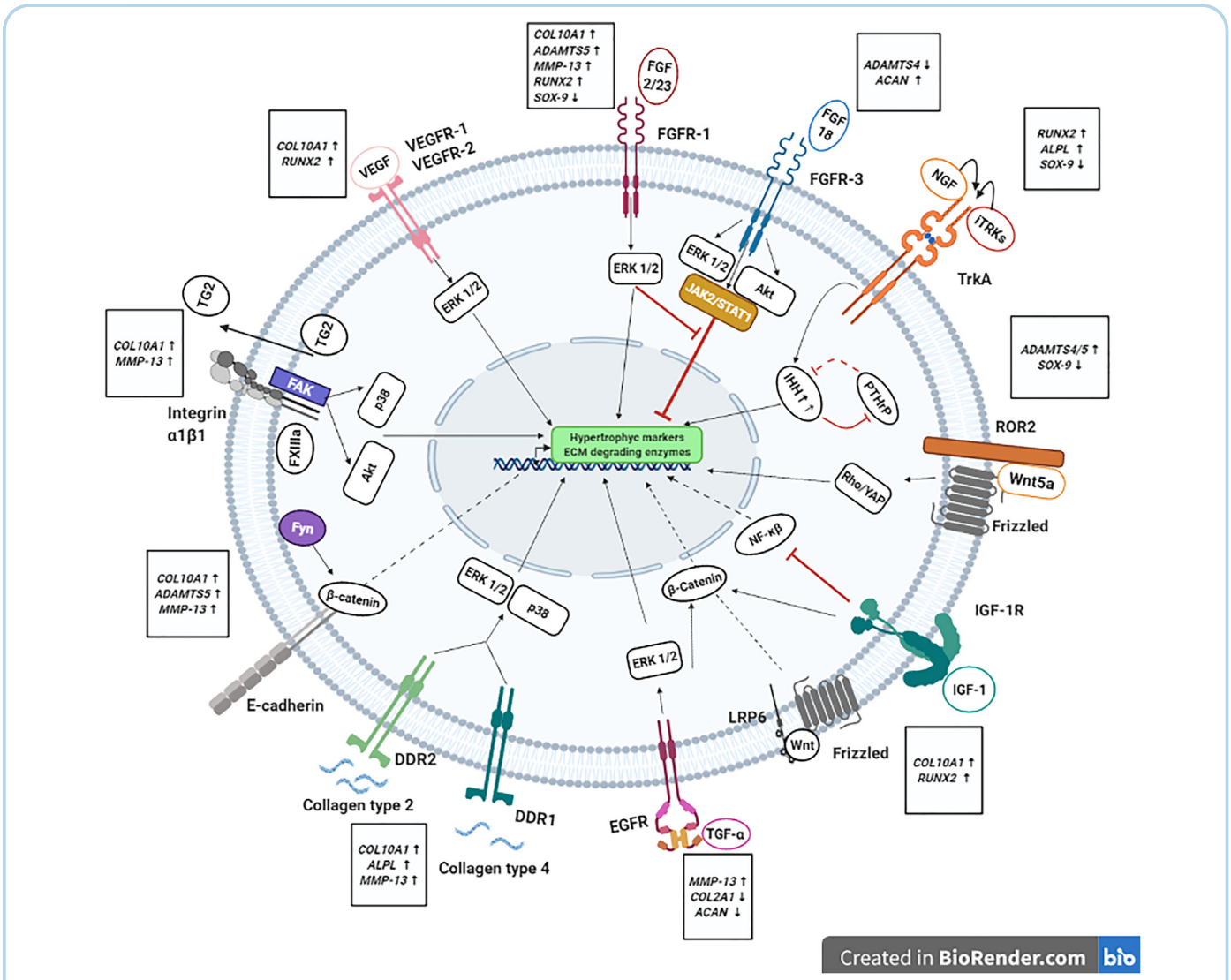

Fig. 3

Overview of the receptor and non-receptor tyrosine kinases that have been associated with osteoarthritis and the pathways through which they regulate chondrocyte hypertrophy. In red, the inhibitory relations are depicted; the black dashed arrows indicate the translocation of the factors to the nucleus and the non-dashed arrows indicate the positive relations for each pathway. In white text-boxes there is written the genes that either are being upregulated or downregulated.

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Drug	Target Tyrosine kinase	Target Disease	Side effects
Fedratinib	JAK2	Myelofibrosis	Anemia Diahrrea Fatigue
Ruxolitinib	JAK2	Myelofibrosis	Anemia Thrombocytopenia
Nintedanib	VEGFRs, FGFR1-3 and PDGFRs	Pulmonary fibrosis	Diarrhea

Table II Overview of FDA-approved tyrosine kinase inhibitors with potential interest for Osteoarthritis

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FAK is able to activate PI3K, which in turns inhibits BAPX1 and that allows the transcription of *Col10a1* and *Mmp13*[41]. In spite of these findings further studies investigating the association between FAK and hypertrophy are required.

Fyn

Fyn is one of the nine members of the Src family, well-known proto-oncogenes that regulate cell growth and are frequently mutated in cancer[92]. Fyn is a tyrosine-specific phospho-transferase activated by autophosphorylation at Tyr416, upon stimulation of diverse transmembrane receptors including RTKs, G protein-coupled receptors, integrins and cytokine receptors[93]. Thus, Fyn mediates multiple signal transduction pathways such as PKCδ/MAPK/NF-κβ, STAT3 or mTORC1, which regulate a large spectrum of biological processes involved in inflammation, fibrosis and cellular survival, respectively[94]. Recently, Fyn has been found to accumulate in human OA cartilage, thereby suggesting a potential role of Fyn also in the pathogenesis of OA[3]. Concretely, Fyn deficiency in mice protected against age-related or trauma-induced cartilage degradation and the development of OA. Furthermore, they revealed that Fyn phosphorylation led to the increased expression of hypertrophic markers such as *COL10A1, MMP13 and ADAMTS5* in mice and human chondrocytes, through the stabilization of β-catenin in a Wnt-independent manner. Given the close relation between Fyn and hypertrophy of articular chondrocytes[3], more pre-clinical studies about Fyn role in OA pathophysiology would be of high interest.

Discussion

In recent years, there has been increasing interest in the roles of TKs in the pathophysiology of various diseases, since these are essential players in cellular signaling processes[3]. In this review it was highlighted that some members of the tyrosine kinase family, including FGFR-1, DDR1, DDR2, EGFR, VEGFR, TrkA, ROR2, FAK and Fyn, have been found to induce chondrocyte hypertrophy in articular cartilage (Fig. 3)[2,8,39,41,66,95]. Thus, the use of inhibitors that impair the activity of these TKs could be considered as a potential treatment for OA. On this regard, repositioning drugs is an attractive alternative to lower development costs and shorten bench to bedside timeline. Three molecules that have been approved for clinical use for the treatment of Myelofibrosis and Pulmonary fibrosis (Table II) might be interesting for drug repurposing as disease-modifying osteoarthritic drugs. Nintedanib is an oral tyrosine kinase inhibitor with specificity against VEGFRs, FGFRs and PDGFRs. Fedratinib and Ruxolitinib, both orally bioavailable, selectively inhibit JAK2, however, considering the pivotal role of JAK2 in chondrocyte hypertrophy, caution should be taken. Of note, the adverse effects associated with these medications are often related to systemic application and could be decreased by dose-scalation or by intra-articular injection. The TKs FGFR-3 and IGF-1 receptor may be playing a protective role in cartilage homeostasis, therefore, its inhibition could lead to hypertrophy in articular chondrocytes (Fig. 3)[30,32] Currently, some companies are working on agonist against FGFR-3 due to their potentiality to treat OA in an efficient manner.

Despite these findings, a limitation of this review is that only few studies exist for each of these proteins. Moreover, some of these studies used growth plate chondrocytes which may behave differently from articular chondrocytes[96]. Hence, it would be interesting to further study the role of TKs in human articular chondrocytes. Furthermore, since tyrosine kinase inhibition could affect other joint structures, such as the synovium and the subchondral bone, the role of TK in these tissues should also be evaluated. To improve the specificity of the treatment, the use of non-competitive inhibitors is recommended since these molecules do not target the active site of the TKs and thereby have less promiscuity in binding[97]. Since TKs do not only modulate chondrocyte hypertrophy but also play a role in inflammation and autophagy[98], TKs inhibitors could significantly help in elaborating treatments that can hinder the progression of OA.

In conclusion, articular cartilage homeostasis is highly susceptible by changes in signaling pathways regulated by TKs that, when dysregulated, lead to chondrocyte hypertrophy. Unrevealing the mechanisms by which TKs influence cartilage hypertrophy could further provide new pharmacological targets for the elaboration of more effective treatments.

Declaration of conflicting interests

The authors declared no conflicts of interest.

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