Journal of Orthopaedic Translation 47 (2024) 116–124

Contents lists available at ScienceDirect

Journal of Orthopaedic Translation

journal homepage: www.journals.elsevier.com/journal-of-orthopaedic-translation

Review Article

The involvement of signaling pathways in the pathogenesis of osteoarthritis: An update

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ARTICLE INFO

Keywords:
Osteoarthritis
Signaling pathways
Signaling crosstalk
Targeted therapy

ABSTRACT

Osteoarthritis (OA) is one of the most common disabling pathologies, characterized by joint pain and reduced function, significantly worsening the quality of life. Even if important progresses have been made in OA research, little is yet known about the precise cellular and molecular mechanisms underlying OA. Understanding dysregulated signaling networks and their crosstalk in OA may offer a strong opportunity for the development of combined targeted therapies. Hence, this review highlights the recent findings on the main pathways involved in OA development, including Wnt, Notch, Hedgehog, MAPK, AMPK, and JAK/STAT, providing insights on current targeted therapies in OA patients' management.

The translational potential of this article: The identification of key signaling pathways involved in OA development and the investigation of their signaling crosstalk could pave the way for more effective treatments and improved management of OA patients in the future.

1. Introduction

Osteoarthritis (OA) represents an evolutive degenerative joint entity that causes progressive damage to articular cartilage, involving all the tissues of the joint, including subchondral bone, synovium, and surrounding capsula, ligament and muscles [1]. It is an extremely common disease, with an estimated 25 % lifetime risk to develop symptoms in people aged 85 [2], and shows an incidence in the overall population that is grown from 1.83 to 2.41 to 6.83–7.78 per 1000 person-years from 2008 to 2019, with a prevalence in females [3].

OA is one of the most important contributors to global disability affecting the elderly [4]. Moreover, it carries an increasing health burden with notable implications for the affected individuals since it is associated to a non-negligible expected increase in socioeconomic costs for the National Health System [5].

OA is a complex chronic disease, in which mechanical stresses and elevated pro-inflammatory cytokines play causative roles in disrupting cartilage homeostasis in the joint. Clinically, it is usually characterized by pain and joint restriction that limit patients autonomy in daily life, with inescapable pain as the most important symptom determining disability [6]. Even if recent technological improvements allow the performance of total bone replacement with minimally invasive techniques [7], a novel therapeutic approach to avoid the surgery is needed. Indeed, nowadays, pharmacological therapy for OA can only control and contrast the amplification of the degenerative cascade of local inflammation. Most commonly, at the initial stages, patients may benefit from off the shelf medications including non-steroidal anti-inflammatory drugs (NSAIDs) and acetaminophen, which in most cases limit pain without slowing the progression of the disease [8]. Hence, surgery remains the most effective tool to improve the quality of life for patients with OA.

At present, despite current effort in research, the ability to counteract OA progression is limited, and most patients develop end stage OA requiring surgery [9].

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https://doi.org/10.1016/j.jot.2024.06.002
Received 6 June 2023; Received in revised form 9 April 2024; Accepted 2 June 2024

To prevent advanced stage OA, the disease has to be detected during an appropriate therapeutic window, before becoming visible at conventional imaging techniques, including the more sophisticate MRIs [10].

For this reason, a deep knowledge of the molecular mechanisms and signaling cascades in the different stages of cartilage degeneration of OA is required. In particular, the structural changes that characterize OA are mediated by several cellular mechanisms (inflammation, cellular senescence, and apoptosis) involving multiple signaling pathways. Wnt, Hedgehog (Hh), Notch, AMP-activated protein kinase (AMPK), mitogen-activated protein kinase (MAPK) and Janus kinase/signal transducers and activators of transcription (JAK/STAT) axis are among those frequently dysregulated [11].

The identification of correct timing of action may allow the development of new drugs based on potential therapeutic targets and more effective than currently available drugs, including chondrogenesis inducers, osteogenesis inhibitors, matrix degradation inhibitors, apoptosis inhibitors, and anti-inflammatory cytokines [10].

In this review, after a brief overview of clinical aspects of OA onset and progression, a comprehensive and updated summary of the relevance of the major signaling pathways involved in cartilage homeostasis and subsequent OA progression will be displayed. Additionally, particular emphasis will be addressed to novel strategies targeting those signaling networks that could eventually lead to innovative therapies for the treatment of OA [see below Table 1]. In conclusion, the multiple crosstalk among these pivotal pathways will be discussed to clarify the intricate direct and/or indirect circuits at different levels behind the onset and progression of OA [Fig. 1].

2. Wnt signaling pathway

The Wnt pathway is a conserved signaling network involved in several physiological mechanisms, including development, differentiation, adult tissue homeostasis, and stem cell maintenance. It follows that the dysregulation of this axis is strictly connected to different pathologies, including cancer and degenerative diseases [12]. The Wnt family is a group of 19 secreted cysteine-rich glycoproteins that act on three different signaling pathways named β-catenin-independent, "non-canonical" β-catenin-dependent, and "canonical" β-catenin-dependent, which is the most known.

In the canonical axis, the translocation of β-catenin to the nucleus triggers the activation of several target genes, regulating cell fate, differentiation, proliferation, apoptosis, and cell polarity. Under physiological conditions, canonical Wnt cascade is tightly and efficiently tuned through multiple feedback mechanisms which guarantee a correct expression of all the components.

It has been well established that cartilage development is finely regulated by signaling networks, including the canonical Wnt pathway, that can push the skeletal mesenchymal cells toward chondrogenic or osteogenic lineage [13]. Indeed, this pathway acts as a key regulator of development and homeostasis of bone, cartilage, and joint, starting from the initial step of cartilage formation to the last process of endochondral ossification. It also regulates the differentiation of both osteoblasts and chondrocytes, which play a fundamental role in bone growth and remodelling, and the production of catabolic proteases [13].

Moreover, this axis controls the maintenance of mature articular cartilage phenotype, the hypertrophic maturation during the process of endochondral ossification, and the tissue degeneration and regeneration [14].

Even though the fine cellular mechanisms underlying OA are still unclear, mutations of Wnt ligands or β-catenin-dependent genes have a fundamental role in OA pathogenesis, suggesting that this degenerative pathology may be, at least partially, "Wnt addicted." To maintain cartilage development as well as a normal function, canonical Wnt pathway has to be strictly regulated [15]. Consequently, both hyper- and hypo-activation may cause chondrocyte pathologies and cartilage loss, as demonstrated in mice as well as human models [16]. For instance, the dysregulation of expression levels of canonical Wnt axis components leads to impairment of cartilage growth, as confirmed by independent studies on animal models [17]. Indeed, the activation of β-catenin in articular chondrocytes have been demonstrated to lead to an OA-like phenotype in mice, establishing a foundational model for understanding β-catenin's role in OA development. Moreover, human genetic studies showed that up-regulation of the canonical β-catenin-dependent network represents a risk factor for OA [18]. It has been demonstrated that the knockdown of Frizzled (FRZ), the transmembrane-domain receptor that binds Wnt, displays severe cartilage loss in OA mice compared to wild type (WT) [19]. Notably, both gain and loss of function of β-catenin in cartilage induce OA [16,18], demonstrating that the canonical Wnt signaling pathway could be required to finely maintain the articular cartilage homeostasis and, at the same time, to activate OA.

Moreover, the Wnt pathway relies on a complex intracellular signaling cascade involving the activation of specific proteins, including protein tyrosine kinases (PTKs) and phosphatases, which are pivotal activators of several signaling pathways implicated in several pathologies, such as cancer, neurological disorders, and degenerative diseases, as OA [20]. The dysregulation of these enzymes can impact on Wnt signaling pathway, disrupting the balance of signaling events that contribute to tissue homeostasis and repair. For instance, it has been demonstrated that the progression of the disease may be reduced through the inhibition of a specific PTK named Fyn, which is related to Wnt pathway in articular chondrocytes during OA [21]. In particular, Fyn interacts directly with and phosphorylates β-catenin, promoting its nuclear translocation and inducing OA-related gene expression. An inhibitor of PTK, AZD0530, and an inhibitor of Fyn, PP1, may hinder the development of OA, becoming a possible innovative target for the treatment of this disease [21].

It is important to note that recent studies have highlighted the critical involvement of Wnt pathway in the morphogenesis of OA across multiple joints including knee, temporomandibular joint (TMJ), hip, and facet joints through genetically engineered mouse models and human tissue analyses. Investigations have revealed that activation of β-catenin

Table 1
Targetable pathways for the treatment of OA.

Drug	Altered signaling pathway	Target	Clinical Trial	Ref
Lorecivivint (SM04690)	Canonical Wnt	CLK/ DYRK1A	NCT03122860 NCT03706521 NCT04520607	[25]
SOST (Sclerotin)	Canonical Wnt	Wnt LRP5/6	NCT01971931	[30, 31,92]
XAV-939	Canonical Wnt	Wnt		[33]
DAPT	Notch	Notch		[40]
Ihh-N	Hedgehog	Hedgehog		[93]
KOR agonist	Hedgehog	Hedgehog		[51]
Metformin	AMPK	AMPK	NCT05034029 NCT05638893 NCT04767841	[61, 63]
Vitamin D	AMPK	AMPK		[64]
ALA-24A	AMPK	AMPK		[66]
SVS (Stevioside)	MAPK	MAPK NF-κB	NCT05149053	[80]
OLT1177 (Dapansutrile)	MAPK	NLRP3	NCT02104050	[81]
U0126	MAPK	ERK		[76]
SP600125	MAPK	JNK		[76]
SB203580	MAPK	p38		[76]
Tizoxanide	MAPK	P38 JNK		[79]
Omentin-1	JAK/STAT			[86]
ACT (Acteoside)	JAK/STAT	IL-6 IFN-γ		[90]
ART (Artesunate)	JAK/STAT	JAK STAT		[91]

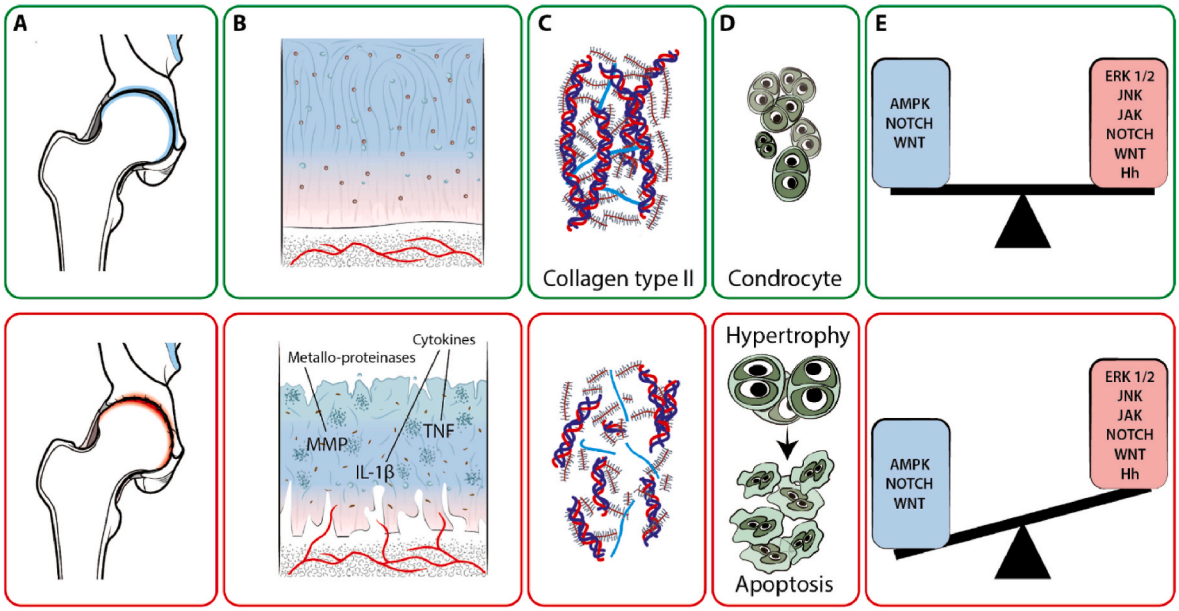

Figure 1. Comparison of articular cartilage composition, structure, and signaling pathways in healthy and osteoarthritic condition. The upper side (green) depicts a physiological joint, while the lower side (red) shows an altered OA joint. A. Anatomy of a healthy and an osteoarthritic hip. B. Extracellular matrix changes in healthy and osteoarthritic joint. MMP, metalloproteinases; IL-1β, interleukin 1β; TNF, tumor necrosis factor. C. Collagen type II fibers orientation in healthy and osteoarthritic samples. D. Chondrocytes in healthy patients compared to hypertrophic and apoptotic osteoarthritic chondrocytes. E. Dysregulated signaling pathways in healthy and osteoarthritic cells. ERK 1/2, extracellular signal-regulated kinase; JNK, Jun N-terminal kinase; JAK, Janus kinase; Hh, Hedgehog.(For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

signaling within aggrecan-expressing cells in the TMJ results in significant morphological defects and altered cellular activities, such as decreased chondrocyte proliferation, increased apoptosis, and upregulated expression of matrix-degrading enzymes, simulating an OA-like phenotype [22]. Similarly, in the hip joint, overexpression of β-catenin was correlated with severe cartilage degeneration, subchondral sclerosis, and osteophyte formation, which are hallmark features of hip OA, indicating β-catenin's pivotal role in hip OA pathogenesis [23]. Further, in spinal degeneration, elevated β-catenin signaling was associated with increased pain sensitivity and transcriptional activation of osteoarthritic pain-related factors, suggesting its involvement in spinal tissue homeostasis and degeneration [24]. Collectively, these findings underscore the pathological contribution of aberrant β-catenin signaling to OA development across different joints and propose targeting β-catenin as a potential therapeutic strategy for managing OA-related degeneration and pain.

Concerning the direct inhibition of Wnt pathway, an inhibitor of the Wnt pathway, named SM04690 or Lorecivivint, has been tested for the treatment of knee OA [25]. Interestingly, SM04690 has entered early-phase clinical trials for knee OA [NCT03122860, NCT03706521, NCT04520607]. It appears to be safe and well tolerated, and it clinically displays significant improvements in pain and function in patients with knee OA compared to healthy subjects [25].

Other endogenous and pharmacological small molecules that inhibits the Wnt pathway have just been tested in combination with innovative therapies, including platelet-rich plasma (PRP) and mesenchymal stem cells (MSCs) [26]. For instance, it has been suggested that PRP may protect activated interleukin-1 beta (IL-1β) chondrocytes through a block of the Wnt pathway, even if the precise mechanism has not yet been clarified [27].

Another Wnt inhibitor tested for OA treatment is sclerostin (SOST), a potent endogenous inhibitor of canonical Wnt signaling pathway (expressed by osteocytes), that, through the binding to LRP5/6 receptor [28] can increase cartilage area, thickness and proteoglycan levels in human transgenic mice [29]. In late-stage OA, SOST expression is reduced, suggesting its important role in bone and cartilage remodelling. In particular, its protective role has been demonstrated in sheep and mouse surgically-induced OA models [30]. SOST-knockout mice showed a severe OA phenotype associated to an increase of subchondral bone without affecting osteophyte formation and an increase of cartilage damage, probably due to a disrupted anabolic-catabolic balance in chondrocytes [31]. Additionally, SOST acts in chondrocytes both via Wnt network inhibition and via the non-canonical Jun N-terminal kinase (JNK) pathway inhibition. Importantly, SOST has also been tested as biomarker of severity of disease in OA patients given that lower SOST levels have been found in plasma from OA patients compared to healthy controls, in a severity score manner [32]. Based on these findings, SOST has been investigated in a clinical trial for the treatment of OA [NCT01971931].

Lastly, XAV-939, a small-molecule inhibitor of Wnt pathway, has been tested on both in vivo and ex vivo human chondrocytes, demonstrating an improvement of OA severity associated with a reduced cartilage degeneration and synovitis [33].

Overall, these findings underscore the critical role of Wnt pathway in the pathogenesis of OA and highlight its potential as a therapeutic target for mitigating the progression of the disease via its inhibition. Indeed, the inhibition of Wnt signaling cascade seems to restore a chondrocyte–protective profile, reverses the catabolic cascade following Wnt activation on synovial fibroblasts and chondrocytes, demonstrating a potential efficacy against OA. Clearly, further studies are needed to elucidate proof of concept, safety, and usefulness of all these drugs.

3. Notch pathway

Notch signaling is a juxtacrine pathway in which the receptor's intracellular domain is cleaved following the ligand binding. Next, it may translocate into the nucleus, where it activates target gene expression [34].

Notch is involved in different cellular processes regulating proliferation, differentiation, and apoptosis. It is known that several tissues express Notch1 receptor on the cell surface (including chondrocytes of articular cartilage [35], controlling the fine balance to maintain the

cartilage homeostasis in lifespan [36]. Indeed, it has been reported that Notch pathway is required in terminal differentiation and maturation of chondrocytes and during osteochondral ossification [37].

Very interestingly, Notch pathway is activated in OA cartilage [36] and Notch1 is aberrantly expressed in OA compared to healthy articular cartilage [36]. Moreover, downstream Notch signaling pathway, are upregulated in OA cartilage. In turn, the dysregulated Notch axis affects the expression of other several OA-associated genes, including IL-8, lubricin, CD10, matrix metalloproteinase 9 (MMP-9) and bone morphogenetic protein (BMP)-2, exacerbating the inflammatory environment in osteoarthritic joints [38].

Of note, Notch pathway has a dual role in the onset and progression of OA. From one side, Notch gain of function mice developed OA-like phenotype [39], as also demonstrated through a pharmacological approach. Indeed, injection of a Notch inhibitor, called DAPT, prevented OA development in the mouse knee joint [40]. On the other hand, Notch seems to have a protective role for chondrocytes. Indeed, in a Notch1 antisense transgenic mouse model, the total abrogation of this signaling cascade leads to the removal of OA phenotype [41]. Given these conflicting findings and to better explain the dual role of Notch signaling in OA development, Liu et al. developed two mouse models, showing that sustained Notch activation in adult joint cartilage leads to severe progressive OA phenotype, while transient Notch activation results in increased cartilage extracellular matrix synthesis and joint maintenance under physiological conditions [42].

Hence, in human articular cartilage, Notch signaling has to be finely balanced and regulated in lifespan to maintain articular cartilage homeostasis and joint integrity. Further studies are needed to fully understand the complex interplay between Notch signaling and OA pathogenesis, paving the way for the development of targeted interventions for this invalidating joint disease.

4. Hedgehog pathway

The conserved Hedgehog (Hh) pathway is involved in several physiological processes, including embryonic development, cell fate regulation, differentiation and proliferation. In mature organisms, it is commonly inhibited or even quiescent [43] and it can be activated under particular conditions, including tissue recovery in bone [44], and muscle [45].

Three different counterparts of Hh have been discovered, named sonic hedgehog (SHh), Indian hedgehog (IHh) and desert hedgehog (DHh) [43].

It has been well established that Hh positively regulates both the differentiation of periarticular chondrocytes into columnar chondrocytes expressed and secreted by chondrocytes at the growth plate modulating the development of the growth plate cartilage, and later in life during adult bone turnover [46] and chondrogenesis, as reported both in vivo and in vitro [47]. Indeed, its inhibition decreases chondrogenic potential and differentiation [47].

Interestingly, Hh pathway is aberrantly activated in OA chondrocytes, leading to chondrocyte hypertrophy and cartilage damage [48]. Indeed, mice with a hyper-activation of Hh signaling develop OA, also correlating with the severity of the disease [49].

Furthermore, significant advancements have been made in understanding the therapeutic potential of targeting the Hh pathway in OA treatment. Studies involving both pharmacological interventions (Hh inhibitor, named Ihh-N) and genetic inactivation of the Hh pathway in mice, as well as experiments using human osteoarthritic cartilage explants, have consistently shown a remarkable reduction in levels of OA markers [50].

Moreover, recent research suggests that the use of kappa opioid receptor (KOR) agonist, involved in mediating analgesic effects and modulating pain perception, can modulate Hh signaling by preventing the progression of cartilage damage in a model of rat knee OA [51].

Overall, these findings strongly suggest that modulation of the Hh pathway could serve as a promising therapeutic strategy for preventing or delaying cartilage degeneration in OA.

5. AMPK pathway

The AMPK pathway plays a key role in linking metabolism and energy homeostasis to signal transduction cascades involved in cell growth, proliferation, survival, and autophagy [52]. Under hypoxia or exercise conditions (then following variations of cellular ATP levels), AMPK is triggered by the phosphorylation on Thr172 on the catalytic subunit. In turn, activated AMPK acts on several substrates that switches on alternative catabolic network to generate more ATP, and simultaneously switches synthetic pathways to further prevent ATP consumption through anabolic biosynthetic axis [52].

It has been demonstrated that AMPK activation is induced by endoplasmic reticulum (ER) stress, leading to chondrocyte apoptosis [53]. Moreover, AMPK has an inhibitory effect during chondrogenic differentiation of primary chondrocyte precursors [54], causing chondrocyte dysfunction, hypertrophy, and fibrotic differentiation [55].

Accumulate evidence reported that inhibition of AMPK activity sustains OA development by enhancing IL-1β-stimulated response [56]. In particular, AMPK phosphorylation at Thr172 has been markedly decreased in the articular cartilage of OA patients and joint tissues of aging-related and surgically induced OA mice [57]. Similarly, other studies have reported that AMPK expression is significantly reduced in human knee OA and mouse OA articular chondrocytes [58]. As a result, higher levels of AMPK are linked to a lower risk of hip OA, but not at the knee [58].

All these data strongly support that restoring AMPK activity may be essential for reducing inflammation and preventing OA development. Great efforts have been done to develop drugs activating the AMPK pathway, as metformin.

As well-known, metformin is an antidiabetic agent having potentially multiple beneficial effects for several age-related and cardiovascular system pathologies. It effectively counterbalances excessive blood lipids, promotes angiogenesis and tissue regeneration, while exhibiting anti-inflammatory, anti-oxidant, and anti-aging effects [59]. Seen the pleiotropic effects of metformin and the involvement of overweight, inflammation, oxidative stress, and aging in OA onset and progression, different studies have been performed to deepen the role of metformin in OA. Indeed, to date, metformin entered in different clinical trials for knee OA patients [NCT05034029, NCT05638893, NCT04767841].As regards bone tissue, metformin promotes mineralization, osteogenic differentiation, and bone defect regeneration through activation of the AMPK signaling network [60].

It has been reported in OA animal models that metformin attenuates the pathological manifestations of the disease by preventing the degeneration of articular cartilage and maintaining the fine balance between the catabolism and anabolism of articular cartilage [61]. In support of this, it has been demonstrated the efficacy of metformin in mitigating cartilage degradation and synovial hyperplasia in both murine and non-human primate models of post-traumatic OA, highlighting the essential role of AMPK signaling in its chondroprotective effects [62]. However, the protective effects of metformin on the progression of OA are not detected in AMPKα1 KO mice [62]. This finding further proves that AMPK signaling is an intermediary of the chondroprotective impact of metformin [62], even if the underlying mechanism is still unclear. Besides, it has also been demonstrated that metformin acts on subchondral bone by inhibiting osteoclast activation through AMPK/NF-κB/extracellular-signal-regulated kinase (ERK) signaling pathway, which results in a delayed OA development [63].

Therefore, metformin could be considered as a promising therapeutic drug for OA even if, recently, further studies highlighted that some antioxidant compounds may have beneficial effects on the treatment of OA, acting on AMPK pathways regulation.

Vitamin D is well-known for its role in maintaining bone health

through its involvement in calcium metabolism and bone mineralization. Moreover, vitamin D regulates autophagy and cellular apoptosis, thereby influencing AMPK pathway and promoting its protective effect [64]. However, it is important to note that clinical trials evaluating the impact of vitamin D on OA patients have yielded side effects, such as headache, nausea and constipation, making its benefits a subject of ongoing debate. Similarly, a natural plant product known as berberine has been demonstrated to activate AMPK signaling in chondrocytes, leading to significant reductions in OA severity and associated pain in mouse models. This effect is mediated through the phosphorylation of AMPKα by liver kinase B1 (LKB1), and its beneficial impact is absent in AMPKα1 knockout mice, emphasizing the critical role of AMPK signaling in berberine's therapeutic action [65].

Introducing another promising antioxidant agent, alisol A 24-acetate (ALA-24A), an active triterpene, is able to attenuate oxidative stress and suppress inflammatory responses by inducing the upregulation of AMPK pathway [66] presenting a potential agent for mitigating the detrimental effects of OA on matrix degradation.

Certainly, further research and clinical investigations are warranted to fully elucidate the therapeutic potential of targeting the AMPK pathway in osteoarthritis management.

6. Metabolic pathways

It is well known that joint cartilage and bone are maintained in a balance between anabolism and catabolism mechanisms. Any disturbance in this fine equilibrium, also triggered by aberrant activation of different signaling pathways, may induce the degradation of extracellular matrix of the articular cartilage. For instance, chondrocytes may shift from a resting regulatory state to a metabolically active state to preserve energy homeostasis and cell survival, leading to the activation of proinflammatory cytokines, oxidative stress and signaling pathways [67]. All these mechanisms may be described as "chondrosenenscence", a condition characterized by a strong procatabolic imbalance, and an increase of senescent secretory and inflammatory effectors, that further disrupt the homeostasis and metabolism of cartilage [68].

Recent studies have highlighted the association between dysregulated cellular energy metabolism, mitochondrial dysfunction, and OA development and progression. Notably, the compromised energy demand observed in chondrocytes of OA joint tissues can be attributed, at least in part, to a decrease of AMPK activity [54]. The reduction in AMPK activity is known to also influence signaling pathways such as NAD-dependent protein deacetylase sirtuin 1 (SIRT1) and mechanistic target of rapamycin (mTOR) [67]. Indeed, interesting findings suggest that the inhibition of SIRT1 is responsible for a catabolic response to IL 1β and TNF in OA chondrocytes [69,70].

Therefore, the imbalance of metabolism related to the development of OA may suggest that OA can be approached as a "metabolic disorder" [71], and the activation of AMPK is corroborated as potential approach to counter the OA degenerative progression of joint cartilage damage.

7. MAPK pathway

The MAPK pathway is an evolutionarily conserved axis which acts through receptor tyrosine kinase (RTK), regulating several critical cellular mechanisms, such as cell proliferation, migration, differentiation, and senescence [72]. It follows that dysfunction or mutation of this signaling is involved in the onset and progression of several pathologies.

Some consecutive steps characterize the MAPK pathway: MAPK is phosphorylated by MAPKK, which, in turn, is activated by MAPKKK [72]. Once activated, MAPK regulates different substrates in the cytosol and nucleus, where it binds to transcription factors, thus activating gene expression. The MAPK are subdivided in 3 main families: ERK, JNK, and p38/stress-activated protein kinase (SAPK).

ERK and p38/SAPK pathways act as positive regulators of osteogenic and chondrogenic differentiation in bone marrow mesenchymal stem cells (BMSCs) [73], while JNK has a minor role in chondrogenesis [74].

Interestingly, MAPK expression levels have been shown to be higher in OA cartilage compared to physiological tissue, suggesting a key role in cartilage degeneration [75]. Indeed, as shown in OA animal models, the use of specific compounds that inhibits ERK expression is sufficient to reduce OA lesions concomitantly with a decrease in MMPs levels [76].

Accumulating evidence suggests that MAPK pathway plays a crucial regulatory role in mediating inflammatory and matrix degrading processes that lead to joint tissue destruction in OA. Cytokines, such as IL-1β, tumor necrosis factor α (TNFα), and MMPs are involved in MAPK signaling network, mediating OA progression. As a result, the inhibition of ERK, JNK and p38/SAPK can prevent their expression, which is required for the OA development [77]. Indeed, an interesting study have revealed that a novel cartilage specific molecule called Small Novel Rich in Cartilage (Snorc) plays a crucial role in OA pathogenesis by simultaneously activating the phosphatidylinositol-3-kinase/serine threonine kinase (PI3K/AKT) and JNK/c-Jun signaling pathways in IL-1β-stimulated chondrocytes [78]. Furthermore, the findings demonstrate that down-regulating SNORC effectively mitigates chondrocyte damage induced by IL-1β by suppressing both PI3K and JNK signaling pathways, highlighting the intricate relationship between SNORC and these key molecular pathways in OA progression [78]. Similarly, tizoxanide (Ti), the main active metabolite of nitazoxanide, has proved its anti-inflammatory activity by preventing IL-1β-induced PI3K/AKT and P38/JNK phosphorylation in chondrocytes of rat OA [79].

Hence, the inhibition of MAPK signal transduction has been described as a potential therapeutic strategy in OA. Much attention has been focused on JNK and p38/SAPK targets since these are commonly activated under stress and inflammation conditions. On the contrary, a systemic inhibition of ERK is considered potentially harmful, given the involvement in different cellular mechanisms, such as the response to growth factors and mitogenic factors and the induction of cell growth and differentiation [75].

A number of JNK inhibitors, that can act directly or indirectly, have been evaluated for OA treatment. Current evidence demonstrated the beneficial effect of stevioside (SVS), a naturally diterpenoid glycoside, in preventing inflammation and apoptosis of chondrocytes, through the downregulation of MAPK and NF-κB pathways, ameliorating the OA phenotype in vivo [80]. Similarly, the use of OLT1177, also named dapansutrile, a specific inhibitor of the NOD-like receptor protein 3 (NLRP3) for the treatment of inflammatory diseases, has proved his antidegenerative and ant-inflammatory action through inhibition of MAPK pathway in both rat OA chondrocytes and in rat OA model [81]. Recently, it entered phase II clinical trials for the treatment of OA [NCT02104050].

Additionally, a recent study showed that MAPKs play a crucial role not only in regulating apoptosis, but also autophagy in chondrocytes [76]. In an OA rabbit model, the impact of specific inhibitors, including U0126 (an inhibitor of ERK), SP600125 (an inhibitor of JNK), and SB203580 (an inhibitor of p38), were examined. Interestingly, inhibition of each MAPK target resulted in a significant increase of autophagic marker levels, which in turn showed a protective effect on OA [76].

Moreover, emerging research has shed light on additional new targets for OA treatment, such as a long non-coding RNA (LncRNA) SNHG7, which is able to mitigate OA chondrocytes inflammation and apoptosis by effectively inhibiting p38 MAPK signaling pathway [82]. Overall, these findings suggest that MAPK signaling could be a promising target to reverse cartilage degeneration in OA.

8. JAK/STAT pathway

JAK/STAT is an evolutionarily conserved signaling pathway which plays an important role in different cellular processes, including proliferation, differentiation, migration, apoptosis, senescence and cell survival [83]. The binding of extracellular ligands leads to the activation of intracellular JAK which in turn phosphorylates several downstream

substrates and activates numerous target genes. It has been observed that JAK dysregulation and/or mutations may induce the development of several pathologies, including immune deficiency, myeloproliferative disorders and cancer [83].

The JAK/STAT network has been firstly identified as a receptor-activated axis mainly responsive to interferon γ (IFN-γ) and members of the IL-6 family. Indeed, JAK/STAT cascade can be triggered by inflammatory factors which are crucial for coordinating the inflammatory responses in both physiological and pathological conditions.

Regarding bone and cartilage, it has been reported that JAK/STAT axis is involved in the regulation of osteoblasts and osteoclasts proliferation and differentiation [84].

As previously reported, OA is an inflammatory disease characterized by the progression of cartilage degradation. Among inflammatory factors, IL-1β, IL-6 and TNF represent the main pro-inflammatory players involved in the pathogenesis of OA. Given that these factors are considered stimulators of the JAK/STAT signaling [83], the progression of OA is intimately connected to JAK/STAT pathway [85].

Several compounds that inhibit JAK/STAT signaling have been tested for the treatment of OA, displaying good therapeutic efficacy. For instance, omentin-1, an anti-inflammatory adipokine, inhibits the JAK/STAT pathway in human chondrocytes, avoiding cartilage matrix destruction by regulating MMPs [86]. Indeed, JAK/STAT pathway is an important regulator of MMPs and, in chondrocytes, JAK/STAT3 activation can be induced by IL-1β, which in turn induces MMPs expression [87].

Of note, JAK/STAT network is connected to the activating effects of IL-6 in up-regulating the expression of MMP1, MMP3, and MMP13 in human chondrocytes [88]. All the MMPs have crucial functions in the irreversible destruction of cartilage due to the cleavage of collagen II [89]; particularly, MMP-13 is only expressed in the cartilage of OA patients.

Interestingly, Chinese herbal medicines have demonstrated their efficacy for treating OA for a long time. Among them, acteoside (ACT), a natural inhibitor, has been shown to have anti-inflammatory properties by preventing the up regulation of cytokines by decreasing the expression of JAK/STAT pathway in both in vitro and in vivo models [90]. Similarly, artesunate (ART) prevents inflammation and osteoclastogenesis through the inactivation of JAK/STAT signaling in a rat-induced OA model [91]. Even if the therapeutic effectiveness of these medications for the treatment of OA is not well understood, these seem important as coadjuvant for other drugs [Table 1].

9. Signaling crosstalk in OA development

All the signaling networks previously discussed can generate various and complicated connections at different levels, triggering a whole range of downstream key molecules which are crucial participant in the onset and progression of OA [Fig. 2].

The comprehension of this possible and complex crosstalk is necessary to further clarify the cellular mechanisms of the disease. More importantly, these pathways dynamically interact, influencing each other's activity that may have detrimental effects, limiting the efficacy of targeted therapies using single treatment, as reported to a large range of pathological conditions, including OA. For instance, it has been reported that in chondrocytes, Wnt pathway may interact with other signaling networks, including BMP/TGFβ [94], Hh [95], retinoid [96] and epidermal fibroblast growth factor receptor (EGFR) [97] pathways that, of note, are pivotal players in regulating cartilage and skeletal development. In turn, MAPK also regulates Wnt/β-catenin signaling in chondrogenesis [98], affecting cartilage development both positively and negatively.

Other evidence suggests that, in adult mouse models of OA, the balance between Hh and β-catenin signaling has a critical role in cartilage degeneration by activating inflammatory response [99]. As a matter of fact, Hh signaling is a negative regulator of chondrocyte differentiation and of selected β-catenin genes, via negative regulators of β-catenin-mediated and TCF-dependent transcription, including

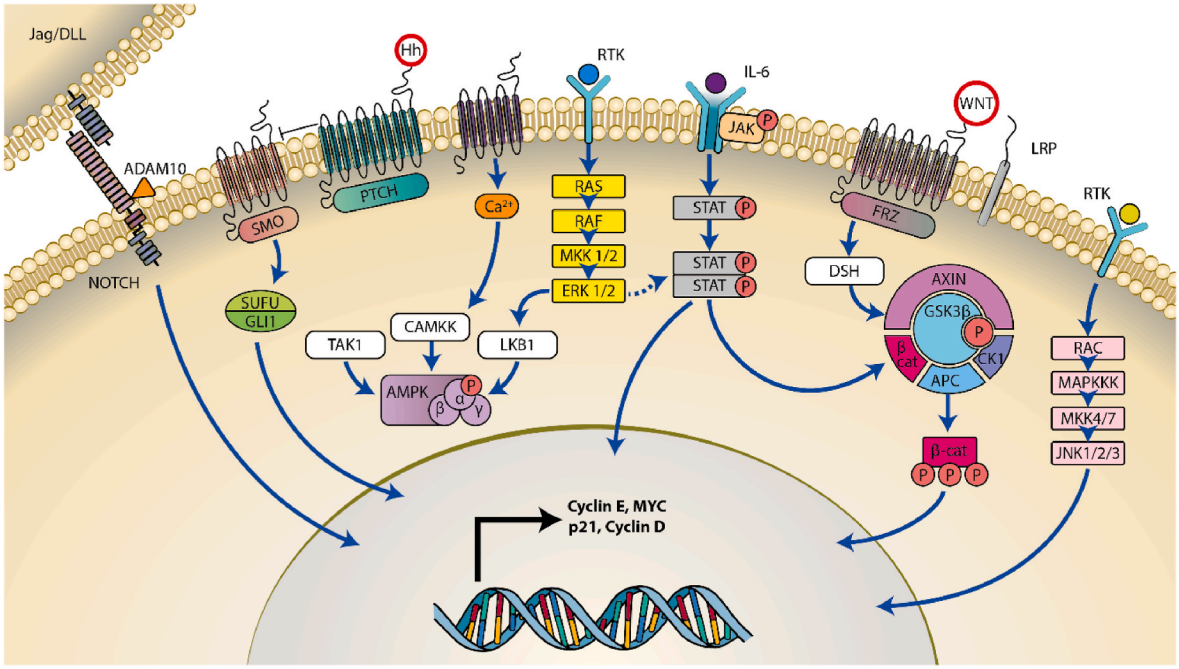

Figure 2. Most common signaling pathways involved in OA and the related crosstalk. ADAM10, A Disintegrin and metalloproteinase domain-containing protein; AMPK, AMP-activated protein kinase; APC, Adenomatous Polyposis Coli; β-cat, β-catenin; CAMKK, Calcium/calmodulin-dependent protein kinase kinase; DLL, Delta like ligand; DSH, Dishevelled; ERK, extracellular-signal-regulated kinase; FRZ, Frizzled; GSK3, Glycogen synthase kinase 3; Hh, Hedgehog; IL-6, Interleukin-6; Jag, Jagged; JAK, Janus kinase; JNK, Jun N-terminal kinase; LKB1, Liver Kinase B1; LRP, lipoprotein receptor-related protein; MAPKKK, mitogen-activated protein kinase kinase kinase, MKK, Mitogen-activated protein kinase kinase; PTCH, Patched; RTK, receptor tyrosine kinase; SMO, Smoothened; STAT, signal transducers and activators of transcription; SUFU, suppressor of fused; TAK1, Transforming growth factor beta-activated kinase 1.

TCF7L2/TCF4 isoforms [100]. Besides, Hh and Notch signaling are likewise closely intertwined and monitor each other.

Previous studies showed that the inhibition of Notch signaling results in the up regulation of Hh target genes, suggesting that the activation of the Notch pathway can counteract the dysregulation of Hh pathway in OA [41]. Consequently, this network leads to chondrocyte hypertrophy and osteophyte formation, confirming the typical phenotype of OA [41].

Another evidence reported that metformin treatment, activating the AMPK signaling, alleviates abnormal osteoclast-mediated bone resorption via MAPK signaling, thereby slowing OA progression [63]. Moreover, the activation of AMPK signaling is shown to inhibit β-catenin signaling in chondrocytes, offering a molecular mechanism by which AMPK activation decelerates OA development [101].

According to a wide number of studies, JAK/STAT pathway is implicated in the reduction of collagen II in chondrocytes by promoting MMPs expression [102]. Consistently, the interaction of JAK/STAT with ERK pathway is associated with loss of matrix, and their inhibition results in a protective mechanism in OA development [88].

Overall, several signaling pathways collectively contribute to the degenerative progression of OA by causing the breakdown of extracellular matrix, chondrocyte dysfunction, and cartilage degeneration. Therefore, understanding the intricate signaling crosstalk in OA development is crucial for identifying potential therapeutic targets and developing effective interventions to reduce or slow down the progression of this debilitating joint disease.

10. Conclusions

OA affects about 7 % of worldwide population and it is one of the most widespread diseases whose bearing is gradually increasing because of aging of the population. OA is a total joint disease that mainly affects articular cartilage, a tissue characterized by a very low reparative capacity, major determinant of tissue degeneration.

Unfortunately, at present OA treatment may only reduce pain and joint stiffness and, despite scientific progresses, viable therapeutic alternatives are still not available. Surgery remains the gold standard for advanced or end stage disease; therefore, the development of novel effective therapeutic strategies is fundamental to limit the requirement of preservative or prosthetic surgery. Consequently, efforts are directed towards the development of novel pharmacological approaches that may counteract, reverse or at least decrease OA degenerative changes, increasing the regenerative potential of articular chondrocytes or progenitors at an early stage of disease.

As we have summarized in this review, several pathways are involved in the onset and progression of OA, including the canonical Wnt, Notch, Hh, AMPK, MAPK, and JAK/STAT axis. All these signaling cascades play a significant role in cartilage development and homeostasis, and are aberrantly altered in human OA chondrocytes. Consequently, targeting signaling networks could become a potential and promising therapeutic approach that can delay or even reverse the progression of this degenerative pathology.

Of note, signaling transduction in OA pathogenesis is very complex and tangled, and the identification of the precise mechanisms underlying the development of the disease would accelerate the development of efficient therapeutic strategies for OA. Indeed, all the above-mentioned networks not only independently contribute to the onset and progression of OA, but also show intricate direct and/or indirect interactions between multiple circuitries and feedback loops at different levels, triggering a whole range of downstream key molecules, crucial participants in OA development. Considering this, the efficiency of targeted therapies using single drugs may have a limited success in treating OA. In this perspective, designing multi-target drugs against the above-mentioned signaling networks, through the combination of compounds that individually target each cascade, appears to be an innovative therapeutic approach for the development of potential therapeutic protocols for the treatment of OA.

Overall, a furthered knowledge of different and complex interplays among signaling pathways could pave the way for clarifying in detail involved cellular and molecular mechanisms and facilitate the identification of novel efficient therapeutic targets for OA.

Funding

The work was supported by: Ministero dell'Istruzione, dell'Università e della Ricerca-PRIN 2017 (to CE); Fondazione Cassa di Risparmio Bologna, Bologna, Italy (to SR).

Author contributions

All of the authors contributed to the writing and editing of the article.

Declaration of competing interest

All the authors declare that they have no conflict of interest.

Acknowledgments

The authors thank Davide Gamberini for graphical assistance.

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