Metformin prevents the onset and progression of intervertebral disc degeneration: New insights and potential mechanisms (Review)

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Abstract. Metformin has been the go-to medical treatment for addressing type 2 diabetes mellitus (T2DM) as a frontline oral antidiabetic. Obesity, cancer and bone deterioration are linked to T2DM, which is considered a metabolic illness. Numerous diseases associated with T2DM, such as tumours, cardiovascular disease and bone deterioration, may be treated with metformin. Intervertebral disc degeneration (IVDD) is distinguished by degeneration of the spinal disc, accompanied by the gradual depletion of proteoglycans and water in the nucleus pulposus (NP) of the IVD, resulting in lower back pain. The therapeutic effect of metformin on IVDD has also attracted much attention. By stimulating AMP-activated kinase, metformin could enhance autophagy and suppress cell senescence, apoptosis and inflammation, thus effectively delaying IVDD. The present review aimed to systematically explain the development of IVDD and mechanism of metformin in the treatment and prevention of IVDD to provide a reference for the clinical application of metformin as adjuvant therapy in the treatment of IVDD.

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Contents

1. Introduction

Intervertebral disc degeneration (IVDD) is a disease characterized by degeneration of the spinal disc, accompanied by the gradual loss of proteoglycans and water in the nucleus pulposus (NP) of the IVD (1). IVDD can lead to changes in the structure of the spine, which can adversely affect the normal physiological function of the spine (2). In addition, IVDD is the prerequisite and pathological basis for degenerative disease of the lumbar spine, including lumbar disc herniation and stenosis (3). Epidemiological studies have shown that IVDD is the primary factor of lower back pain (LBP), which is a common clinical symptom (4). In 2019, the global adult incidence of LBP was 7.34% (5,6). As the leading factor in the global disability burden, LBP-related disability cases increased by 47% from 1990 to 2019 (5). The lifetime instances of LBP in Americans amounts to ~80% (7). A notable burden is imposed on society when people struggle with recurrent LBP that causes harm to their physical and mental health (8,9). However, the exact pathogenesis of IVDD is not clear and there is a lack of effective treatment options for IVDD and secondary LBP.

Oral metformin is a prescription medication most frequently used for treating diabetes, including type 2 diabetes mellitus (T2DM). Patients with T2DM who use metformin have excellent safety profiles, along with satisfactory blood glucose control. This drug rarely causes adverse reactions

such as hypoglycaemia (10-12). The primary cause of fasting hyperglycaemia in patients with T2DM is increased liver glucose production. Various studies have noted that metformin controls hyperglycaemia by inhibiting gluconeogenesis in the liver (13-16). Metformin is efficient at alleviating a wide range of conditions, such as obesity (17), eating disorders (18), tumours (19), cardiovascular disease (20,21), polycystic ovary syndrome (22), kidney disease (23), cell senescence (24), osteoporosis (OP) (25) and osteoarthritis (OA) (26). DM is significantly associated with IVDD (27). DM can promote IVDD by abnormal blood glucose regulation and damaging IVD metabolism, which makes IVDD a complication of diabetes (28). Due to the association between IVDD and DM, metformin has been demonstrated to alleviate IVDD (29-34). Recent research has revealed that metformin may boost its curative effect on IVDD by encouraging the release of mesenchymal stem cell (MSC)-derived extracellular nanovesicles (30).

The hypoglycaemic effect of metformin is primarily mediated by inhibiting the mitochondrial respiratory chain in the liver and decreasing the production of ATP, which leads to the activation of AMP-activated kinase (AMPK) and increases the ratio of AMP/ATP, thereby inhibiting liver glycogenolysis and glucose production (35,36). Improvement of IVDD is associated with the AMPK signalling pathway (29,37). Autophagy is an internal recycling mechanism that has been preserved throughout evolution to degrade damaged or old cytoplasm in order to preserve cells in a state of equilibrium (38). Impaired autophagy in IVD cells is a primary contributor to IVDD. Appropriate induction of autophagy can slow progression of IVDD, but excessive autophagy can lead to IVD cell death (3,39). Hypoxia can decrease excessive autophagy by limiting reactive oxygen species (ROS) production and inactivating the AMPK/mTOR signalling pathway, thereby contributing to the survival of IVD cells (37). Metformin induces autophagy in NP cells of IVD in an AMPK-dependent manner, which suggests the importance of the AMPK pathway for the treatment of IVDD with metformin (29). The present review summarizes research advances on metformin and IVDD and potential mechanisms of metformin in controlling the onset and progression of IVDD. These results may provide fresh perspectives on how metformin is clinically used to prevent and treat IVDD and facilitate further research into its mechanism of action.

2. IVDD

Composition of IVD. The IVD is a key structure in the human spinal joint, connecting the upper and lower cones to perform movements and carry the load of the spine. The IVD is made up of three components: NP, anulus fibrosus (AF) and the cartilage endplate (CEP) and cone endplate on the upper and lower surfaces (40) (Fig. 1). The NP is composed of chondrocyte-like cells and has a large number of randomly distributed collagen fibres and radially arranged elastic fibers, the most important component of which is aggregated proteoglycan (aggrecan) (41,42), which provides the osmotic properties needed to resist compression and maintain the height of the IVD (43). The AF is composed primarily of water, collagen, aggregated and non-aggregated proteoglycan and non-collagenous protein (44). The AF is structurally composed of 15-25 concentric rings or lamellae composed of type I collagen fibres that are reversed in their alignment between consecutive cones (45). The AF is used to accommodate the NP and maintain NP pressurization under compressive loading (43,46). The CEP mostly is composed of type II collagen, glycosaminoglycan (GAG), and water (47) in similar proportions to those found in articular cartilage (48). The CEP exists in a highly hydrated proteoglycan matrix reinforced by collagen fibres, which fibers may contribute to the connection between the CEP and AF (49), while elastic fibers are key for the connection between the NP and adjacent CEP (50). CEP can separate the IVD from the adjacent cone while accommodating the NP (51). CEP can act as a semipermeable membrane that facilitates material exchange between cone layers (52), which provides nutrients to the cone and eliminates metabolites. CEP can act as a cushion and evenly distribute the load (53).

Mechanisms of IVDD. Aberrant cell-mediated response to increasing collapse of structure is a prevalent route of IVDD (Fig. 1). Degenerative IVD is characterized by structural failure accompanied by accelerated cellular senescence (45). Although mechanical overload is hypothesized to be the direct cause of IVDD, it is only an aggravating factor. The premature weakening of IVD is one of the key factors leading to disc-related issues. This degeneration can be caused by various factors, including genetic predisposition, age, insufficient transport of metabolites, and loading history (45).

A key stage in the development of the IVDD is the degradation of the extracellular matrix (ECM) by NP and AF cells. In NP cells, the matrix exhibits a decrease in GAG, aggrecan and elastin, an alteration in collagen and an increase in fragmented aggrecan, fibronectin and advanced glycosylation end products (AGES) (54). The decrease in type II collagen expression in NP cells is accompanied by an increase in type I and III collagen, ensuring that the absolute amount of collagen remains constant. However, there is an association between changes in type X collagen and calcification of the CEP. High expression of type X collagen may stimulate preosteoblasts to undergo osteogenesis in chondrocytes, leading to calcification of CEP (55,56). In addition, fragments of aggrecan are filtered out of the IVD (57), and the decrease in aggrecan is hypothesized to be closely related to a decrease in the hydration capacity and ability to withstand load of NP cells (58). Additionally, the formation of AGES can increase stiffness and fragility of collagen fibres (54). This may lead to a decrease in the elasticity of the IVD, making it more susceptible to injury and degeneration, which in turn affects the stability and function of the spine (54). In addition, syndecan-4, a transmembrane acetyl heparan sulfate proteoglycan, may mediate degradation of ECM and aggregated proteins via thrombospondin motif 5 and MMPs (59,60). The small leucine-rich proteoglycans are also impaired by degradation (61). This may lead to a loss of elasticity and stability of the ECM, affecting cell-matrix interactions and, consequently, the physiological function and structural stability of the tissue (62). Decrease expression of islet amyloid polypeptide, calcitonin receptor and receptor activity-modifying proteins in AF cells disrupts ECM homeostasis, thereby promoting IVDD (61). Furthermore, the

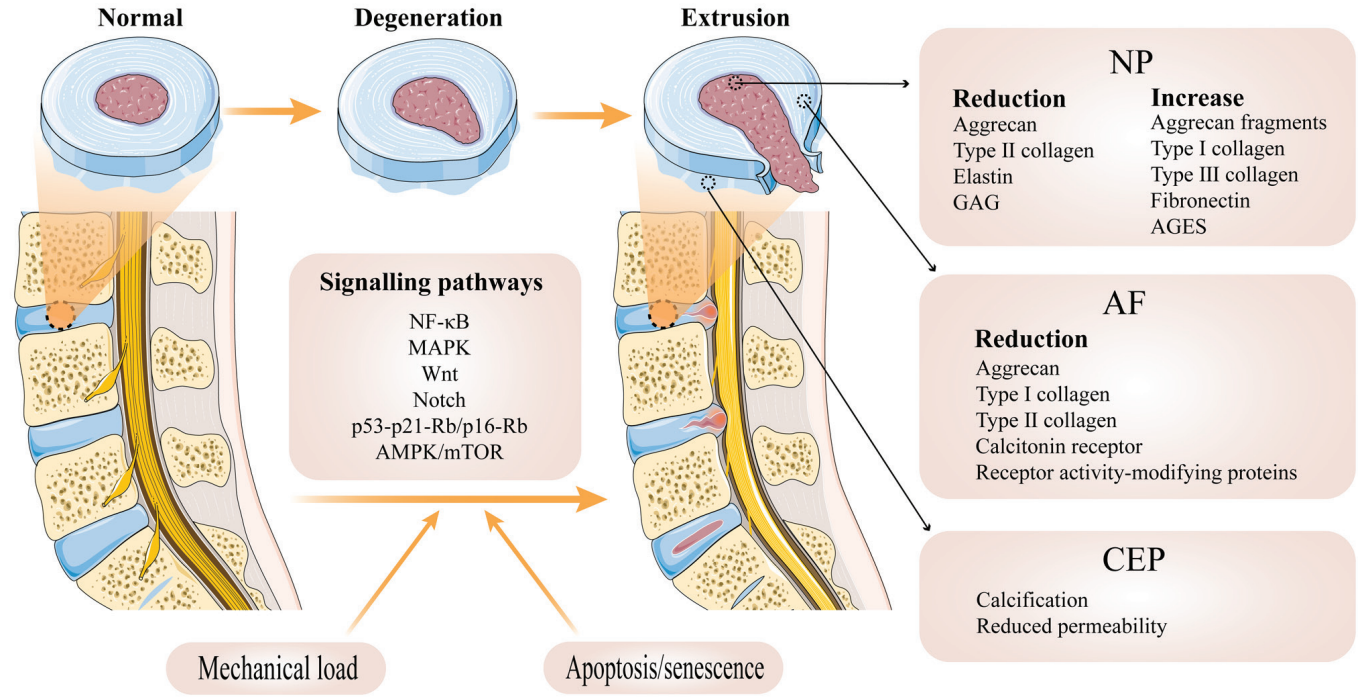

Figure 1. Process of IVDD. The IVD degenerates through signalling pathways such as NF-κB, MAPK and Wnt under mechanical load and senescence. AF of the IVD gradually ruptures and the NP breaks through the posterior longitudinal ligament to reach the epidural. In NP, aggrecan fragments, type I collagen, type III collagen, fibronectin, AGES increase, while aggrecan, type II collagen, elastin, GAG decrease. In AF, aggrecan, type I collagen, type II collagen, calcitonin receptor, receptor activity-modifying proteins decrease. In CEP, there is calcification and reduced permeability. Figure adapted from Server Medical Art (smart.servier.com/). AF, anulus fibrosus; CEP, cartilage endplate; IVDD, intervertebral disc degeneration; NP, nucleus pulposus; GAG, glycosaminoglycan; AGES, advanced glycosylation end products.

reduction of type I and type II collagen and lectins in AF cells leads to a transition of their load-bearing form to an inner annular elastic cushioning. Prolonged transition may result in compressed pain and AF damage (51). Alterations in the activity of certain enzymes, such as histoproteinases, matrix proteases and polymerase, may indirectly trigger degeneration of the disc (55). For example, MMPs may cause apoptosis in NP cells and calcification of the CEP (63,64).

Numerous signalling pathways are thought to be closely associated with IVDD, such as the NF-κB and MAPK pathways, which advance the progression of IVDD by regulating proinflammatory mediators (65) such as TNF-α, IL-1β and IL-6. In addition, initiation and development of disc disease may be dependent on the Wnt and the Notch pathways. Activation of the Wnt pathway accelerates NP cell senescence (66), while the hypoxia-activated Notch pathway can influence the course of IVDD via proinflammatory factors (66). DNA damage also plays a role in IVDD, leading to the loss of proteoglycans, decreased disc height and increased cellular senescence (67,68). DNA damage and telomere shortening induce replicative senescence in IVD cells primarily through activation of the p53/p21-retinoblastoma (Rb)/p16-Rb signalling pathway (69,70). In addition, oxidative stress serves an important role in IVDD and ROS damage the structure and function of IVD by damaging lipids, DNA and protein (71). Hydrogen peroxide promotes senescence by activating the senescence pathway, which stops NP cells at the G0/G1 phase of the cell cycle (72). The effects of oxidative stress also indicate activation of the p53/p21-Rb/p16-Rb signalling pathway (73). Inflammation promotes senescence in IVD cells and the inflammatory factors TNF-α and IL-1α, -1β, -6 and -17 enhance catabolism of the ECM (73).

Sirtuin (SIRT)1 can protect against IVD by inhibiting the p53-dependent and p16-dependent cellular senescence pathways and it can activate the Akt signalling pathway to protect NP cells from apoptosis (73,74). Moderate autophagy can mitigate apoptosis and delay IVDD in response to oxidative stress (75). Most autophagy is activated via AMPK/mTOR-dependent pathways (76). Overactivated autophagy may adversely affect IVD and hypoxic conditions can inactivate the AMPK/mTOR signalling pathway by limiting ROS production and decreasing activation of autophagy in NP cells through a pathway involving hypoxia-inducible factor 1α (75). In addition, autophagy safeguards CEP cells against calcification brought on by the intermittent, cyclic mechanical strain (77).

Apoptosis is associated with IVDD in NP cells, which are associated with activation of caspase-3 activity, oxidative stress and SIRT1 (37). Extrinsically, TNF-α regulates Fas/Fas ligand levels and thus activates caspase-3/9 activity (66), and may cause a significant increase in a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family of disintegrins and ADAMTS-7 in degenerating NP cells (78). In addition, in degenerating IVD, microRNAs (miRs) are differentially expressed, including downregulation of miR-155, production of miR-10b and miR-27a, and elevation of miR-21, and downregulation of miR-155 also activates caspase-3 activity to promote apoptosis (37). Additionally, p38 MAPK, JNK1/2 and ERK1/2 in the MAPK family may damage the IVD (78). It has been shown that the MAPK family exerts proapoptotic effects on senescence

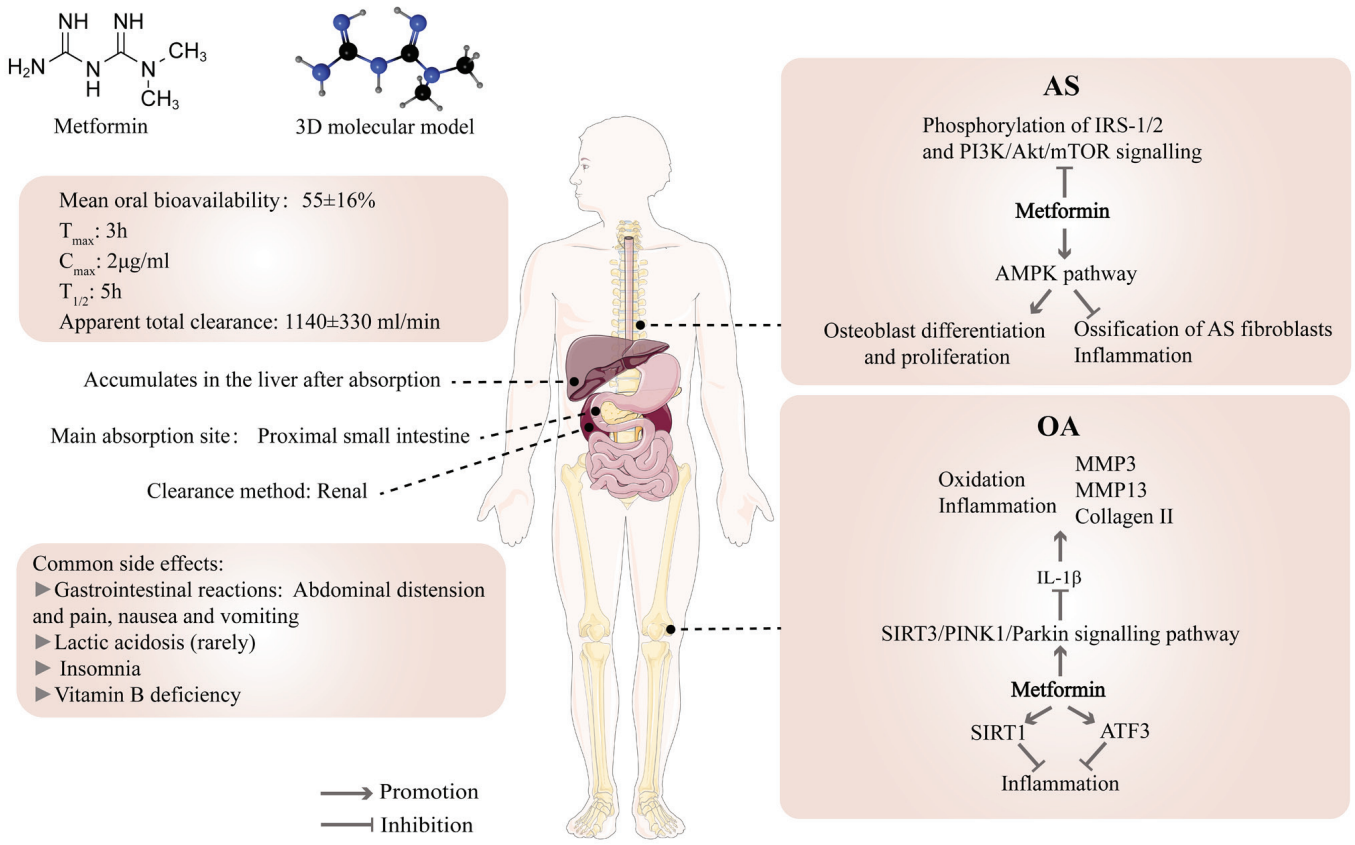

Figure 2. Role of metformin. In AS, metformin activates the AMPK pathway and inhibits IRS-1/2 phosphorylation and PI3K/Akt/mTOR signalling; in OA, metformin enhances the SIRT3/PINK1/Parkin, SIRT1 and ATF3 signalling pathways. Figure adapted from Server Medical Art (https://smart.servier.com/). AS, ankylosing spondylitis; ATF, activating transcription factor; IRS, insulin receptor substrate; OA, osteoarthritis; PI3K, phosphoinositide 3-kinases; PINK, PTEN-induced kinase; SIRT, sirtuin; T_{max}, Peak Time; C_{max}, Peak Concentration; $T_{1/2}$, Elimination half-life.

via direct or indirect activation of the p53/p21-Rb/p16-Rb pathway (73). In addition, apoptosis in AF and CEP cells is associated with the presence of the MAPK family and caspase-3, -8 and -9 (37).

3. Metformin

Physical and chemical properties. The structural formula of metformin (N, N-dimethylbiguanide) is $C_4H_{11}N_5$ (molecular weight, 129.16 g/mol); it is composed of a single protonated planar molecule in a non-polar methyl group and two imino groups. Metformin hydrochloride (molecular weight, 165.6 g/mol), which is widely used in the clinic, is constructed of dimethylamine hydrochloride and dicyandiamide (79). It is a white crystalline powder at room temperature. The density is 1.28 g/cm^3, the melting point is 223-226˚C and the boiling point is 224.1˚C (79). This substance is almost insoluble in chloroform and ether. It can be dissolved in water at a ratio of 1:2, and in ethanol at a ratio of 1:100 (80,81). Metformin has low lipophilicity, so it cannot quickly and passively diffuse through the cell membrane (24,82,83). As a hydrophilic base, metformin has an acid-base constant of 2.8 and 11.5. It has numerous tautomeric structures, is monoprotonated at neutral pH (84) and exists as a cation at physiological pH. The concentration of metformin in physiological fluids is represented by the free base (molecular weight, 129.16 g/mol) (83) (Fig. 2).

Pharmacokinetics. Following oral administration, metformin is dispersed in the gastrointestinal tract, liver and kidney (85). The mean oral bioavailability of metformin is 55±16%. Absorption is mainly limited to the proximal small intestine, and it accumulates in the liver after absorption (83). The peak plasma concentration is reached ~3 h after oral administration, and the peak in the liver appears within 1 h of administration (83,86). The plasma concentrations of metformin are 1-4 mg/l in patients who received 1-2 g/day metformin oral treatment (12). At 4 h after drug intake, the liver absorbs 80% of metformin and the body eliminates 98% within 24 h (87). Metformin is primarily excreted through the kidneys. Elimination half-life and apparent total clearance in individuals with normal renal function are 5 h and 1,140±330 ml/min, respectively (83). The absorption, liver utilization and renal excretion of metformin are primarily mediated by organic cation transporters (OCTs). Studies have shown that proton inhibitors decrease metformin transport by inhibiting OCTs (24,83).

To lessen gastrointestinal adverse reactions such as diarrhoea, malignant vomiting and so on, metformin should be taken with meals. Eating can affect drug absorption, but the decreased rate of absorption is not clinically significant for most patients (24,83,88). The faecal recovery of metformin is 20-30% of the oral dose, and metformin is not readily detectable in faeces after intravenous administration, suggesting that faecal metformin is not absorbed or utilized (83).

General mechanisms of metformin in degenerative bone disease. Degenerative skeletal diseases include OP, OA and IVDD, as well as diseases such as ankylosing spondylitis (AS) and periodontitis (24) (Fig. 2). The use of metformin in the treatment of degenerative bone diseases has been verified to be beneficial (24,89). The incidence of OP is lower among patients who have diabetes with carcinoma *in situ* who are treated with metformin than those not receiving metformin (25). Metformin improves the long-term outcome for people who are significantly overweight and have knee OA (90). To the best of our knowledge however, there have been no clinical studies showing the effectiveness of metformin in the treatment of IVDD. Since the NP is similar to articular cartilage, which consists of water, collagen and proteoglycans, (91-93) and NP and bone tissue have common components and origins (94,95), the mechanisms by which metformin affects them are similar.

Many studies have shown that activation of AMPK has a notable effect on musculoskeletal disorder (96-98). AMPK activity is significantly downregulated in human and mouse chondrocytes with OA and significantly elevated following metformin administration (82). Metformin can lead to osteoblast differentiation *in vitro* by activating AMPK, promoting bone matrix synthesis and osteoblast proliferation (99). Metformin also stimulates the regenerative process in bone lesions in diabetic and non-diabetic rats (99). Metformin serves an effective role in inhibiting ossification and inflammation in AS fibroblasts by activating the AMPK pathway (100). The aforementioned studies suggest an important role of the AMPK signalling pathway in treatment of degenerative skeletal diseases with metformin. However, the direct targets of metformin that act via the AMPK pathway in these diseases remain unclear. Recent studies have shown that metformin binds presenilin Enhancer 2 (PEN2) and initiates a signalling pathway that intersects with the AMPK/activated lysosomal glucose sensing pathway via ATP6AP1 (101-103). Whether metformin acts through this signalling pathway in skeletal degenerative disease remains unclear.

Phosphoinositide 3-kinase (PI3K)/Akt is one of the key pathways that regulates cell behaviour (including apoptosis, proliferation and differentiation); previous studies have shown that this pathway is a key regulator of osteoblasts and is associated with fracture healing (104-107). Metformin ability to regulate the PI3K/Akt pathway could contribute to its efficacy in the treatment of AS (100). Metformin reduces insulin and insulin-like growth factor-1 (IGF-1) levels, thereby inhibiting phosphorylation of insulin receptor substrate 1/2 (IRS-1/2) and PI3K/Akt/mTOR signalling (24), which is key for the control of skeletal degenerative lesions.

SIRT family is a group of antiaging genes (108) that are closely associated with the aetiology of degenerative bone disease (109). Studies have demonstrated that metformin inhibits IL-1β-induced oxidative and osteoarthritic inflammatory changes by enhancing the SIRT3/PINK1/Parkin signalling pathway. In addition, metformin attenuates IL-1β-induced expression of catabolic genes, such as MMP3 and 13 and enhances the anabolic factor collagen II, which is beneficial for treatment of OA (110-113). In addition, metformin-induced activation of SIRT1 decreases inflammatory mediators and matrix degradation substances, inhibits accumulation of receptor for AGE (RAGE) and protects articular cartilage, thus preventing the development of OA (114). Another study confirmed that metformin inhibits lipopolysaccharide (LPS)-mediated enhancement of IL-6, IL-1β and TNF-α production in human gingival fibroblasts (HGFs) by increasing activating transcription factor-3 (ATF3) expression. Decreases in TNF-α and IL-1β protein expression suggest that metformin mitigates LPS-induced inflammatory responses via the AMPK/NF-κB pathway (115). However, whether this mechanism of metformin is involved in IVDD requires further experimental verification.

Side effects. Metformin is an efficient and typically well-tolerated drug with notable hypoglycaemic effects but there are some adverse events (Fig. 2); the most prevalent negative reactions include gastrointestinal discomfort, including abdominal distension and pain, nausea and vomiting. These symptoms are typically mild, but medication may need to be suspended and the dose adjusted (24,88,116,117). In rare cases, metformin is related to lactic acidosis. Studies have shown that cardiovascular disease and liver and kidney damage following treatment are likely to be the main causes of lactic acidosis and there is no causal relationship with metformin (24,118,119). It has been reported that 1.4% of patients treated with metformin have insomnia, which may be associated with changes in blood glucose levels (120). DM with depression often suffer from insomnia (121,122). In addition, long-term metformin treatment may lead to vitamin B12 deficiency and require regular monitoring to decrease the incidence of side effects (123-125). Researchers determined the milk-to-plasma ratio of metformin in lactating patients and showed that metformin was safe during lactation and did not cause side effects (10,24). However, maternal and infant risks and individual differences should be considered and metformin is not recommended for pregnant patients (24).

4. Mechanism of metformin in IVDD

Metformin induces autophagy through the AMPK pathway and inhibits senescence and apoptosis in myeloid cells. As a highly evolutionarily conserved serine/threonine protein kinase, AMPK is commonly expressed in eukaryotic cells (126). The role of AMPK in the development of IVDD is becoming more obvious (Table I). Activation of the AMPK signalling pathway has been observed in human IVDD (127). AMPK phosphorylation is increased in rat IVDD. IVDD is a complex process involving both apoptosis and senescence (29). In degenerating rat NP cells, AMPK activation further dephosphorylates mTOR and inhibition of AMPK-mediated mTOR facilitates apoptosis and senescence while suppressing cell proliferation and cell cycle progression (34,128). Meanwhile, human AF cell apoptosis is influenced by AMPK (129).

Several *in vivo* studies have demonstrated a significant increase in the concentration of oxidative products in aging and degenerating IVD (130-132). Oxidative stress has been shown to induce apoptosis via the mitochondrial pathway and to induce premature cellular senescence (72,133). Chen *et al* (29) modelled apoptosis and senescence in medullary cells by inducing oxidative stress using tert-butyl hydroperoxide (TBHP) (29). The aforementioned study investigated the effects of metformin on apoptosis and senescence

Table I. Preclinical study of metformin in the treatment of IVDD.

Treatment groups	Experimental model	Results	Conclusion	(Refs.)
100 and 200 μM metformin	TBHP-induced senescence in NP cells/ puncture-induced rat IVDD model	Autophagy, as well as its upstream regulator AMPK, is activated by metformin in NP cells in a dose- and time-dependent manner. Metformin promotes expression of anabolic genes such as COL2A1 and Acan expression while inhibiting expression of catabolic genes such as MMP3 and ADAMTS5 in nucleus pulposus cells. *In vivo*, metformin ameliorates IVDD	Metformin activates autophagy via the AMPK pathway to protect NP cells from apoptosis and senescence and improve IVDD *in vivo*	(29)
EVs, EVs + metformin (1 mM, 24 h)	Puncture-induced rat IVDD model	Metformin increases EV markers CD63 and Alix proteins, autophagy markers and the EVs transport-related proteins total and phosphorylated SNAP29, increases expression of ITIH4 in EVs and ameliorates senescence of NP cells	Metformin enhances release of EVs from MSCs via autophagy-associated pathways and ameliorates IVD cell senescence both *in vitro* and *in vivo*	(30)
10, 50 and 100 μM metformin	TBHP-induced senescence in NP cells/ puncture-induced rat IVDD model	Metformin inhibits TBHP-induced expression of cell senescence markers P16, P21. With increasing concentrations of metformin, ratio of autophagy marker LC3-II/LC3-I increases, level of p62 decreases and autophagic vesicles in cytoplasmincrease. Metformin activates autophagy and decreases level of DNA damage marker γ-H2AX	Metformin ameliorates IVDD by inactivating the cGAS/STING pathway via autophagy	(31)
Metformin (100 mM Metformin for 4 h	Mechanical stretch and IL-1β-induced inflammation in rat AF cells *in vitro*	Metformin increases COL1, COX-2 and MMP13 gene expression, decreases PGE2 production and NF-κB nuclear translocation following 4 h IL-1β treatment	Anti-inflammatory effect of metformin in IVD cells may be mediated by the NF-κB pathway; effect of metformin is enhanced by mechanical stretch	(33)
Metformin (0-10 mM for 7 days)	LPS-induced inflammation in rabbit AFSCs *in vitro*	Metformin inhibits LPS-induced release of HMGB1 from AFSC nuclei, upregulation of certain inflammation-associated genes (IL-1β and -6, COX-2 and TNF-α) and proteins (IL-1β, COX-2 and TNF-α) as well as catabolic genes (MMP3 and 13) and decrease in anabolic gene (collagen I and II) expression	Metformin exerts an anti-inflammatory effect by blocking HMGB1 translocation, catabolic production and senescence in AFSCs	(32)

Table I. Continued.

Treatment groups	Experimental model	Results	Conclusion	(Refs.)
20 μg/ml metformin for 24-72 h	*In vitro* culture of IVD cells from patients undergoing lumbar microdiscectomy and sequestrectomy	Extracellular matrix structure is disrupted by metformin treatment, with decreased reproductive capacity at 72 h, and inhibited the expression of CHAD and COMP and decreased the expression levels of IL-1β, MMP7 and MMP19.	Metformin adversely affects IVD tissue cell culture, including inhibition of cell. proliferation and alteration of gene expression associated with extracellular matrix synthesis and degradation	(34)

IVDD, intervertebral disc degeneration; TBHP, Tert-butyl hydroperoxide; NP, Nucleus pulposus; COL2A1, Collagen type II alpha 1 chain; ADAMTS, A disintegrin and metalloproteinase with thrombospondin motifs; EV, Extracellular vesicle; MSC, Mesenchymal stem cell; SNAP, Synaptosome associated protein; ITIH, Inter-alpha-trypsin inhibitor heavy chain; cGAS, cyclic GMP-AMP synthase; STING, Stimulator of interferon genes; AFSC, Anulus fibrosus stem cells; PGE, Prostaglandin E; LPS, Lipopolysaccharide; HMGB, High mobility group box; CHAD, Chondroadherin; COMP, Cartilage oligomeric matrix protein.

of myeloid cells under oxidative stress and examined expression of anabolic and catabolic genes in myeloid cells following metformin treatment; metformin had a significant protective effect against TBHP-induced apoptosis. Metformin pretreatment inhibits the TBHP-induced increase in cleaved-caspase3 and Cyclin-dependent kinase inhibitor 2A (p16INK4a) protein content in a dose-dependent manner. In addition, TBHP treatment decreases mRNA levels of the key ECM synthesizing gene collagen type II alpha 1 chain (Col2a1), whereas metformin significantly inhibits this downregulation. The effects of metformin are realized through the activation of autophagy by the AMPK pathway. Metformin significantly decreases Bax and caspase expression and increases BCL-2 under oxidative stress conditions via the mitochondrial pathway. Metformin also significantly decreases the senescence marker senescence-associated β-galactosidase (SA-β-gal) activity and expression of the senescence mediators p16INK4a, p21WAF1 and phosphorylated-p53, which results in the inhibition of oxidative stress-induced senescence in NP cells. Following metformin treatment, expression of catabolic enzymes MMP3 and ADAMTS-5 is inhibited and synthesis of type II collagen and aggrecan is increased, maintaining NP cell homeostasis from an ECM perspective (29). However, more investigation is necessary to show how metformin triggers autophagy via the AMPK pathway. Metformin binds to PEN2 and starts a signalling cascade that interacts with the AMPK-activated lysosomal glucose sensing pathway via ATP6AP1 (101). Low doses of metformin activate the PEN2/ATP6AP1/AMPK pathway, while high concentrations of metformin directly block mitochondrial respiratory chain complex I, thereby lowering ATP and increasing AMP to enable AMPK activation (102). To the best of our knowledge, however, there have been no studies that directly show that metformin improves IVDD by activating AMPK by binding to PEN2 (Fig. 3).

Metformin promotes extracellular vesicle (EV) production and inactivates the cGAS/STING signalling pathway via autophagy, thereby improving myeloid cell senescence. EVs are vesicular secretions produced by MSCs that mediate the biological effects of MSCs (134-136). Compared with MSC-based cell treatment, EV therapy provides a number of potential benefits, including decreased manufacturing costs, greater stability and ease of sterilization, storage and infusion therapy (134). EV therapy has been considered a promising alternative strategy for treatment with MSCs. Autophagic activity is associated with EV biogenesis and secretion and the regulation of autophagy may influence EV production (137,138). Metformin, which is an AMPK activator, is associated with autophagy activation (139). In a recent study, Liao *et al* (30) showed that metformin treatment effectively promotes the secretion of EVs *in vitro* and that this process is associated with autophagy activation (30). Metformin activates autophagy in MSCs primarily via AMPK signalling and promotes formation of multivesicular bodies (MVBs) or amphipathic bodies, which is followed by membrane fusion and EV release. In addition, metformin treatment induces phosphorylation of synaptosome associated protein (SNAP29), a key SNARE protein that mediates fusion of MVBs with the plasma membrane, thereby facilitating the release of EVs (30). Liao *et al* (30) also hypothesized that metformin facilitates

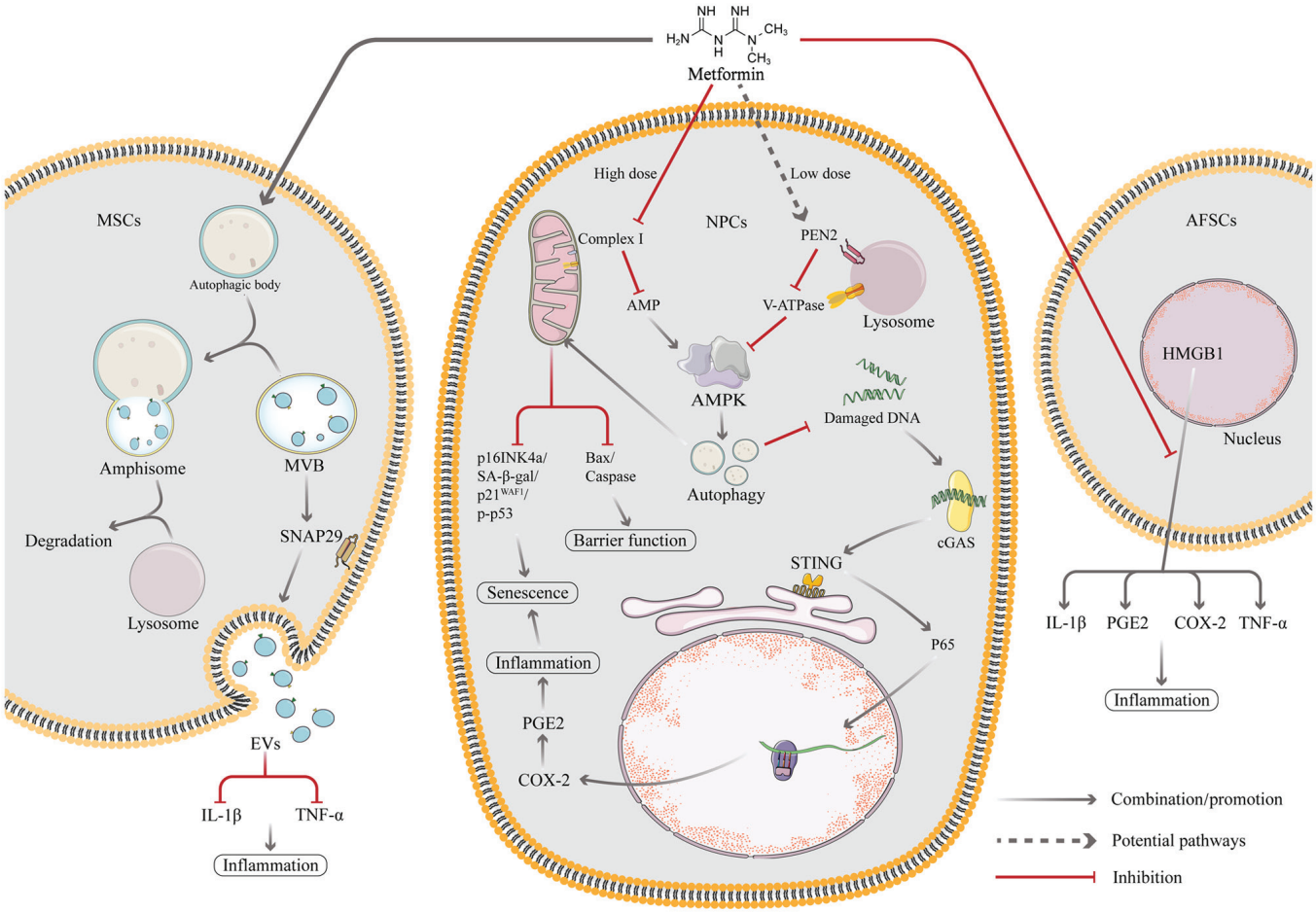

Figure 3. Potential mechanism of metformin in intervertebral disc degeneration. In MSCs, metformin promotes formation of MVB or amphipathic bodies, induces phosphorylation of SNAP29 and promotes EV release. In the NP, high doses of metformin reduce AMP by inhibiting mitochondrial complex I; low doses of metformin may bind to PEN2, and both pathways activate AMPK thereby enhancing autophagy. Autophagy reduces the expression of p16INK4a, SA-β-gal, $p21^{WAF1}$ and p-p53 and inhibits senescence; autophagy also inhibits Bax, Caspase and leads to barrier function. In addition, autophagy reduces the activation of cGAS/STING signaling pathway and phosphorylation of P65, thereby reducing COX2 and the inflammatory mediator PGE2. In AFSCs, metformin inhibits extracellular migration of HMGB1 and decreases expression of inflammatory mediators. Figure adapted from Server Medical Art (https://smart.servier.com/). AFSC, anulus fibrosus stem cells; cGAS, cyclic GMP-AMP synthase; COX2, cyclooxygenase-2; EV, extracellular vesicle; HMGB1, high mobility group box 1; MSC, mesenchymal stem cell; NP, nucleus pulposus; PINK, PTEN-induced kinase; SNAP, synaptosome associated protein; STING, stimulator of interferon genes; MVB, multivesicular body; p-, phosphorylated; SA-β-gal, senescence-associated β-galactosidase; WAF1, wild-type p53 activated fragment-1; PGE2, prostaglandin E2.

release of EVs by altering the expression of inter-α-trypsin inhibitor heavy chain 4, which serves a key role in the regulation of cell proliferation and inflammatory responses. The secretome of MSCs (including EVs) exerts antioxidant and anti-inflammatory effects to rescue resident senescent cells in the IVD; EV treatment reduces the rate of senescent NP cells and delays the progression of IVDD (140).

One of the key indicators of the innate immune response is cyclic GMP-AMP synthase (cGAS)/stimulator of interferon genes (STING) signalling and its primary function is to recognize and respond to cytoplasmic DNA. After cGAS recognizes and binds DNA in the cytoplasm, STING initiates downstream responses (31). Senescent cells secrete a variety of cytokines called the senescence-associated secretory phenotype (SASP). SASP factors affect the intracellular environment and induce local or systemic dysfunction. However, it has been demonstrated that cGAS/STING signalling can stimulate production of inflammatory factors that lead to SASP factor secretion by senescent cells and accelerate ageing (141). Ren *et al* (31) demonstrated that metformin inhibits the activation of the cGAS/STING pathway by activating autophagy, thereby inhibiting the inflammatory response and NP cell ageing and ultimately delaying IVDD (31). During IVDD, release of inflammatory factors is the primary cause of excessive senescence in myeloid cells (31). Several studies have proven that oxidative stress is present in degenerative discs and ROS production disrupts intracellular homeostasis and leads to inflammation and accelerated senescence in NP cells (142-144). The cGAS/STING signalling axis consists of the second messenger loop cGAS of the interferon gene and circulating GMP-AMP receptor-stimulating factor. The inflammatory response and cellular senescence both rely on this pathway, which is part of the innate immune system. cGAS sequesters damaged DNA fragments in the cytoplasm in senescent myeloid cells, which then recruits downstream STING to activate the NF-κB signalling pathway, promoting the release of inflammatory cytokines (145). In addition, the cGAS/STING signalling pathway is a pathway that is responsive to cytoplasmic DNA.

Under normal conditions, intracellular DNA is stable and present in the nucleus and mitochondria (146-148). When DNA is damaged, histone γ-H2AX (DNA damage marker) is phosphorylated and damaged DNA fragments enter the cytoplasm and are recognized by cGAS, thus activating STING and its downstream molecules (149). NF-κB has been identified as a downstream molecule of cGAS-STING. Damaged DNA fragments induce cGAS/STING activation, which phosphorylates P65 and promotes its translocation to the nucleus (150,151). This promotes the production of inflammatory cytokines, which then induce oxidative stress, leading to degradation and destruction of ECM and accelerated cellular senescence (152). The study has also shown that silencing STING gene inhibits senescence and that overexpression of STING promotes senescence in myeloid cells (152), which suggests that cGAS/STING signalling may serve as a novel therapeutic target to inhibit senescence and mitigate the progression of IVDD (Fig. 3).

Metformin inhibits myeloid cell senescence and apoptosis by suppressing the inflammatory response. NF-κB is a key mediator of cellular responses to inflammation; the downstream factor prostaglandin E2 (PGE2) disrupts IVD cell matrix homeostasis, thereby destroying the disc (153,154). There is substantial evidence that inhibiting NF-κB inhibits the inflammatory response and the production of catabolic factors in IVD cells (153,155), thus providing a potential strategy for inhibiting disc degeneration. Unlike conventional non-steroidal anti-inflammatory drugs, metformin does not inhibit COX-2 (PGE2 synthase) through an enzymatic response but rather regulates COX-2 expression via NF-κB (33). Metformin results in a large decrease in nuclear ectopic NF-κB and a significant decrease in PGE2 levels following dual stimulation of mechanical and inflammatory stress (33). The effect of metformin on proinflammatory gene expression is likely due to blockade of NF-κB translocation to the nucleus via the SIRT1/FOXO/Peroxisome proliferator-activated receptor gamma coactivator 1 (PGC1) pathway induced by activated AMPK (33,156).

High mobility group box 1 (HMGB1) is a nuclear protein that binds to DNA and is a cofactor for gene transcription (157). Under normal conditions, HMGB1 is in the nucleus and serves a role in regulating DNA stability and transcription, but it is released into ECM in response to certain stimulatory conditions (such as LPS) (158), which promotes expression of PGE2 and some inflammatory factors (TNF-α, IL-1β and IL-6) (159,160). Metformin exerts antiaging and anti-inflammatory effects by inhibiting HMGB1 translocation and catabolic products of AFSCs. HMGB1 also significantly decreases the levels of SA-β-gal activity, inflammatory factors (TNF-, IL-1 and IL-6), and certain catabolic enzymes (MMP3 and 13) in AFSCs (32) (Fig. 4).

IL-1, which is an inflammatory factor, is key to the development of IVDD (161). AGEs amplify the inflammatory response via the NOD-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome/IL-1β inflammatory response axis, which can lead to IVDD (162,163). In addition, IL-1β leads to a significant increase in ADAMTS and MMP expression, which promotes ECM degradation and leads to IVDD (164,165). Additionally, IL-1β can induce senescence and apoptosis in myeloid cells and inhibit their proliferation (161). It has also been shown that IL-1β increases intracellular ROS levels in murine vertebral MSCs, thereby exacerbating oxidative stress in IVD cells (166). Metformin inhibits IL-1β production by the SIRT3/PINK1/Parkin and the AMPK/SIRT1 pathway (110,114) and inhibits LPS-mediated enhancement of IL-1β in HGFs by increasing ATF3 expression (115). In addition to decreasing IL-1β production, metformin also reduces the effects of existing IL-1β by inhibiting activation of the NLRP3 inflammasome (167). Therefore, metformin may inhibit the proinflammatory effect of IL-1β via several pathways, thus exerting a protective effect against IVDD. In addition, resveratrol inhibits IL-1β-mediated apoptosis in the NP by regulating the PI3K/Akt pathway (168); metformin regulates the PI3K/Akt pathway (169), suggesting that metformin may also inhibit IL-1β-mediated apoptosis in myeloid cells by modulating the PI3K/Akt pathway; nonetheless, additional experiments are required to confirm this hypothesis (Fig. 4).

Metformin can act on IVDD by activating the SIRT signalling pathway. SIRT family is composed of NAD^+-dependent histone deacetylases, which participate in biological processes, such as cell survival, senescence, proliferation, apoptosis, DNA repair, metabolism and caloric restriction (170). SIRT1-7 are considered attractive therapeutic targets for ageing-associated diseases such as T2DM and inflammatory and neurodegenerative disease (171). SIRT1 inhibits senescence and apoptosis in myeloid cells via autophagy. SIRT1 blocks the expression of MMP1 and 3 at the mRNA and protein levels in human dermal fibroblasts (172) and enhances the expression of cartilage-specific ECM genes, such as type II collagen and aggregated proteoglycan (173). SIRT1 inhibits matrix metalloproteinase (MMP) expression and activity, and SIRT1 also promotes the synthesis of matrix components, delaying IVDD through these pathways (174,175). SIRT1 acts on the disc via the PI3K/Akt pathway (74,176). There has been much evidence that metformin has a strong activating effect on SIRT1 (177,178). To the best of our knowledge, however, few studies have shown that metformin affects IVDD through SIRT1 signalling and further investigations still need to be conducted (179,180).

SIRT3 is a key factor for IVDD. A key mechanism of AGE-induced oxidative stress and secondary human NP apoptosis is impairment of SIRT3 activity and the mitochondrial antioxidant network (181). Nicotinamide mononucleotide (NMN) activates SIRT3 via the AMPK/PGC-1α pathways (181,182). By preventing compression-induced mitochondrial division, SIRT3 expression improves mitochondrial function, decreases the formation of ROS (183) and activates the SIRT3/FOXO3/SOD2 signalling pathway; SIRT3 activates transcription factor FOXO3a, which in turn promotes superoxide dismutase 2 (SOD2) synthesis and ROS catabolism (184), which reduces NP cell stress and the degradation of ECM. The activating effect of metformin on SIRT3 is evident, and metformin significantly increases the expression of Histone H3 Lysine 79 trimethylation (H3K79me3) in the SIRT3 promoter region by stimulating AMPK production, thereby increasing levels of SIRT3 (185). In addition, metformin inhibits the inflammatory response in myeloid cells via the SIRT3/PINK1/Parkin signalling pathway (110).

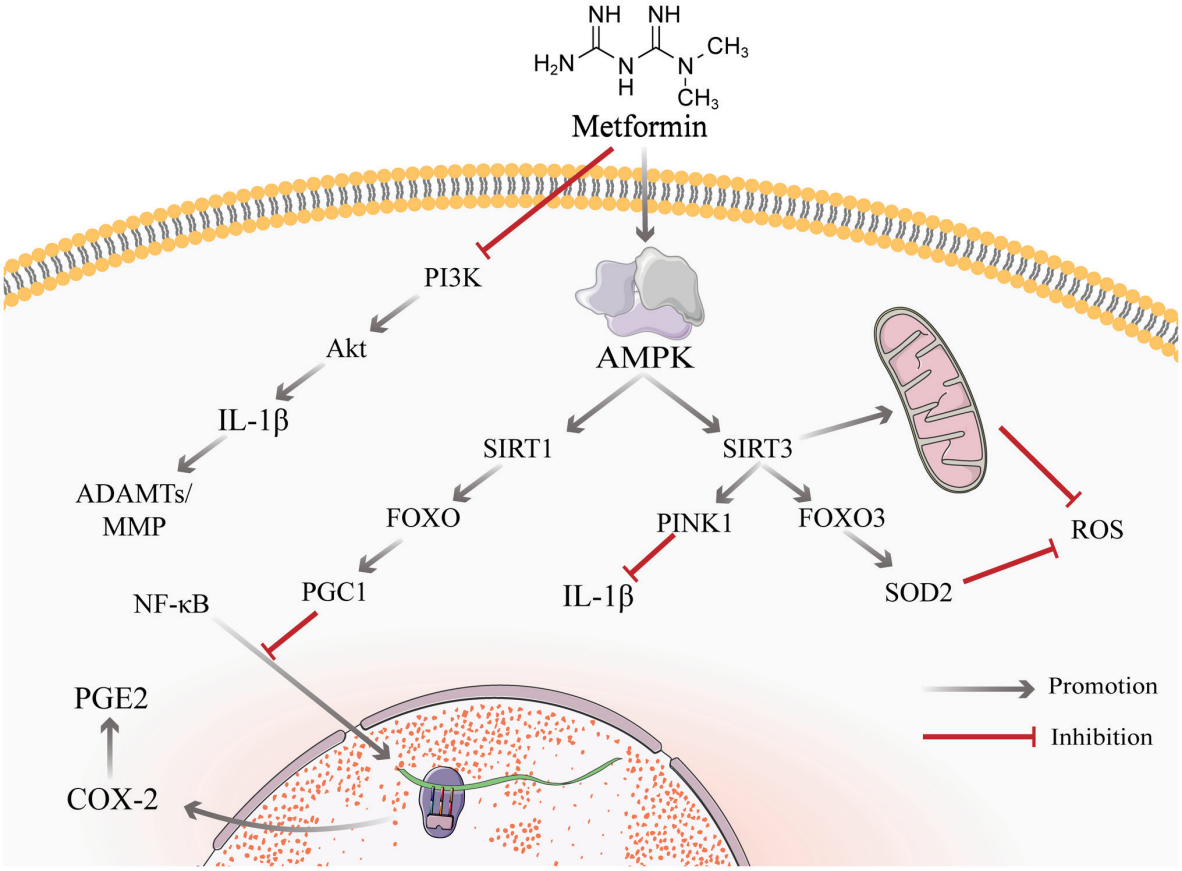

Figure 4. Metformin likely acts on intervertebral disc degeneration through SIRT-related signalling pathways. Metformin reduces ADAMTs and MMPs by inhibiting the PI3K/Akt signaling pathway. Activation of AMPK by metformin reduces COX2 expression by activating the SIRT1 and activates the SIRT3 pathway on the other hand, thereby increasing PINK1, FOXO3, and mitochondrial activity, and consequently decreasing IL-1β and ROS levels. Figure adapted from Server Medical Art (https://smart.servier.com/). COX2, cyclooxygenase-2; PI3K, phosphoinositide 3-kinase; ROS, reactive oxygen species; SIRT, sirtuin; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; AMPK, AMP)-activated protein kinase; SOD, superoxide dismutase; PINK, PTEN) induced putative kinase.

When NAD is used to inhibit the effect of SIRT3, a significant decrease in the effect of metformin also occurs (186). This finding suggests that metformin may act via SIRT3 to improve IVDD.

In senescent NP cells, expression of SIRT6 is decreased, and SIRT6 decreases levels of the replicative senescence marker SA-β-gal, which inhibits IL-1β-induced apoptosis and activates autophagy in NP cells by inhibiting phosphorylation of mTOR; activation of autophagy inhibits stress-induced apoptosis in NP cells and regulates the expression and activity of certain mechanistic catabolic enzymes (187). For example, MMP-3 (Matrix Metalloproteinases) and MMP-13 are enzymes that are frequently regulated during autophagy, and they play important roles in extracellular matrix degradation and interstitial cell remodelling (188). ADAMTS-4 (A Disintegrin and Metalloproteinase with Thrombospondin Motifs) and ADAMTS-5 are typical examples of autophagy-regulated enzymes, and their activity and expression change significantly during articular cartilage and intervertebral disc degeneration (189). Autophagy may affect cell metabolism and survival by regulating the number and function of lysosomes to influence the activity of endogenous mechanolytic enzymes (190). Metformin augments the expression of SIRT6 *in vitro* (191). It has also been shown that SIRT2 can decrease the expression of P21 and P53 and thus inhibit ageing and oxidative stress in NP cells (192), and there is a relationship between metformin and SIRT2 (193). However, whether metformin ameliorates IVDD directly by acting on SIRT-related signalling pathways requires further study.

5. Clinical studies

To the best of our knowledge, the use of metformin in the treatment of IVDD has not been validated by clinical data. However, in clinically relevant conditions, such as obesity, aging and diabetes, that may be a direct or indirect inducer of IVDD, metformin has been used in clinical treatment or trials (24,194,195). Patients experiencing insulin sensitivity and resistance who are overweight exhibit increased weight loss with metformin (196). To the best of our knowledge, few clinical trials directly examine the effects of metformin on human aging (197,198). A randomized placebo-controlled clinical trial of metformin is underway to prevent frailty in elderly patients with poor glucose tolerance and examine the effects of metformin on several markers of aging (199). However, multiple preclinical experiments have established that metformin can be beneficial in treating IVDD (29-34). By promoting autophagy or controlling release of EVs from bone marrow MSCs, metformin prevents apoptosis and senescence in NP cells (29,30). In addition, a randomized, double-blind,

placebo-controlled, crossover trial showed that metformin delayed senescence in the elderly population via metabolic and non-metabolic effects (200), suggesting that metformin plays a role in ageing-related diseases. The Targeting Aging with Metformin (TAME) trial was approved by the Food and Drug Administration (FDA, America) in 2015 (199,200). The TAME trial may make metformin the first approved anti-aging drug; as it is not testing metformin against a single disease, but rather a collection of age-related diseases, it establishes aging as a medical condition that can be intervened in or treated, rather than an irreversible process outside of human control. This shift in the concept of aging may make it easier to conduct future anti-aging clinical trials and the increase in the number of clinical trials associated with cellular senescence as a major contributor to IVDD may facilitate the clinical scope of investigating metformin for IVDD in future (201,202). Metformin is the first-line drug for T2DM, especially for obesity-associated and gestational diabetes (203). Metformin positively regulates obesity, aging and diabetes through AMPK pathway signaling, PI3K/AKT/mTOR pathway phosphorylation, oxidative stress, autophagy and other pathways to alleviate and improve disease progression (194,204). Ramanathan *et al* (33) confirmed that the inflammatory response in IVD treated with metformin is significantly inhibited. In addition, studies have shown that metformin promotes angiogenesis in the injured spinal cord by activating enzyme pathways, thereby improving neurological function after spinal cord injury in aged mice and promoting recovery of motor function following injury (205,206). Although metformin has not yet been used for clinical trials of IVDD, the aforementioned preclinical and clinical trials of diseases closely related to IVDD suggest potential mechanisms.

In clinical practice, metformin is often used as an adjunctive therapeutic agent. For example, metformin has anti-inflammatory properties (165). When combined with drugs that also have certain anti-inflammatory effects, metformin produces significant synergistic anti-inflammatory effects. Metformin and pioglitazone have different mechanisms of action on oxidative stress; combination of these two drugs is more effective in improving oxidative stress (161). Sildenafil + metformin + leucine and metformin + atorvastatin combination showed higher efficacy in lowering oxidative stress and decreasing inflammatory markers compared with each drug alone (162,163). IVDD often occurs in conjunction with DM, aging and inflammation (207). Especially in elderly patients with DM, chronic inflammation and IVDD, metformin can be used to treat DM as well as to slow down the further progression of IVDD. There have been numerous clinical studies of metformin in the treatment of OA (ICTRP, https://trialsearch.who.int/) (Table II), and there are many similarities between IVDD and OA, both in terms of the mechanism of occurrence and at the preventive and therapeutic levels (7,208-210). Specifically, for example, both IVDD and OA involve chronic inflammation and apoptotic processes, and elevated inflammatory markers such as IL-1β, IL-6, and TNF-α, to name a few, are present in patients with both IVDD and OA (211,212). Metformin may have potential for the treatment of IVDD. In the future, whether metformin can be used independently to delay IVDD or as an adjunctive drug to delay IVDD while assisting in the treatment of other diseases, and in what ways its benefits can be increased, need to be corroborated by basic research studies and clinical trials.

6. Perspectives

Although metformin affects IVDD in numerous molecular processes, there are still notable difficulties in its therapeutic use. The first is that the mechanism by which metformin acts on IVDD has not been fully elucidated and confirmed. Metformin has been shown to induce autophagy via AMPK, control MSC EV release and activate the cGAS/STING pathway to prevent apoptosis and senescence in NP cells (29-31). More research is required to understand additional distinct anti-inflammatory and autophagy-activating mechanisms of metformin in IVD cells. In addition, existing studies are cell culture and preclinical studies; to the best of our knowledge, there is a lack large-scale, randomized, double-blind, placebo-controlled clinical trials (29-33). Designing controls is challenging due to the number of potential confounding variables, including sample size, age, presence of other common conditions (particularly diabetes), adherence to treatment and follow-up accuracy. The drug concentration of oral metformin in the target area, feasibility of the local route of administration, the half-life of the drug and effect of the combined drug on efficacy are also factors that need to be explored in clinical experiments.

There are many studies showing that metformin does not lead to lower blood glucose when treating other conditions in non-diabetic patients because metformin does not stimulate insulin secretion (213-215). In recent years, metformin has been studied for the treatment of other clinical conditions, including cancer, heart disease and neurodegenerative disease such as IVDD (24). However, when metformin is used in non-diabetic patients, doctors may closely monitor their blood glucose levels to ensure they are in the normal range as other medications or health conditions may interact with metformin to affect blood glucose (216). Therefore, structure-activity relationship (SAR) or quantitative SAR studies could be considered to enhance the effect of metformin by modifying its structure to some extent and ensuring that it does not lower blood glucose levels.

Following oral administration, bioavailability of metformin is relatively low as it is primarily absorbed from the upper part of the small intestine (217). Incomplete absorption may be ameliorated by use of convenient drug delivery systems, such as bioadhesion and gastric retention drug delivery systems (218). In addition, due to the short biological half-life of metformin, repeated high doses of metformin are required, which usually results in decreased patient compliance and an increased incidence of side effects, such as diarrhoea, nausea and weight loss. Metformin also causes lactic acidosis, which is sometimes fatal (217). It is also necessary to develop new formulation strategies for metformin to improve its bioavailability, reduce the frequency of administration and minimize gastrointestinal side effects and toxicity. Drug delivery systems (such as particles, nanoparticles, liposomes and lipoid) are useful systems to overcome difficulties associated with conventional dosage forms. These systems offer advantages over conventional dosage forms, such as effective protection of the drug from enzymatic degradation, decreased

Table II. Clinical trials using metformin.

Trial ID no.	Title	Recruitment status	Phase	Primary sponsor	Condition	Primary outcome
NCT06328426	Vitamin D With omega-3 or metformin in osteoarthritis	Not recruiting	N/A	Assiut University (Assiut, Egypt)	Osteoarthritis	Effusion-synovitis volume on musculoskeletal ultrasound
IRCT20200522047538N2	Evaluation of the effectiveness of metformin in WOMAC index of overweight knee osteoarthritis patients	Recruiting	3	Mashhad University of Medical Sciences (Mashhad, Iran)	Knee. osteoarthritis	WOMAC index of osteoarthritis before the study and 4 months later
NCT06231758	Metformin safety and efficacy in osteoarthritis	Recruiting	3	PhD Mostafa Bahaa (Damietta, Egypt)	Knee osteoarthritis	Change of VAS pain score from baseline to post-treatment
NCT06126029	Effect of metformin as add-on therapy to ibuprofen on disease activity in knee osteoarthritis patients	Recruiting	2	Bangabandhu Sheikh Mujib Medical University (Dhaka, Bangladesh)	Knee osteoarthritis	KOOS
PACTR202311507335269	Effects of metformin phonophoresis and exercise therapy on knee pain, range of motion, and function in knee osteoarthritis	Not recruiting	4	Cairo University (Cairo, Egypt)	Musculoskeletal disease	Knee pain and function
NCT06096259	Preventing injured knees from osteoarthritis: Severity outcomes	Not recruiting	2	Brigham and Women's Hospital (Boston, United States)	Knee osteoarthritis, post-traumatic osteoarthritis, ACL tear	KOOS; modified MOAKS cartilage score
NCT05638893	Metformin as adjuvant therapy in obese knee osteoarthritis patients	Not recruiting	2/3	Tanta University (Tanta, Egypt)	Knee osteoarthritis	WOMAC, weight
NCT05034029	Effect of metformin on patients with osteoarthritis	Not recruiting	N/A	Guangzhou First People's Hospital (Guangzhou, China)	Osteoarthritis	Change of tibiofemoral cartilage volume and WOMAC from baseline to 24-month follow up
ACTRN12621000710820	Effectiveness of metformin for knee osteoarthritis with obesity-a randomised, double-blind, placebo-controlled trial of a potential disease modifying therapy	Not recruiting	3	Prof Flavia Cicuttini (Melbourne, Australia)	Knee osteoarthritis, obesity	Change in VAS knee pain from baseline to 6-month follow-up

Table II. Continued.

Trial ID no.	Title	Recruitment status	Phase	Primary sponsor	Condition	Primary outcome
NCT04767841	The AMPK modulator metformin as a novel adjunct to conventional therapy in patients with knee osteoarthritis	Recruiting	1/2	Sadat City University (Sadat, Egypt)	Knee osteoarthritis	VAS assessment of pain
RCT20150202020908N2	Evaluation of the molecular effects of metformin and improvement of symptoms in osteoarthritis patients	Not recruiting	2/3	Shiraz University of Medical Sciences (Shiraz, Iran)	Knee osteoarthritis	WOMAC questionnaire score at the beginning of the study and 16 weeks after starting metformin treatment
ChiCTR2000037081	Clinical study of autologous fat glue alone or combined with metformin in the treatment of knee osteoarthritis	Recruiting	0	Xinhua Hospital Affiliated to Medical College of Shanghai Jiaotong University (Shanghai, China)	Osteoarthritis	Weight, MRI, DNA methylation, knee function score, telomerase
ChiCTR2000036958	Clinical study of autologous adipose derived mesenchymal stem cells alone or in combination with small molecule drugs in the treatment of osteoarthritis	Recruiting	0	Xinhua Hospital Affiliated to Medical School of Shanghai Jiaotong University (Shanghai, China)	Osteoarthritis	MRI, DNA methylation, knee function score, telomerase

WOMAC, Western Ontario and McMaster Universities osteoarthritis index; VAS, visual analog scale; KOOS, knee injury and osteoarthritis outcome score; MOAKS, Magnetic resonance imaging osteoarthritis knee score; ACL, Anterior cruciate ligament.

drug side effects, improvement of patient comfort and compliance, control or site of drug release and improvement of relative bioavailability of the drug (219). In particulate dosage form, nanoparticle drug delivery systems are unique and may enhance targeted delivery of metformin to specific sites of action, which has been used as a promising approach for the treatment of cancer (220). However, whether metformin in the form of particles and nanoparticles as carriers is potentially important for its goal of delaying IVDD needs to be further investigated. Additionally, adverse medication reactions, particularly gastrointestinal symptoms, need to be detected and assessed further before metformin is used in the clinical treatment of IVDD. Besides, the combination of metformin with other drugs also needs to be considered in terms of drug interactions and adverse effects, as well as whether metformin can be used clinically for the treatment of IVDD. Specific experiments need to be designed to validate this on a case-by-case basis, as well as more basic research.

7. Conclusion

Globally, IVDD has notable health implications. For example, IVDD can lead to low back or neck pain (212), IVDD may lead to reduced disc height and spinal deformity, which can affect a patient's motor function (221), and IVDD may also lead to nerve compression, causing neurological symptoms such as sciatica and myelopathy (222). Metformin, as a first-line drug for the treatment of T2DM, may also contribute to the treatment of IVDD. The present review summarizes potential mechanisms of metformin for the treatment of IVDD. Metformin induces autophagy via the AMPK pathway and inhibits senescence and apoptosis of NP cells (29). Metformin promotes the production of EVs through autophagy and inactivates the cGAS/STING signalling pathway, improving the senescence of myeloid cells (30,31). Moreover, it has been shown that metformin inhibits senescence and apoptosis of medullary cells by suppressing inflammatory responses (32,33). Finally, metformin may act on IVDD by activating the SIRT signalling pathway, providing a theoretical basis for more basic and clinical studies of metformin in IVDD and associated disease. For elderly patients with both diabetes and IVDD-related diseases, metformin may serve as an adjunctive medication for the treatment of IVDD. The present review provides direction and for future preclinical and clinical studies of metformin in IVDD.

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Authors' contributions

HT, JL and WY conceived the study. WY, YY, YW and ZG wrote and edited the manuscript. ZG and WY constructed figures and tables. JZ, WG and YC performed the literature review. YL, HW, LZ and YW revised the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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