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The Role of COX-2 in Knee Osteoarthritis: A Comprehensive Analysis of Cytokines, Inflammation, And Signalling Pathways

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ABSTRACT

Knee osteoarthritis (KOA)of the knee is a prevalent joint disorder closely associated with multiple factors, among which cyclooxygenase-2 (COX-2) plays a pivotal role in inflammatory responses and cytokine release. This review aims to elucidate the role of COX-2 in the pathogenesis of knee osteoarthritis, analyze its interplay with key cytokines, and examine the signaling pathways involved in this process. By employing immunohistochemical techniques, we intend to gain a deeper understanding of the expression patterns of COX-2 and its functions within the inflammatory microenvironment, thereby providing new insights for the treatment of knee osteoarthritis.

INTRODUCTION

Knee osteoarthritis (KOA) is a prevalent degenerative joint disease characterized by the progressive deterioration of articular cartilage, subchondral bone remodeling, and synovial inflammation. Epidemiological studies indicate that KOA affects approximately 10% of men and 18% of women over the age of 60 globally, with a significant burden observed in the Middle East and North Africa (MENA) region, where the prevalence has increased markedly from 1990 to 2019 Hoveidaei et al. (2023) Clinically, KOA presents with symptoms such as joint pain, stiffness, swelling, and decreased range of motion, significantly impairing the quality of life and functional capacity of affected individuals Spitaels et al. (2020) The disease's multifactorial nature, influenced by age, obesity, joint injury, and genetic predisposition, necessitates comprehensive understanding and management approaches Driban et al. (2020).

Cyclooxygenase-2 (COX-2) is an enzyme that plays a crucial role in the inflammatory response and pain pathways. It is induced during inflammatory processes and is responsible for the conversion of arachidonic acid into prostaglandins, which mediate inflammation and pain Ahmadi et al. (2022). In the context of KOA, elevated COX-2 expression has been associated with increased levels of inflammatory cytokines, contributing to the synovial inflammation characteristic of the disease Fischer et al. (2024). This highlights the potential of COX-2 as a therapeutic target, as selective COX-2 inhibitors have been shown to alleviate pain and improve function in KOA patients Clark et al. (2023). Cytokines, particularly pro-inflammatory cytokines such as interleukins (IL-1β, IL-6) and tumor necrosis factor-alpha (TNF-α), play a pivotal role in the pathophysiology of KOA. These cytokines are involved in the inflammatory cascade that leads to cartilage degradation and synovial inflammation Bafrani et al. (2019) The infrapatellar fat pad, a significant source of inflammatory cytokines, contributes to the local inflammatory environment in KOA, further exacerbating joint damage Zhou et al. (2022). Understanding the intricate interplay between COX-2 and cytokines in KOA is essential for developing targeted therapies aimed at mitigating inflammation and preserving joint function. The objective of this review is to elucidate the epidemiology and clinical manifestations of KOA, examine the biological functions of COX-2 in inflammation, and explore the critical role of cytokines in the disease's progression. By synthesizing current literature, this review aims to highlight potential therapeutic avenues and inform clinical practice in managing KOA effectively.

THE RELATIONSHIP BETWEEN COX-2 AND KOA

The expression of COX-2 and its regulatory mechanisms

COX-2 is a key enzyme involved in the inflammatory process and is significantly upregulated in osteoarthritis (OA). The expression of COX-2 is primarily regulated by various pro-inflammatory cytokines, such as interleukin-1 (IL-1) and TNF-α, which are often elevated in OA. Studies have shown that the activation

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of signaling pathways, including NF-κB and MAPK, plays a critical role in inducing COX-2 expression in chondrocytes and synovial cells under inflammatory conditions. For instance, the aberrant expression of COX-2 correlates with the downregulation of microRNA-758-3p in synovial tissues of OA patients, indicating a complex regulatory network involving both transcriptional and post-transcriptional mechanisms Liu et al. (2021). Additionally, the role of oxidative stress in modulating COX-2 expression has been highlighted, where reactive oxygen species (ROS) can activate transcription factors that enhance COX-2 gene expression Ke et al. (2022). Understanding these regulatory mechanisms is crucial for developing targeted therapies aimed at reducing COX-2 levels and mitigating inflammation in OA.

The pathological role of COX-2 in osteoarthritis

The pathological role of COX-2 in KOA is multifaceted, primarily contributing to inflammation, cartilage degradation, and pain. Elevated COX-2 levels are associated with increased production of prostaglandins, which are mediators of pain and inflammation in OA Cho et al. (2022). The persistent expression of COX-2 in the joint leads to a vicious cycle of inflammation and cartilage destruction, exacerbating the disease progression. Moreover, selective COX-2 inhibitors have been shown to possess chondroprotective effects, suggesting that managing COX-2 activity could be a viable therapeutic strategy Timur et al. (2020). Research indicates that COX-2 not only influences inflammatory pathways but also interacts with other signaling molecules that contribute to chondrocyte apoptosis and extracellular matrix degradation. Therefore, targeting COX-2 could potentially halt or reverse the degenerative processes associated with KOA.

THE ROLE OF CYTOKINES IN KOA

Classification and function of major cytokines

Cytokines are pivotal in the pathophysiology of knee osteoarthritis, with both pro-inflammatory and anti-inflammatory cytokines playing significant roles. Major pro-inflammatory cytokines involved in OA include IL-1, TNF-α, and IL-6, which are known to promote inflammation, cartilage degradation, and pain Fischer et al. (2024). These cytokines act by stimulating the production of matrix metalloproteinases (MMPs) and inhibiting the synthesis of cartilage matrix components, leading to the destruction of articular cartilage. Conversely, anti-inflammatory cytokines, such as IL-10 and transforming growth factor-beta (TGF-β), are crucial for maintaining cartilage homeostasis and promoting repair mechanisms Zhou et al. (2022). The balance between these cytokines is essential for joint health, and dysregulation can lead to the progression of OA.

The interaction between cytokines and COX-2

The interaction between cytokines and COX-2 is a critical aspect of the inflammatory response in knee osteoarthritis. Pro-inflammatory cytokines, particularly IL-1 and TNF-α, have been shown to upregulate COX-2 expression in chondrocytes, thereby enhancing the production of inflammatory mediators such as prostaglandins Li et al. (2021). This positive feedback loop amplifies the inflammatory response and contributes to the symptoms of OA. Furthermore, studies suggest that the interplay between cytokines and COX-2 can influence chondrocyte survival and apoptosis, affecting cartilage integrity Zhao et al. (2024). Understanding these interactions provides insights into potential therapeutic targets, as modulating the effects of specific cytokines or inhibiting COX-2 could help alleviate inflammation and slow the progression of osteoarthritis.

MECHANISMS OF INFLAMMATORY RESPONSE IN KNEE OSTEOARTHRITIS

Initiation and Maintenance of Inflammation

The inflammatory response in KOA is a complex process that involves the activation of various immune cells and the release of pro-inflammatory cytokines. This process is initiated by mechanical stress and damage to the cartilage, which leads to the release of damage-associated molecular patterns (DAMPs) that activate pattern recognition receptors (PRRs) on synovial cells and immune cells within the joint. The activation of these receptors triggers a cascade of inflammatory signaling pathways, including the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, which plays a crucial role in the transcription of pro-inflammatory cytokines such as IL-1 and TNF-α Wang et al. (2020).

Moreover, the maintenance of inflammation in knee OA is sustained by the continuous presence of inflammatory mediators and the recruitment of additional immune cells, such as macrophages and T cells, to the joint. These cells not only produce inflammatory cytokines but also secrete MMPs that contribute to cartilage degradation Udomsinprasert et al. (2019). Recent studies have highlighted the role of galectin-3 in promoting synovial inflammation through the activation of the phosphatidylinositol-3-kinase/Akt pathway, indicating a potential therapeutic target for managing inflammation in OA Udomsinprasert et al. (2023). Additionally, the presence of chronic inflammation in the synovial fluid of OA patients has been associated with the severity of the disease, further emphasizing the need for effective anti-inflammatory strategies Rolle et al. (2019).

Role of COX-2 in the Inflammatory Response

COX-2 is a key enzyme involved in the inflammatory response in knee osteoarthritis, primarily responsible for,

the conversion of arachidonic acid into prostaglandins, which are potent mediators of inflammation. Elevated levels of COX-2 have been observed in the synovial tissue and fluid of OA patients, correlating with increased levels of prostaglandin E2 (PGE2), a pro-inflammatory mediator that exacerbates pain and inflammation in the joint Ahmadi et al. (2022). The inhibition of COX-2 has been shown to reduce the levels of inflammatory cytokines and alleviate symptoms in OA patients, making it a target for therapeutic intervention Liu et al. (2024).

Moreover, recent research has indicated that COX-2 not only contributes to the inflammatory process but also plays a role in the resolution of inflammation. Specialized pro-resolving mediators derived from omega-3 fatty acids can modulate COX-2 activity, promoting the resolution of inflammation and restoring tissue homeostasis Jordan et al. (2022).

The dual role of COX-2 in both promoting and resolving inflammation highlights the complexity of targeting this enzyme in therapeutic strategies. Inhibition of COX-2 has been associated with adverse effects, including gastrointestinal complications, necessitating a careful consideration of the therapeutic approaches used in managing KOA Sahu et al. (2023). Understanding the precise mechanisms by which COX-2 influences inflammation in KOA will be critical for developing effective treatments that balance pain relief and inflammation resolution.

THE ROLE OF SIGNAL TRANSDUCTION PATHWAYS IN COX-2 REGULATION

Overview of Major Signal Transduction Pathways

Signal transduction pathways are crucial for cellular communication and play a significant role in regulating various biological processes, including inflammation and cancer progression. The primary pathways involved in the regulation of COX-2 include the mitogen-activated protein kinase (MAPK) pathway, phosphoinositide 3-kinase (PI3K)/Akt pathway, and NF-κB signaling. The MAPK pathway, which consists of extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK, is activated by various extracellular stimuli and is involved in regulating gene expression, cell proliferation, and apoptosis.

The PI3K/Akt pathway is essential for cell survival and growth, and its activation leads to the upregulation of COX-2 expression in response to inflammatory signals. NF-κB is a transcription factor that, when activated, translocates to the nucleus to promote the transcription of pro-inflammatory genes, including COX-2. The interplay among these pathways is complex, and their dysregulation can lead to pathological conditions such as cancer and chronic inflammation. Nagaraju et al. (2022), A Najafi et al. (2020), Kontomanolis et al. (2022).

Interaction Between Signal Transduction Pathways and COX-2

The interaction between signal transduction pathways and COX-2 is pivotal in mediating inflammatory responses and tumorigenesis. For instance, TNF-α, a pro-inflammatory cytokine, activates the NF-κB pathway, leading to increased COX-2 expression in various cell types, including macrophages and epithelial cells. Hosokawa et al. (2022), Okamoto et al. (2024).

Additionally, studies have shown that the PI3K/Akt pathway can enhance COX-2 expression by promoting the stability of COX-2 mRNA and increasing its translation Eo et al. (2019). Furthermore, the activation of the MAPK pathway, particularly ERK, has been implicated in the upregulation of COX-2 during inflammatory responses, indicating a multifaceted regulatory mechanism Li et al. (2022), Liu et al. (2021). In cancer, COX-2 is often overexpressed, and its regulation by these signaling pathways contributes to tumor progression and resistance to apoptosis. Targeting these pathways may provide therapeutic strategies for conditions characterized by elevated COX-2 levels, such as colorectal cancer and other malignancies Han et al. (2021), Okamoto et al. (2024). Understanding the intricate relationship between COX-2 and signal transduction pathways is essential for developing novel anti-inflammatory and anticancer therapies.

IMMUNOHISTOCHEMISTRY IN RESEARCH APPLICATIONS

Basic Principles of Immunohistochemistry

Immunohistochemistry (IHC) is a critical technique in biomedical research and clinical diagnostics, leveraging the specificity of antibodies to detect particular antigens in tissue sections. The fundamental principle of IHC involves the binding of an antibody to its target antigen, followed by visualization through various detection systems, which can be chromogenic or fluorescent. This process typically begins with the fixation of tissue samples to preserve cellular structures, followed by embedding in paraffin or freezing for cryosectioning. The antibodies used in IHC can be monoclonal or polyclonal, each with unique advantages depending on the specificity and sensitivity required for the study. The use of secondary antibodies conjugated to enzymes or fluorophores enhances the detection signal, allowing for the visualization of antigen distribution and expression levels within the tissue microenvironment. Recent advancements have introduced multiplexed IHC techniques, enabling the simultaneous detection of multiple antigens in a single tissue section, which is particularly beneficial for understanding complex biological processes and cellular interactions in situ Ginnis et al. (2021), Sato et al. (2021). Furthermore, the integration of imaging technologies with IHC has opened

new avenues for tissue analysis, providing high-dimensional data that can elucidate the spatial organization and heterogeneity of cellular populations within tissues Maiques et al. (2019), Schlecht et al. (2021).

Applications of Immunohistochemistry in KOA Research

In the context of KOA, immunohistochemistry has emerged as a powerful tool for elucidating the pathophysiological mechanisms underlying this degenerative joint disease. IHC allows researchers to visualize and quantify the expression of various biomarkers associated with inflammation, cartilage degradation, and bone remodeling in affected tissues. For example, studies have utilized IHC to investigate the localization and expression levels of pro-inflammatory cytokines, MMPs, and other mediators involved in the OA process Fang et al. (2021), Wang et al. (2024). By employing IHC techniques, researchers have been able to identify specific cellular populations, such as macrophages and T-cells, that contribute to the inflammatory milieu in OA-affected joints Zhang et al. (2023). Additionally, the application of multiplex IHC in OA research has facilitated the simultaneous assessment of multiple markers, providing insights into the interplay between different cellular pathways and their contributions to disease progression Shin et al. (2020), Chiu et al. (2024) This comprehensive approach not only enhances the understanding of OA pathogenesis but also aids in the identification of potential therapeutic targets and biomarkers for disease monitoring and progression Moon et al. (2020), Cheng et al. (2019). Overall, the use of immunohistochemistry in KOA research underscores its significance in advancing our knowledge of this complex condition and improving clinical outcomes for patients.

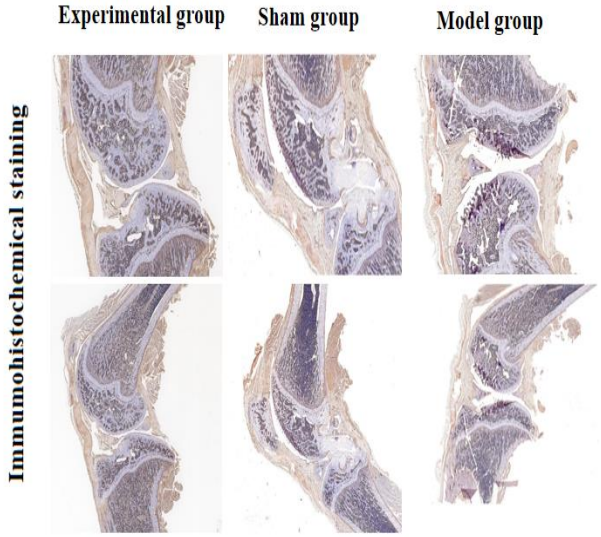
The immunohistochemical expression of COX-2 in rat KOA

To determine the immunohistochemical expression of COX-2 in KOA, 30 male SD rats aged 8.5 weeks were selected from the Animal Experiment Center of Guangxi Medical University (License No: SYXK Gui 2020-0004, Ethical Approval No: 202105004). The experimental group (10 rats) underwent no surgical intervention. Twenty SD male rats were anesthetized with 10% chloral hydrate (3 ml/kg) via intraperitoneal injection, and once satisfactory anesthesia was achieved, the rats were placed in a supine position and fixed to the surgical table, followed by routine disinfection and draping. The skin of the left and right hind limbs was shaved with a surgical razor, and the hind limbs were cleaned with gauze soaked in new chlorhexidine, followed by disinfection with iodine. In the model group (10 rats), a 2 cm incision was made parallel to the medial collateral ligament at the left and right knees, with the skin, muscle, and fascia separated in sequence, the joint capsule incised, and the patella displaced at a 90° flexion to open the joint cavity. The anterior cruciate ligament was located and cut with scissors, and a drawer test was performed to ensure complete transection of the anterior cruciate ligament. The joint cavity was flushed with 0.9% sodium chloride solution, and the joint capsule and skin were sutured. In the sham group (10 rats), only the joint capsule was incised without any further treatment, and then sutured. After all rats recovered, they were returned to their cages. One month later, the articular cartilage tissues from each group of rats were dewaxed and activated for enzyme assays, followed by routine paraffin embedding and sectioning.

Knee joint immunohistochemical staining: The paraffin sections were dewaxed to water, followed by antigen retrieval, and placed in a 3% hydrogen peroxide solution for 25 minutes at room temperature in the dark. The slides were washed 3-5 times in PBS (pH 7.4) on a decolorizing shaker, with each wash lasting 5 minutes, and then blocked with serum for 30 minutes. After removing the blocking solution, the prepared primary antibody was added to the sections, which were then incubated in a humid box at 4°C for 12 hours. The corresponding secondary antibody was applied at room temperature for 50 minutes. The slides were washed 3 times in PBS, with each wash lasting 5 minutes. DAB chromogenic solution was added, and the chromogenic time was controlled under a microscope; a brown-yellow color indicated a positive result, after which the slides were rinsed with running water to stop the reaction. The cell nuclei were stained with hematoxylin, dehydrated, and mounted. The expression of COX-2 related antibodies was detected.

In this study, in the control group, COX-2 antibody showed positive expression in immunohistochemical staining.

Figure 1: Immunohistochemical manifestations of osteoarthritis pathology in SD rats.



CONCLUSION

In conclusion, the role of COX-2 in KOA is a pivotal element that warrants further investigation. COX-2 is not only an enzyme involved in the inflammatory response but also a key player in the pain pathways associated with OA. The modulation of COX-2 activity could potentially provide therapeutic benefits, as evidenced by the varying responses observed in different studies. As such, it is crucial to balance the emerging viewpoints surrounding COX-2 inhibitors, considering both their analgesic properties and their potential adverse effects.

The intricate relationship between cytokines and the inflammatory response in KOA further complicates the therapeutic landscape. Cytokines such as IL-1β and TNF-α are known to exacerbate joint inflammation and degrade cartilage. However, there is a growing body of evidence suggesting that the inhibition of specific cytokine pathways may alleviate symptoms and slow disease progression. This highlights the need for a nuanced understanding of the inflammatory milieu in OA, as targeting one cytokine may not yield the desired results without considering the broader context of cytokine interactions.

Moreover, the exploration of signaling pathways as potential therapeutic targets presents an exciting avenue for future research. Pathways such as NF-κB, MAPK, and JAK/STAT are integral to the inflammatory process in OA. Identifying specific inhibitors that can selectively modulate these pathways could lead to innovative treatment strategies that minimize side effects while effectively managing symptoms. Looking ahead, future research should focus on the integration of findings from diverse studies to establish a more comprehensive understanding of OA pathology. Multi-target approaches that consider the interplay between COX-2, cytokines, and signaling pathways could enhance therapeutic outcomes.

DECLARATIONS

Ethics approval and consent to participate

This paper and accompanying images have been published with the consent of the Hospital and Animal Ethics.

Consent for publication

The publication of this paper has been approved by Guangxi Bone Injury Hospital.

Availability of data and materials

The data and materials are authentic and available.

Competing interests

None.

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Authors' contributions

Study concept/design: all. Data collection: all. Writing the paper: all. Critical revision: all.

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