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Review Article

The Contributive Role of IGFBP-3 and Mitochondria in Synoviocyte-Induced Osteoarthritis through Hypoxia/Reoxygenation Injury: A Pathogenesis-Focused Literature Review

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Osteoarthritis (OA), one of the most common joint disorders, is characterized by chronic progressive cartilage degradation, osteophyte formation, and synovial inflammation. OA lesions are not only located in articular cartilage but also in the entire synovial joint. Nevertheless, most of the early studies done mostly focused on the important role of chondrocyte apoptosis and cartilage degeneration in the pathogenesis and progress of OA. The increased expression of hypoxia-inducible factors (HIF-1α and HIF-2α) is known to be the cellular and biochemical signal that mediates the response of chondrocytes to hypoxia. The role of the synovium in OA pathogenesis had been poorly evaluated. Being sensitive to hypoxia/reoxygeneration (H/R) injury, fibroblast-like synoviocytes (FLS) play an essential role in cartilage degradation during the course of this pathology. Insulin-like growth factor binding protein 3 (IGFBP-3) acts as the main carrier of insulin-like growth factor I (IGF-I) in the circulation and remains the most abundant among the six IGFBPs. Synovial fluids of OA patients have markedly increased levels of IGFBP-3. We aim to discuss the interconnected behavior of IGFBP-3 and synoviocytes during the course of osteoarthritis pathogenesis, especially under the influence of hypoxia-inducible factors. In this review, we present information related to the essential role that is played by IGFBP-3 and mitochondria in synoviocyte-induced osteoarthritis through H/R injury. Little research has been done in this area. However, strong evidences show that the level of IGFBP-3 in synovial fluid significantly increased in OA, inhibiting the binding of IGF-1 to IGFR 1 (IGF receptor-1) and therefore the inhibition of cell proliferation. To the best of our knowledge, this is the first paper providing a comprehensive explanatory contribution of IGFBP-3 and mitochondria in synovial cell-induced osteoarthritis through hypoxia/reoxygenation mechanism.

1. Introduction

Osteoarthritis (OA), also known as degenerative joint disease or osteoarthrosis, is the most common form of arthritis and the leading source of physical disability with severely impaired quality of life in people in industrialized nations [1]. OA was first differentiated from other forms of joint disease at the beginning of the 20th century, on the basis of the hypertrophic changes seen in bone [2], encouraging scientists to focus more on osteology with the aim of providing further insights into the disease [3]. Osteoarthritis, although derived from the Greek words *osteon* for bone, *arthron* for joint, and the suffix *-itis* for inflammation, the site of the most pronounced structural alterations is not the bone but the joint cartilage, and severe inflammation is seen in only few patients [1]. Biochemical processes involving tissues, ligaments, bones, and muscles

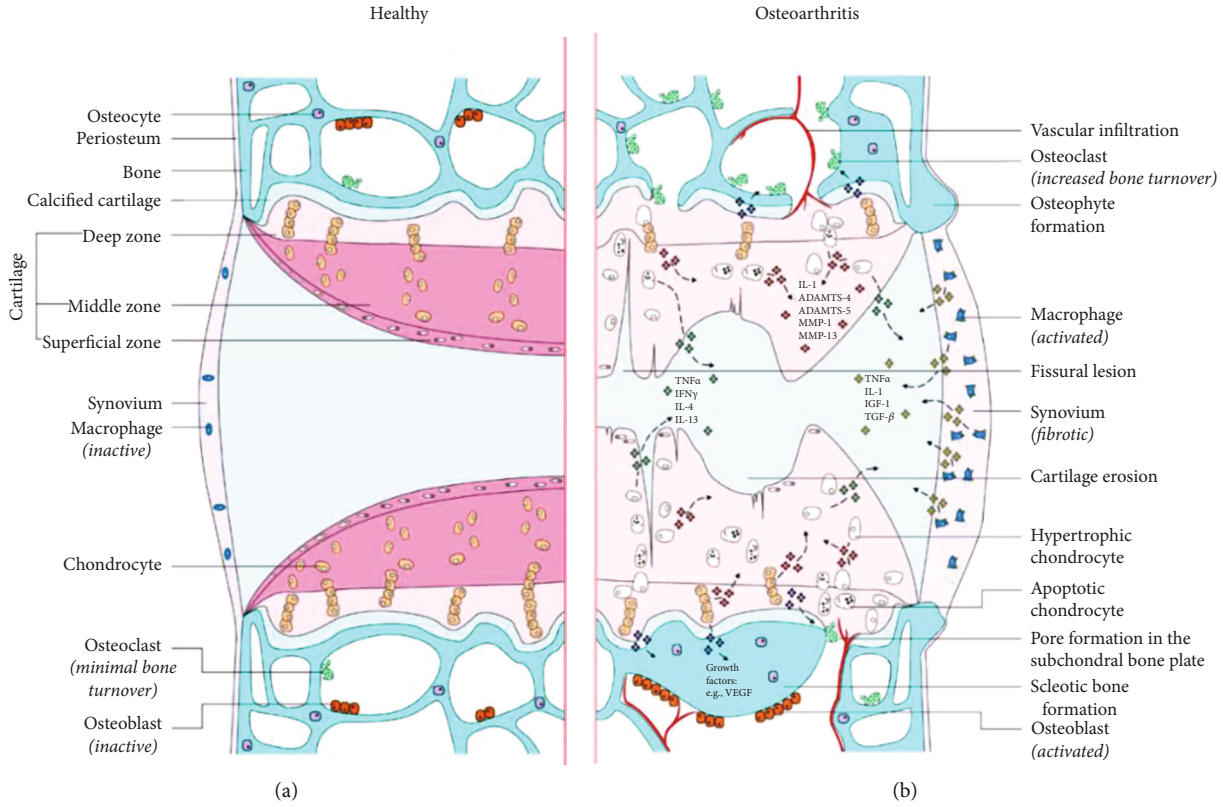

Figure 1: Schematic representation of the differences between (a) a healthy joint and (b) an osteoarthritic joint (*reprinted from Glyn-Jones* et al. [5] *and reused with permission from Elsevier under license number 4820090120408*). (a) In a healthy condition, chondrocytes produce substances that provide lubrication and reduce friction during joint articulation. (b) In osteoarthritic state, the joint changes pathologically due to alterations of multiple cell types and there is activation of synovial macrophages. Abbreviations: ADAMTS = a disintegrin and metalloproteinase with thrombospondin-like motifs; IL = interleukin; MMP = matrix metalloproteinase; TNF = tumor necrosis factor; IFN = interferon; IGF = insulin-like growth factor; TGF = transforming growth factor; VEGF = vascular endothelial growth factor.

eventually intertwine and collectively damage all joint compartments [1], resulting in a cascade of events including alterations of the synovium on both morphologic and biochemical levels as shown in Figure 1 and very well depicted by Glyn-Jones et al. in one of their relevant publications [1, 4, 5]. Generally, the process of joint destruction can always be evaluated for the pathogenesis ("typing"), for its extent ("staging"), and for the degree of the most extensive focal damage ("grading") [1]. While the "typing" of the disease is either idiopathic ("primary") or posttraumatic ("secondary"), its "grading" and "staging" have been much under debate [1]. This ongoing debate has brought forth proposal of several systems by eminent scientists or research groups including Pritzker and colleagues [6], Outerbridge [7], Otte [8], and Gelse et al. [9].

Systemic and local biomechanical factors affect the likelihood for a joint to develop OA [3]. Strong and irrefutable evidences show that osteoarthritis is a disease with a variety of pathophysiologic drivers leading to multiple phenotypes including inflammatory OA, cartilage-driven OA, traumatic/acute OA, and bone-driven OA [10]. In some cases, patients may present an overlap of more than one phenotype during the clinical course of their pathology. However, let us note that each OA phenotype may potentially be treated differently, and this might pave the way for methodologies of developing stratified medicines and phenotypical regimens for OA patients. Although estimates of the OA prevalence and incidences have varied across studies, there is an undeniable fact that adults are the most affected [11].

The quest to understand the pathophysiology of OA had previously focused on cartilage and periarticular bone studies as OA had been principally regarded to be a disease of cartilage. Hypoxia-inducible factors (HIF) play a key role in the breakdown of cartilage during OA. Findings show that the expression of HIF-1α and HIF-2α is significantly upregulated in osteoarthritic cartilages to mediate the response of chondrocytes to hypoxia [12–15]. However, nowadays, mounting and undeniable findings allow us to scientifically and clinically acknowledge that OA does not only affect the cartilage but the whole joint, including cartilage, bone, and synovium, with each of these components playing a critical role in the pathogenesis and the course of the disease [16]. Synovial fluids of OA patients have markedly increased levels of insulin-like growth factor binding protein 3 (IGFBP-3) [17]. The investigation of synoviocytes' behavior during the course of osteoarthritis pathogenesis has become an area of interest for many scientists.

This review presents general knowledge on OA and the sources and functions of reactive oxygen species/oxidative stress in the synovium. It highlights the impact of synovitis in OA, with evidence implicating synovial cell responses to

cytokines in the pathogenesis of OA. We will discuss recent development in the best of our understanding of the role of IGFBP-3 in synoviocyte-induced OA under hypoxia/reoxygenation conditions.

2. Methods

A comprehensive literature review was conducted in order to identify studies and analyze findings that discuss the pathogenic role of IGFBP-3 in synoviocyte-induced OA in a hypoxic state. The methodology used here was adapted from the one previously used by Leonardi et al. [18].

2.1. Search Strategy. We performed an electronic search by looking into several databases including MEDLINE and Embase via OvidSP, Scopus, and Google Scholar, from inception up to January 31, 2020. Electronic search strategy consisted of keywords such as "osteoarthritis AND IGFBP-3" and "Osteoarthritis and Hypoxia/Reoxygenation OR hypoxy-inducible factor" with the following limits activated: synoviocytes and mitochondria. Related publication links from the relevant papers and references of identified citations were manually used to further retrieve additional original articles that were not captured by the primary electronic searches. The search and selection of papers were restricted to documents written in English.

2.2. Selection of Studies. Any published study or paper in English was considered for inclusion especially if in addition to "osteoarthritis" one or more of the following keywords, "IGFBP-3," "Fibroblast-like synoviocytes," "hypoxia/reoxygenation," and "hypoxia-inducible factors," constituted the main focus of the study. Eligible studies were considered if they included a randomized control cohort and case cohort. In case a study was included in more than one publication, only the available full-text publication was considered.

2.3. Eligibility Assessment and Data Extraction. The first two authors (DG and GAB) independently performed the literature search and carried out the data extraction. Agreement by consensus was used to solve any discrepancies between the two authors. Study design, research objectives, osteoarthritis state, and study findings were the main features of data extraction performed by the two authors.

3. Results

3.1. Number of Retrieved Publications. The primary and secondary searches identified 624 articles. Titles of all articles were reviewed, search results were screened, and 110 duplicates were removed. However, 481 publications were excluded for several reasons: articles not published in English, published papers from non-peer-reviewed journals, abstract and posters from conference presentations, editorials, studies without control cases, papers with no access to full text, case reports, clinical trial protocols, studies with wrong comparator, and studies focusing on concepts other than synoviocytes, IGF binding protein 3, and hypoxia-inducible factors. Afterwards, we identified 33 articles, which we considered deemed relevant for the focus on this specific topic.

3.2. Major Joint Tissues Involved in Osteoarthritis. According to the American College of Rheumatology (ACR) criteria, OA is clinically characterized by joint pain, tenderness, crepitus, stiffness and limitation of movement with occasional effusion, and variable degrees of local inflammation [19]. The pain in OA is frequently activity related; and constant pain usually becomes a feature later in the disease [20]. The OA-related pain is not simply attributable to the structural changes in the affected joint but a result of intermovement between structural change, peripheral and central pain processing mechanisms. Additionally, damage to cartilage, chondrocytes, and menisci gives debris to the synovium that in turn initiates the recruitment of inflammatory mediators, which again increase responsiveness to synovial nerve endings to heighten OA pain.

3.2.1. Types of Synovial Joints. The human body is composed of several types of joints. According to their structural classification, they are divided in three types, namely, fibrous, cartilaginous, and synovial joints. However, based on the degree of the movement permitted, they are categorized as synarthrosis (immoveable), amphiarthrosis (slightly moveable), and diarthrosis (freely moveable) [21]. Sutures, gomphoses, and syndesmoses are the three types of fibrous joints. They are joints where the adjacent bones are strongly and directly connected to each other by fibrous connective tissue. The cartilaginous joints are subdivided into two, namely, synchondrosis and symphysis. They lack a joint cavity and involve bones that are joined together by either hyaline cartilage (synchondrosis) or fibrocartilage (symphysis) [21, 22]. Identified as the most common type of joints, synovial joints are associated as the most weight-bearing joints [22]. As intricate structures, these joints are composed of articular cartilage, synovial membranes, ligaments, and an articular capsule that is characterized by the presence of a lubricating synovial fluid. Structurally, they are the most complex and are most likely to develop uncomfortable and crippling dysfunctions. Each of the different types of synovial joints allows for specialized movements that permit different degrees of motion [21]. Based on the anatomical structure of the joints and the synergy of their movement, synovial joints are subclassified into six types: pivot (between C1 and C2 vertebrae), hinge (elbow, knee), condyloid (wrist), saddle (trapeziometacarpal joint), plane (between tarsal bones), and ball and socket (shoulder, hip) [22]. Their mobility makes the synovial joints especially important to the quality of life. The bones of a synovial joint are covered by a layer of hyaline cartilage that lines the epiphyses of joint ends of bone with a smooth and slippery surface [21].

3.2.2. Clinical Features of OA by Joint Site. Classically described as slowly progressive and the most common form of arthritis, OA is an irreversible disease of articular joints leading to pain and loss of joint function. Based on clinical features, the cause and prevalence of osteoarthritis at different joints differ from one site to another (Table 1). Each site-joint osteoarthritis often presents its own distinct features [23, 24].

Table 1: Clinical features of OA by joint site.

Site-joint OA	Characteristics	Ref.
Knee OA	Knee osteoarthritis is very common, comprising the largest proportion of all cases and affecting 12.4 million (33.6%) adults over the age of 65. There are five phenotypes: (a) minimal joint disease phenotype, (b) strong muscle phenotype, (c) nonobese and weak muscle phenotype, (d) obese and weak muscle phenotype, and (d) depressive phenotype.	[23, 25]
Hip OA	Hip osteoarthritis stands for 13% in osteoarthritic patients and a major cause of pain and disability in the elderly population. Three different subtypes (normotrophic, hypertrophic, and atrophic) of hip OA have been considered nowadays.	[24, 26]
Shoulder OA	Shoulder OA is the final diagnosis in 5% of those who report shoulder pain, affecting up to 32.8% of patients over the age of sixty years. Its prevalence increases with age, and women appear to be more susceptible than men.	[24, 27]
Hand OA	Hand OA affects 26% of women and 13% of men over the age of 71.	[28].
Ankle OA	Ankle OA has a prevalence of less than 1% of the world's adult population. Approximately 30% of ankle OA cases are idiopathic and affect a relatively younger population as compared with other OA joint afflictions.	[24, 29]
Elbow OA	OA is far less common at the elbow than at the other upper limb joints and even seems rare. Symptomatic elbow OA is a relatively rare condition that comprises only up to 2% of patients with elbow arthritis and almost exclusive to males. According to the joint side involved, the elbow OA can be categorized as humeroradial OA and humeroulnar OA.	[24, 30]
Lumbar spine OA	Lumbar spine osteoarthritis (OA) is very common, with estimates of prevalence ranging from 40 to 85%. Facet joint osteoarthritis (FJOA) is a common disease widely prevalent in older adults causing low back and lower extremity pain.	[31, 32]
Temporomandibular joint OA	Little focus is given to the incidence of temporomandibular joint (TMJ) OA, although it may lead to dental malocclusion and reduced health-related quality of life. In an age group of 9-90 years, the percentage of TMJ OA ranges from 28% to 38% and the incidence increases with advancing age.	[33, 34]

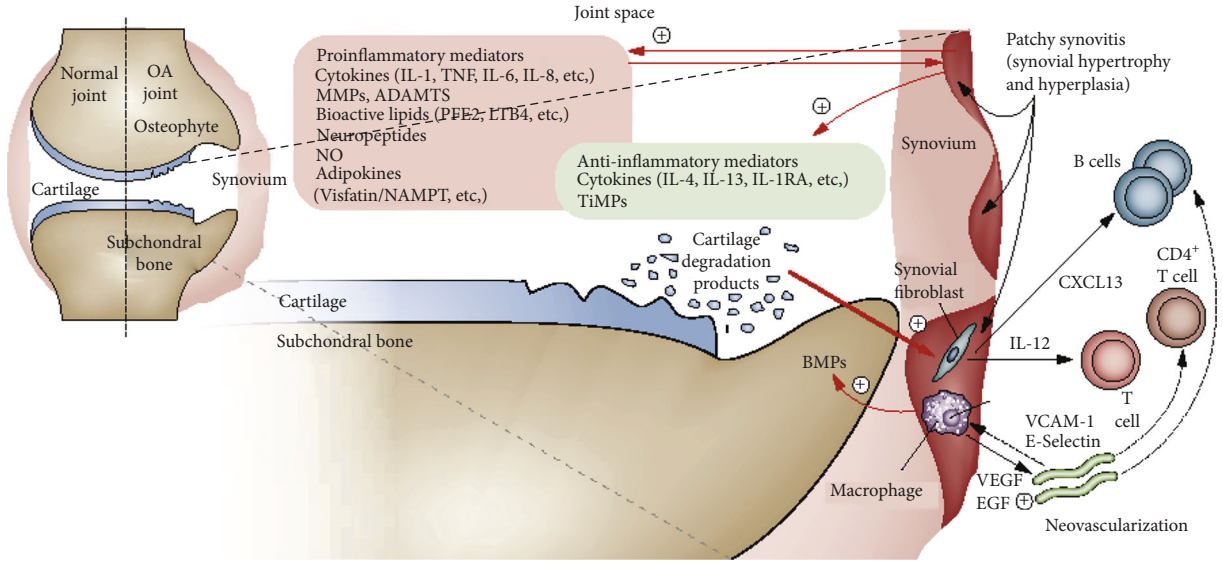

Figure 2: Involvement of the synovial membrane in OA pathophysiology (*reprinted from Sellam and Berenbaum* [4] *and reused with permission from Nature Springer under license no. 4820091329692*). The type A synoviocytes phagocytosed the cartilage breakdown products that are released into the synovial fluid, amplifying synovitis. This process will lead to the production of anti-inflammatory substance and to the formation of osteophytes via the bone morphogenetic protein (BMP). Abbreviations: CCL2: CC-chemokine ligand 2; CXCL13: CXC-chemokine ligand 13; EGF: endothelial growth factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; IL-1Ra: IL-1 receptor antagonist; LIF: leukemia inhibitory factor; LTB4: leukotriene B4; NAMPT: nicotinamide phosphoribosyl transferase; NO: nitric oxide; NGF: nerve growth factor; PGE2: prostaglandin E2; TIMP: tissue inhibitor of metalloproteinase; TNF: tumor necrosis factor; VCAM-1: vascular cell adhesion molecule 1; VEGF: vascular endothelial growth factor.

3.3. Role of Synovium in the Pathology of Osteoarthritis. The synovium is a major part of the joint; therefore, its inflammation plays an essential role in the course of the disease. Undeniable evidences of the role of synovitis in OA are now widely accepted and available in medical literature. Sellam and Berenbaum, in one of their OA-related papers, have well summarized the evidence of the role of synovitis in OA. The findings showed that the role of synovium inflammation

Table 2: Major histopathological features of the four patterns of OA-associated synoviopathy in comparison to each other and to normal synovium (adapted from Oehler et al. [38]).

	Normal	Hyperplastic synoviopathy	Inflammatory synoviopathy	Fibrotic synoviopathy	Detritus-rich synoviopathy
Villous hyperplasia	−	**++(+)**	++(+)	++(+)	++(+)
Synovial lining—proliferation	−	+	++	++	++(+)
Synovial lining—activation	−	+	++	+	+
Fibrinous exudate	−	−	(+)	+	**++(+)**
Capsular fibrosis	−	−	(+)	**+++**	+++
(Macromolecular) cartilage and bone debris	−	−	(+)	−	**+++**
Granulocytic infiltrate	−	−	−	−	+
Lymphoplasmocellular infiltrate—diffuse	−	−	**++**	(+)	+(+)
Lymphoplasmocellular infiltrate—aggregates/follicles	−	−	**++**	(+)	(+)
Stage of the disease		Early stage	Early and late stage	Late stage	Late stage

Note: −: negative; +: positive; ++: moderate; +++: excessive; (+): activated. Bold data indicate key diagnostic criteria.

can be undeniably proven from five levels of evidence: (i) clinical, (ii) imaging, (iii) histological, (iv) molecular, and (v) biological markers [4]. Type A synoviocytes (macrophage-like cells) and type B synoviocytes (synovial fibroblast) are the two major types of cells found within the synovium. The responsibility of the former type of cells lies in the fight against pathogens by producing and releasing specific substances, which in turn are involved in the inflammation and cartilage degradation [35]. Over the course of the pathology, they either exhibit a proinflammatory M1 phenotype (early stage) or anti-inflammatory M2 phenotype (latter stage) [36]. Synovial fibroblasts, along with other cell types such as chondrocytes, are presumably responsible for hyaluronan secretion. They are proven for acting as a barrier that keeps synovial fluid in the joint capsule [35]. These two types of synoviocytes both function as integral players in their physiological state and power to maintain a healthy environment. In OA progression, the alteration of their cellular functions may result from a pivotal role of synovitis in OA pathogenesis [4]. In synovitis, upregulated factors such as interleukin (IL), matrix metalloproteinases (MMPs), and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTs) induce the production of anti-inflammatory mediators. Moreover, there is formation of osteophytes via bone morphogenetic protein (BMPs) (Figure 2) [4].

Synovitis has long been an indicator of rheumatoid arthritis; research findings have now proven its participation and impact in OA [35]. Synovitis is considered to be associated at any stage in OA pathogenesis and therefore considered as a predictor of disease progression [4]. Since the past decade, undisputable evidence shows synovitis to be associated with greater symptoms such as pain and degree of joint dysfunction and may promote more rapid cartilage degeneration in OA [6, 37]. In OA synovial specimens, scientists have identified four patterns of OA-associated "synoviopathy" including (i) hyperplastic, (ii) fibrotic, (iii) detritus-rich, and (vi) inflammatory (Table 2) [38].

3.4. Pivotal Role of Mitochondria in Osteoarthritis Pathogenesis through H/R Injury. Known as the powerhouse of the cell, a mitochondrion is a platform of cell signaling and decision-maker of cell death. It modulates cell metabolism, reactive oxygen species (ROS) genesis, cell apoptosis, and Ca^{2+}. Mitochondria perform oxidative phosphorylation (OXPHOS) via election transport chain (ETC) reaction to synthesize ATP [39]. Ischemia-reperfusion (I-R) and hypoxia/reoxygenation (H/R) mechanisms are two distinct mechanisms that alter mitochondrial functions. These two expressions are sometimes interchangeably used by researchers and scientific writers. However, they are two distinct pathophysiological phenomena, although the clinical outcome from these two distinct events might look the same, resulting in cell death (Figure 3) [39]. Hypoxia is a condition in which the body or one of its regions is deprived of adequate oxygen supply while ischemia is a reduction of blood supply to tissues, causing a limitation of oxygen and glucose required for the metabolism. Ischemia always results in hypoxia; however, hypoxia can occur without ischemia. Outcomes of such insults are variable depending on the type and severity of the insult [40]. For instance, when ischemia is severe and prolonged, the loss of ATP and metabolic alterations induce an inevitable cell necrosis. However, if ischemia is short and transient, activation of prosurvival signals increases myocardial tolerance against subsequent ischemia [40].

Mitochondrial function serves as a key effector in the pathways and a mediator for the protective effect that short periods of hypoxia-reoxygenation and some drugs provide against tissue injury caused by subsequent prolonged hypoxia-reoxygenation (preconditioning) [41]. They induce an array of alterations in mitochondrial metabolic function, and therefore, these changes in mitochondrial structure integrity are widely believed to be important pathogenic factors that underlie ischemic cell injury in various tissues [41]. During hypoxia at mitochondrial permeability transition

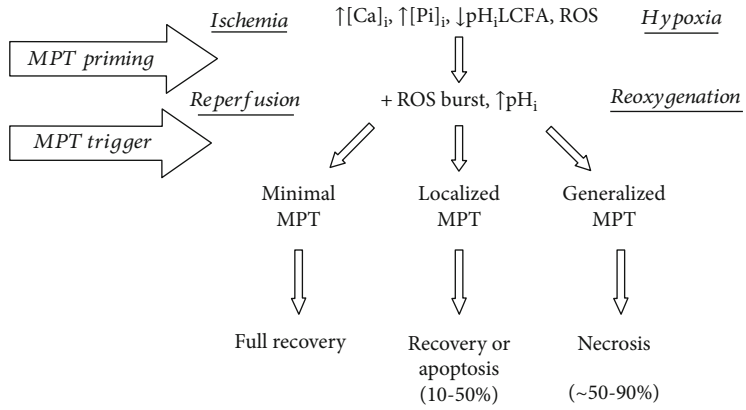

Figure 3: MPT and cell fate during ischemia-reperfusion and hypoxia/reoxygenation (*adapted from Weiss et al.* [39]). MPT occurs during ischemia or hypoxia injury. Cytosolic Ca^{2+} becomes elevated and is driven into the matrix. During mitochondria depolarization, accumulated matrix Ca^{2+} flows down its electrochemical gradient back into the cytoplasm. Cell fate depends on the degree of mitochondria depolarization. Abbreviations: Ca: calcium; LCFA: long-chain fatty acids; MPT: mitochondrial permeability transition; Pi: inorganic phosphate; ROS: reactive oxygen species.

(MPT) priming phase, accumulation of intracellular $Ca2^{2+}$, long-chain fatty acid (LCFA), ROS, and inorganic phosphate (P_i) promotes mitochondrial permeability transition pore (MPTP), which is a high conductance channel in the inner mitochondrial membrane (IMM) (Figure 3).

Because O_2 is used as a substrate by mitochondria, during hypoxia their respiration is inhibited. However, during reoxygenation, rapid restoration of respiration results in increased mitochondrial ROS production [41]. Oxidative stress and especially O_2^- cause synovial cell apoptosis in vitro through mitochondrial injury [42]. Likewise, NO reduces the survival and induces cell death of OA synoviocytes by regulating mitochondrial functionality [43]. However, high NO levels can induce synovial cell apoptosis only when cell capacities to repair DNA damage are exceeded [a], through activation of caspase-3, caspase-9, and MAPK and upregulation of COX-2 expression [44]. In the IMM, there is a high conductance channel known as the mitochondrial permeability transition pore (MPTP), and an increased ROS production and calcium dysregulation are likely to contribute to its opening [45]. Several published data reported that there are four types of K^+ channels localized in the IMM: ATP-sensitive K^+ channel (K_{ATP} channel), Ca^{2+}-activated K^+ channel (K_{Ca} channel), voltage-gated Kv1.3 K^+ channel, and twin-pore domain TASK-3 K^+ channel [46]. Findings have claimed that the ATP-activated K^+ channels (K_{ATP}) and Ca^{2+}-activated K^+ channels (K_{Ca}) are present in the IMM and display changes in activity during H/R injury [47, 48].

ROS and reactive nitrogen species (RNS) have been associated in the process of matrix and cell component degradation in OA and may play a critical role in the pathogenesis of OA. Human chondrocytes cultured from OA patients express inducible nitric oxide synthase (iNOS) and produce significant amounts of NO [1], even though the mechanisms by which NO could contribute to OA pathogenesis are still hypothetical and still under investigation from various clinical and laboratory perspectives. Neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS) are the three recognized isoforms of NOS [49]. The existence of mitochondrial NOS (mtNOS) is still a subject of debate as no specific gene for mtNOS has yet been validated [50]. The essential participation of iNOS expression and the subsequent increase of NO in the pathogenesis of OA are corroborated by *in vivo* experiments demonstrating that specific inhibition of iNOS results in decreased production of catabolic factors such as IL-1β, MMPs, and peroxynitrite [51]. OA synoviocytes produced low nitrite levels spontaneously under basal normoxic conditions, and studies revealed that under H/R conditions, there is an induction of $^{\cdot}NO$ metabolism in OA synoviocytes, which is shown by increased iNOS expression and nitrite production [52–54]. Thus, RNS and ROS are two key areas in which scientists could offer deeper investigation in order to elucidate the pathogenesis and molecular biology of OA.

3.5. Role of IGFBP-3 in Synoviocyte-Induced Osteoarthritis

3.5.1. Overview of IGF and IGFBP Family. The insulin-like growth factor (IGF) signaling pathway is a well-defined system playing an essential role in regulating proliferation, differentiation, and apoptosis in mammalian organisms [55]. This system involves the complex coordination of growth factors (IGF-I and IGF-II), cell surface receptors (IGF-IR, IGF-IIR, and the insulin receptor (IR)), high-affinity binding proteins (IGFBP-1 to 6), IGFBP proteases, and several low-affinity IGFBP-related proteins (IGFBP-rP1 to 10) [55] (Figure 4). IGF-I plays specialized roles at different stages of life. Until pubertal life stage, IGF-1 stimulates the linear growth of bones by increasing the proliferation of epiphyseal chondrocytes and remodeling processes within the growth plate cartilage. At adulthood stage, its role is crucial for maintaining homeostasis in articular cartilage, by stimulating the production of matrix proteins through chondrocytes, counteracting their degradation, and preventing cell death [56, 57].

The bioactivity of IGF is not only dependent on interaction with IGFRs but also by the multifunctional family of

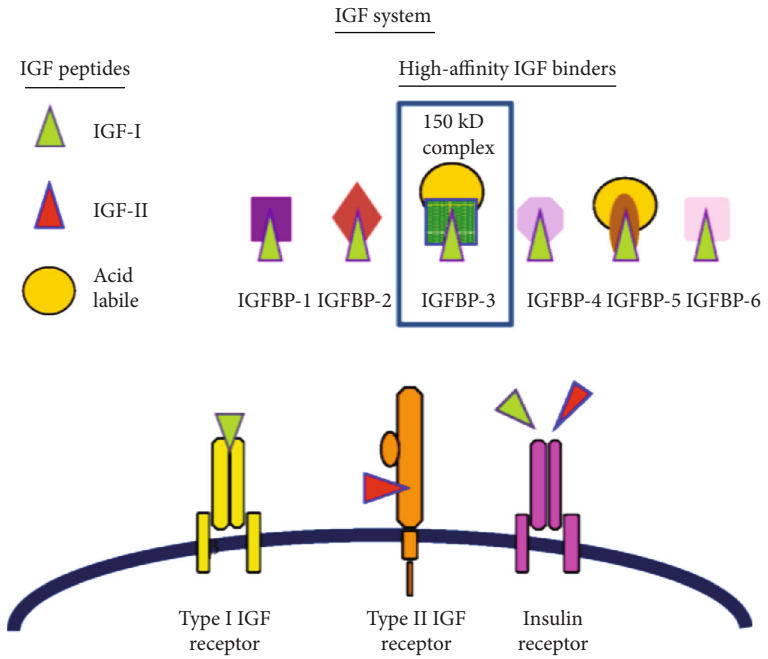

Figure 4: Schematic diagram of the IGF system. The IGF system is composed of several constituents including IGF-I, IGF-II, their respective receptors, and insulin receptor. In addition, there are 6 high-affinity binding proteins. IGFBP-3 binds to IGF-1 in complex with acid labile subunit. Abbreviations: IGF: insulin-like growth factor; IGFBP: insulin-like growth factor binding protein (*reused with permission from Garza* [59]*, author of the dissertation entitled "Insulin-like growth factor binding protein-3 (IGFBP-3) plays an essential role in cellular senescence: molecular and clinical implications"*).

IGFBPs. Based on their primary structure and their post-translational modifications, IGFBPs are differentially tissue targeted. Among the six known IGFBPs, IGFBP-2, IGFBP-3, and IGFBP-4 are known to be secreted by articular cartilage or chondrocytes, with IGFBP-3 being the predominant one and responsible for carrying 75% of IGF-I and IGF-II in the heterotrimeric ternary complex with an acid-labile subunit [58].

3.5.2. IGFBP-3 in Synoviocyte-Induced Osteoarthritis through H/R Injury. Among the high-affinity binding proteins of the IGF system, IGFBP-3 remains the best and extensively studied protein. Strong evidence exists to support the striking versatility of action of this protein, based on the fact that IGFBP-3 can not only act as a modulator of IGF action but also as an independent ligand to promote intracellular signaling [60]. The activity of IGFBP-3 has been studied to a certain extent as this protein has been implicated in the pathogenesis of a number of different pathologies including osteoarthritis [61], asthma [62], cancer [49], fetal trisomy 21 [63], and depressive disorder [64].

Traditionally considered not associated with transient episodes of ischemia and/or hypoxia, osteoarthritis is nowadays receiving great attention as a clinical manifestation of I-R and/or H/R injury [17, 54, 65]. Hypoxia is recognized as an important feature of the joint microenvironment, especially in the perpetuation of joint destruction in OA [54].

Highly sensitive cells to H/R, FLS are considered associated with cartilage degradation during osteoarthritis pathogenesis [17]. Hypoxia-inducible factor (HIF) family members (HIF-1α, 2α, and 3α) are the principal mediators of hypoxic response. Transcription of HIF-1α is highly expressed in OA cartilage, particularly in the late stage of the disease. The expression of HIF-1α and its target genes *Glut-1* and *PGK-1* in OA cartilage is associated with the progression of articular cartilage degeneration [12, 13]. On the other hand, HIF-1α is also a pivotal regulator in cartilage engineering allowing chondrocytes to maintain their function as professional secretory cells in the hypoxic growth plate [66–68]. In osteoarthritic cartilage, the transcription factor HIF-1α is involved in the upregulation of microsomal prostaglandin E synthase 1 (mPGES-1) and may therefore play an important role in the metabolism of OA cartilage [69]. HIF-2α is a key component for hypoxic induction of the human articular chondrocyte phenotype [70]. Evidences suggested that articular cartilage destruction might also be associated with the fact that HIF-2α directly induces the higher expression of catabolic factors including matrix metalloproteinases (MMP1, MMP3, MMP9, MMP12, and MMP13), aggrecanase-1 (ADAMTS4), nitric oxide synthase-2 (NOS2), and prostaglandin-endoperoxide synthase-2 (PTGS2) [14, 66]. Thus, these findings support its implication in OA through cartilage breakdown to be a critical evidence of the participation of this protein in OA pathogenesis [14]. Sound evidence has also indicated that H/R injury participates in various signaling cascade episodes including increased expression of tumor necrosis factor-(TNF-) α-induced IGFBP-3, downregulation of the expression of IGF-1, and release of intracellular ROS, eventually leading to apoptosis (Figure 5) [71].

In hypoxic conditions, HIF-1 activates transcription of the proapoptotic protein IGFBP-3, which blocks IGF-1

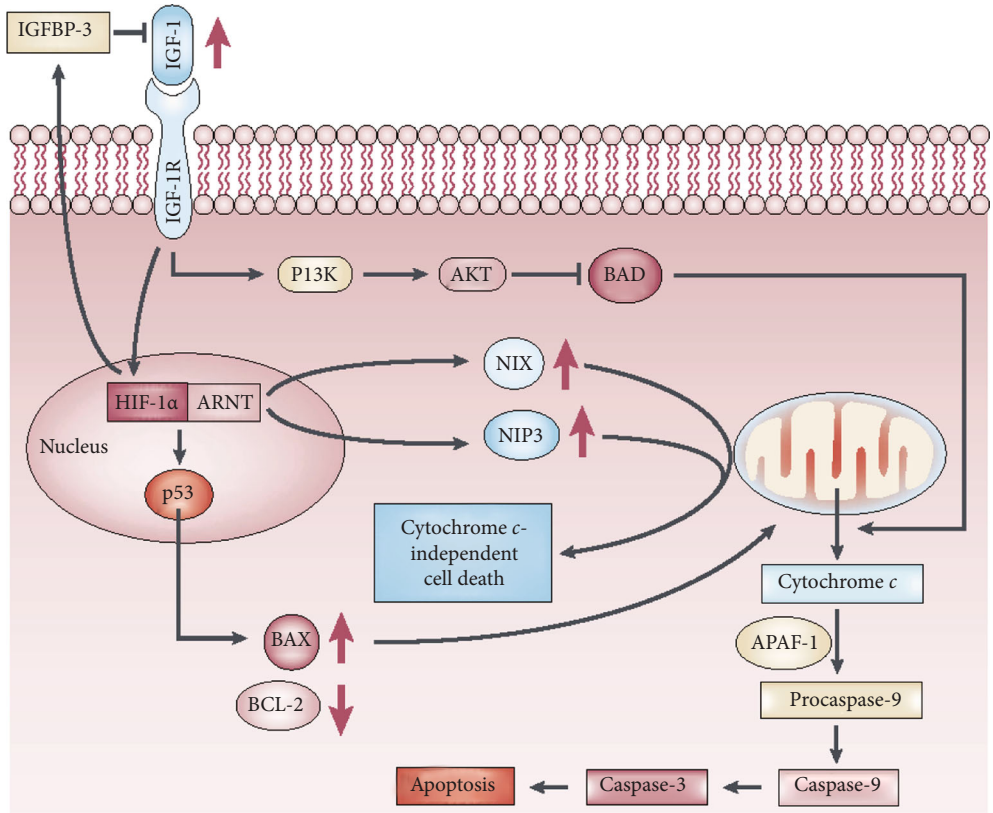

Figure 5: Hypoxia regulation of the cell death pathway (*reprinted from Harris* [72] *and reused with permission from Springer Nature under license number 4820081324341*). HIF-1 (a complex of HIF-1α and ARNT) activates transcription of many proapoptotic genes including IGFBP-3. HIF-1 activates the transcriptional activity of p53 and induces transcription of BAX. In turn, BAX promotes release of cytochrome *c* and promotes apoptosis via cascade reactions. Abbreviations: APAF-1: apoptotic protease-activating factor-1; ARNT: aryl hydrocarbon receptor nuclear translocator; HIF: hypoxia-inducible factor; IGF-1: insulin-like growth factor-1; IGF-1R: IGF-1 receptor; IGFBP-3: IGF binding protein 3; PI3K: phosphatidylinositol 3-OH kinase.

signaling. HIF-1 also activates expression of NIP3 and NIX, which in turn induce a mitochondrial-pore permeability transition and cell death. Studies showed that, compared to healthy subjects, synovial fluids of OA patients have markedly increased levels of IGFBP-3 [61]. Moreover, the increased level of IGFBP-3 in OA has been reported to be directly associated with the severity of the disease. In another study, Zhang and coworkers investigated the regulating effects of IGFBP-3 in inflammation and apoptosis, and conclusive evidence showed that the inflammatory response was reduced by the blockage of the NF-κB pathway and induction of apoptotic in OA FLS by IGFBP-3 [61]. The implication of IGFBP-3 in OA pathogenesis has also been studied under different signaling pathways. For instance, published data showed that IGFBP-3 induced chondrocyte apoptosis through nuclear-mitochondrial translocation of Nur77 [72].

Investigation of the implications of IGFBP-3 in OA pathogenesis under H/R mechanism is becoming an area of interest for scientists nowadays. In a study conducted by Zhang et al., the findings revealed that the expression of IGFBP-3 in FLS was upregulated under H/R conditions; pretreatments with TNF-α before H/R significantly increased the expression of IGFBP-3 [61]. In addition, other results showed that H/R significantly increased the levels of various factors including CCL5, interleukin-1b (IL-1β), and interleukin-6 in cell-free culture supernatants and promoted TNF-α-induced expression of inflammatory cytokines [17]. Overall, findings suggest that under H/R, IGFBP-3 may promote the permeability of the mitochondrial membrane and release of ROS, triggering inflammation in FLS and therefore inducing osteoarthritis.

4. Discussion

4.1. Main Findings. As we used the same search strategy and considered the principal focus on the essential role of IGF binding protein 3 in fibroblast-like synoviocyte-induced osteoarthritis pathogenesis, as well as hypoxia/reoxygenation injury, we found that there are few studies conducted that focus on this specific topic. Although several original studies highlighted their primary focus on either IGF binding protein 3, or synovial cells in osteoarthritis, or on hypoxia and synoviocytes, only two papers had investigated the relation between IGFBP-3, synoviocytes, and hypoxia/reoxygenation in osteoarthritis. However, there was a high proportion of relevant studies that included the investigation of chondrocytes and IGF binding proteins. While the medical literature

stated an undeniable evidence of the involvement of cells such as chondrocytes in osteoarthritis pathogenesis, researches regarding fibroblast-like synoviocyte role in osteoarthritis are still comparatively few. Moreover, the pivotal role of hypoxia/reoxygenation injury in this musculoskeletal disorder has become an area of great interest. Overall, evidence from the scientific literature strongly supports that osteoarthritis is a musculoskeletal disorder affecting the whole joint, and the synovium plays a key role in osteoarthritis pathogenesis.

4.2. Signaling Pathways Involved in Synoviocyte-Induced Osteoarthritis and Future Research Direction. In osteoarthropathies, most of the research has paid more attention to the chondrocytes in terms of understanding the OA pathogenesis. Recently, several reports had indicated that synovitis is the major characteristic of OA and that reducing the number of osteoarthritis synoviocytes (OAS) is one of the key factors for curing the disease. In the quest to understand the mechanisms and nature of signaling pathways involved in synoviocyte-induced OA, several proteins and transcripts have gone through investigations throughout the years. Findings showed that various signaling pathways are involved in synoviocyte-induced OA, including hypoxia signaling [17], NF-κB signaling pathway [73, 74], eicosanoid pathway [75], IL-6/STAT3 signaling pathway [76], Wnt/β-catenin pathway [77], and hedgehog signaling [78].

Liang and colleagues investigated the influence of vasoactive intestinal peptide (VIP) recombinant plasmid on synoviocytes. Findings suggested that VIP recombinant plasmid could inhibit the proliferation of synoviocytes, improve the pathological symptoms of OA disease, and produce a therapeutic effect on OA via the NF-κB signaling pathway [73]. In another study, authors found that follistatin-like protein 1 (FSTL1) functions as an essential proinflammatory factor in the pathogenesis of OA by activating the first pathway and enhancing synoviocyte proliferation [74]. Recently, researchers have assessed the implication of arachidonic acid, linoleic acid, and 20 oxylipins in synovial fluid from 58 knee OA patients and 44 controls. Results showed that levels of three lipoxins (LXs) in synovial fluid were associated with knee OA. The expression of 11,12-DHET and 14,15-DHET was statistically upregulated in affected compared to unaffected knees of people with unilateral disease. In addition, their expression and the expression of 8,9-DHET were also associated with knee OA radiographic progression in the over 3.3 years of follow-up of 87 individuals [75]. Through the IL-6/STAT3 signaling pathway, Li and colleagues investigated the role of lncRNA gastric cancer-associated transcript 3 (GACAT3) in OA [56]. Researchers found that, compared with normal synoviocytes, GACAT3 was significantly highly expressed in OA synoviocytes [76]. In addition, GACAT3 could influence the proliferation of OA synoviocytes. Researchers have investigated the role of this signaling pathway in TMJ OA and facet joint OA. Findings showed that mediators and downstream effectors of Wnt/β-catenin signaling are increased in OA as well other forms of arthritis, suggesting that the Wnt/β-catenin signaling pathway plays a direct role in OA pathogenesis through bone and joint pathology and synovial tissue [77].

Recent advances in osteoarthritis synoviocytes have enabled comprehensive analysis of various cells, proteins, and signaling pathways involved in this musculoskeletal disorder. The association of fibroblast-like synoviocytes in osteoarthritis pathogenesis is a strong and clear evidence that cannot be undermined today. In addition, mitochondria role through hypoxia/reoxygenation mechanism is an area that needs to be highly considered in further researches. Outcomes from these investigations shall definitively provide better, suitable, and targeted therapy to orthopedic patients.

4.3. Limitations. Retrieved papers included diversified types of osteoarthritis, which again varied across the studies. In addition, variation in setting and study population/samples are two main factors that limited the comparability. Publication bias was not assessed, meaning that several published studies we retrieved might have been data reporting only positive findings of IGFBP-3, FLS, and H/R on osteoarthritis pathogenesis. Although this paper has some limitations, it stands as the first study that brings an explanatory contribution role of IGF binding protein 3 and mitochondria in synoviocyte-induced osteoarthritis through hypoxia/reoxygenation injury.

5. Conclusions

OA is an invalidating disease characterized by progressive cartilage degradation. Research findings suggested that OA is a disease with a variety of pathophysiologic drivers leading to multiple phenotypes. Increasing undeniable evidence is now at hand proving that OA is not just a cartilage problem but of the entire joint tissue. Studies have shown the essential role of hypoxia-inducible factors into the course of the disease. Moreover, hypoxia plays a vital role in OA pathogenesis as hypoxia amplifies the NF-κB pathways by inducing synovitis. Strong evidences show that the level of IGFBP-3 in synovial fluid significantly increased in OA, inhibiting the binding of IGF-1 to IGFR 1 and therefore the inhibition of cell proliferation. Published papers related to the implication of the insulin-like growth factor (IGF) signaling pathway as a complete system or including its associated receptors and proteins in inflammatory joint disorders are available in the medical literature. Although some of these papers have shared undeniable knowledge in the light of the mechanistic pathogenesis of the pathology, they often either focus on chondrocytes [72], osteoblast [79], or usually rheumatoid arthritis [80]. Even where IGFBP-3 is put in exert, little is known regarding its pivotal role at the different stages of the disease [17]. Scientists from interdisciplinary background are using novel techniques such as bioinformatics to add to the field the knowledge regarding potential therapy target in order to understand OA development [81]. To the best of our analysis and in the light of the knowledge presented and discussed in this review, compared with other papers, the novelty of our submission lies in the highlights made on the exploration of inflammation mechanisms leading to OA pathogenesis. Although the exact mechanism of OA

pathogenesis, which is surely complex, remains poorly understood, there is no doubt that synovitis is counted to be one of the key pathogenic events during the course of the disease. Our paper not only discusses in depth the implication of hypoxic factors but also highlights the insulin-like growth factor binding protein-3–synoviocyte interaction and interconnectivity in osteoarthritis. Further investigations are needed to strengthen the undeniable evidence of IGFBP-3 and synovial cell interconnectivity in osteoarthritis pathogenesis through H/R injury.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

D.G. and H.L. conceptualized the paper. D.G. and G.A.B. wrote the manuscript. D.G., G.A.B., J.L., and H.L. revised the manuscript. H.L. supervised the entire manuscript. All authors have read and agreed to the published version of the manuscript.

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