ENDOTHELIAL DYSFUNCTION INDUCES JOINT AGING AND OSTEOARTHRITIS IN HYPERTENSIVE RATS

ZHANG YUQI

PhD

The Hong Kong Polytechnic University

2025

The Hong Kong Polytechnic University

Department of Biomedical Engineering

Endothelial dysfunction induces joint aging and osteoarthritis in hypertensive rats

Zhang Yuqi

A thesis submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

December 2024

Certificate of Originality

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it reproduces no material previously published or written, nor material that has been accepted for the award of any other degree or diploma, except where due acknowledgement has been made in the text.

_____ (Signed)

__Zhang Yuqi_____ (Name of student)

Abstract

The intricate relationship between hypertension and knee osteoarthritis (OA) has sparked intense debate, with recent studies suggesting a complex interplay between these two conditions. Systolic and pulse pressures are correlated with an increased incidence of radiographic knee OA. The data implies that hypertension may not only contribute to chronic joint pain but also exacerbate structural damage in the knee joint, potentially through hemodynamic shear stress. However, a large-scale Mendelian Randomization study involving nearly 385,000 participants from the UK Biobank revealed a paradoxical inverse correlation between systolic blood pressure and knee and hip OA, hinting that genetic predisposition to hypertension may unexpectedly lower OA risk. This unexpected finding highlights the need for further investigation into the bidirectional relationship between hypertension and OA, including the cellular and molecular mechanisms that underlie this complex interaction. By elucidating the underlying mechanisms, we can develop more effective therapeutic strategies to mitigate the impact of hypertension on OA and improve patient outcomes.

To study the effects of hypertension on joints, we utilized two hypertensive rat models. In Chapter 2, the deoxycorticosterone acetate (DOCA)-induced hypertension model was employed, while in Chapter 3, we used the spontaneously hypertensive rat (SHR) model. In Chapter 4, OA was induced in hypertensive rats to assess whether hypertension exacerbates OA. In Chapter 5, we used a human cell model to investigate

the crosstalk between endothelial cells and chondrocytes. Blood pressure and joint oxygen saturation levels were monitored, and at 9–10 months of age, rats were sacrificed for aortic and leg tissue analysis. CT scans of the legs and histological staining of aortic and joint tissues were performed to evaluate structural changes. The antihypertensive drug captopril was administered to examine pre- and post-treatment effects on vascular and joint health. To study hypertension's impact on OA, hypertensive rats underwent ACLT surgery, followed by two months of monitoring before histological analysis. Statistical comparisons were performed using one-way ANOVA with Tukey's test.

In Chapters 2 and 3, both DOCA-induced hypertensive rats and SHR models exhibited significant vascular and joint aging, reduced chondrocyte proteoglycan levels, elevated cartilage degradation marker MMP-13, and bone loss compared to controls. SHR rats showed reduced joint oxygen saturation, increased vascular oxidative stress, and accumulation of senescent cells with proteoglycan loss in joints. Captopril demonstrated anti-senescent and chondroprotective effects in SHR and aged chondrocytes but did not prevent bone loss. In Chapter 4, hypertensive OA models did not show worsened blood pressure but indicated increased cardiovascular burden and more severe joint dysfunction. In Chapter 5, co-culture systems revealed that IGFBP6 mediated biochemical crosstalk between endothelial dysfunction and chondrocyte aging.

Our findings demonstrate that hypertension induces vascular dysfunction and endothelial cell senescence, leading to chondrocyte aging and degradation. This study reveals hypertension-induced vascular and joint changes, providing evidence for the impact of vascular health on joint integrity. In the captopril treatment group, both vascular and joint phenotypes improved, suggesting that antihypertensive drugs may offer a novel therapeutic strategy for OA by addressing endothelial dysfunction. These findings underscore the need for further exploration into the vascular-joint connection, potentially broadening therapeutic approaches for patients with co-existing hypertension and OA.

Publications arising from the thesis

Journal articles (published)

1. Chan, L. C., **Zhang, Y.**, Kuang, X., Koohi-Moghadam, M., Wu, H., Lam, T. Y. C., Chiou, J., & Wen, C. (2022). Captopril Alleviates Chondrocyte Senescence in DOCA-Salt Hypertensive Rats Associated with Gut Microbiome Alteration. *Cells*, *11*(19), 3173. https://doi.org/10.3390/cells11193173.

Journal articles (under finial revision)

1. Yuqi Zhang[#], Karen Ching[#], Lanlan Zhang[#], Lin Zhu[#], Zhongyu Liu, ZongWei Cai, Man Ting Au*, Chunyi WEN*. Endothelial dysfunction contributes to chondrocyte senescence associated with Insulin-like growth factor-binding protein-6 in a spontaneously hypertensive rat model. (the Journal of the American Heart Association under finial revision)

2. Hantang Wang, Lanlan Zhang, Yuqi Zhang, Wei Wang, Junguo Ni, Jun Liu, Weiyuan Gong, Man Ting AU, Huiling Cao and Chunyi Wen* Spatially resolved Single-cell-based Histocytomorphometry for Growth Plate Analysis Across Multiple Species. (the Journal of Orthopaedic Translation under finial revision)

Journal articles (under preparation)

1. Siu Ling Yip[#], **Yuqi Zhang[#],** Lanlan Zhang, Man Ting Au, Lin Zhu, Chunyi Wen*. Post-traumatic Osteoarthritis driven pain and neuroinflammation via blood circulation in Wistar Kyoto Rats. (under preparation)

2. Zhou Zou, Weiyuan Gong, Xuan Lu, **Yuqi Zhang,** Manting Au, Junxin Lin, HongWei Ouyang, Guozhi Xiao, Bin Tang*, Chunyi Wen*. Endothelin-1/Endothelin B Receptor Signalling Mediates Prx1+ Skeletal Stem Cells Senescence: A Driver of Osteoporotic Bone Loss. (ready to submit)

Poster presentation

1. **Yuqi Zhang**[1], Chunyi WEN[1,]*.(2023). Anti-hypertensive Medicine Captopril Alleviates Chondrocyte Senescence in Spontaneous Hypertensive Rats. 2023 ASBMR Annual Meeting. ASBMR 2023 Pre-Meeting Young Investigator Travel Grant (presenting author)

2. Lanlan Zhang, **Yuqi Zhang,** Man Ting Au, Chunyi Wen. Hypertension accelerates cartilage maturation and promotes premature senescence in chondrocytes. OARSI 2024.

3. Jun Liu, Hantang Wang, **Yuqi Zhang**, Chunyi Wen. THE RESTORATIVE AND PROTECTIVE EFFECT OF DONEPEZIL IN OSTEOARTHRITIS. OARSI 2024.

#: equal contribution; *: Correspondence

Acknowledgement

During the four years of my PhD studies at the Hong Kong Polytechnic University, there were very happy times and very difficult moments. I received help from many people and encountered many difficulties. The four years of my PhD have given me experiences that I have never had before, which I will never forget.

First of all, I need to thank Dr. Wen, my mentor. I am very grateful that he not only provided me with the opportunity to study for a Ph.D. but also supported my study and life in Hong Kong with a full scholarship. Under his careful guidance, I grew from a novice who knew almost nothing about scientific research to a researcher who can independently design research projects, write academic papers, and successfully apply for research funds. Whenever I encounter problems or bottlenecks in my research, Dr. Wen will not only provide new research directions, but also patiently guide me on how to break through the difficulties. His technical prowess and deep understanding of topics enable him to quickly get to the heart of a problem and collaborate with other academic colleagues to solve it. At every important moment in my doctoral career, Dr. Wen was my strongest supporter. He not only provided me with great help academically but also provided me with continuous spiritual support and encouragement. His enthusiasm for scientific research deeply influenced me, which not only enabled me to achieve academic achievements, but also made great progress in my outlook on life and values. Here, I sincerely thank Dr. Wen for his education and training, which will be the most valuable asset in my study and career.

Secondly, I am very grateful to the Hong Kong Polytechnic University for providing a good learning environment and professional experimental equipment and management. Every time there is a problem with the instrument, the staff will help solve it in time. The instrument management training is also done well. If there are very difficult problems to solve, they will actively help contact the engineers to solve them. This makes me very touched and thanks the school staff for their efforts. I'm also very grateful to the school for providing us with funds to go abroad for exchanges and conferences. During our four-year PHD life, I went to Canada to attend an academic conference. The school reimbursed all the expenses, which solved my worries. Going abroad to attend academic conferences also allowed me to see a lot of scientific research technologies that I had never seen before, and I realized the gap between the top levels of the industry and us, which impressed me deeply and benefited a lot.

Finally, I would like to thank all my collaborators and all members of the laboratory, especially Au Man ting, Zhang lanlan, Yip Siu ling, Ni junguo, Karen Ching, Luo Xiaohe, Kuang Xiaoqin, Lok Chun Chan, Jiang Tianshu, Wang Wei, Zou zhou, Liu Jun, Li Zhan, Cui Yinkun, Dr. Tang Bin, Dr. Zhulin, Wang Hantang. Thanks to them for helping me in my PHD studies. I would also like to thank my family and friends, especially my father, mother, brother, grandmother and grandfather. Thanks to Zhu Zhengting for some of my spiritual pillars.

Table of contents

List of figures

List of Abbreviations

ACE	angiotensin-converting enzyme
ACEI	angiotensin-converting enzyme inhibitor
ACL	anterior cruciate ligament
ACLT	anterior cruciate ligament transection
ADAMTS	a disintegrin and metalloproteinase with thrombospondin motifs
AGEs	advanced glycation end-products
AGT	angiotensinogen
Ang I	angiotensin I
Ang II	ngiotensin II
ARBs	ANG II receptor blockers
ASESC	Animal Subjects Ethics Sub-Committee
a-SMA	alpha smooth muscle actin
BDNF	brain-derived neurotrophic factor
BMD	bone mineral density
BMI	Body mass index
BMLs	bone marrow lesions
BV/TV	Bone volume fraction
CAF	Concentrated Animal Facility
CGRP	calcitonin gene-related peptide
CLR	calcitonin receptor-like receptor

COVID-19	Coronavirus infectious disease
CRP	C-reactive protein
CVD	Cardiovascular disease
DAMPs	damage-associated molecular patterns
DBP	diastolic blood pressure
DDR	DNA damage response
DMM	destabilization of medial meniscus
DOCA	deoxycorticosterone acetate
DRG	dorsal root ganglion
ECM	extracellular matrix
Egr-1	early growth response-1
ENaC	epithelial sodium channels
eNOS	endothelial nitric oxide synthase
ET-1	Increased production of endothelin-1
FLS	Fibroblast-like Synoviocytes
FOXO4	Forkhead Box O4
GWAS	Genome-wide association studies
H2O2	hydrogen peroxide
HIF-1α	hypoxia-inducible factor-1α
HR	heart rate

HRV	Heart Rate Variability
IGF-1	Insulin-like growth factor-1
IGF-1R	IGF-1 receptor
IGFBPs	Insulin-like growth factor binding proteins
IL-1β	interleukin-1β
IL-6	interleukin-6
iNOS	inducible nitric oxide synthase
JAK	Janus kinase
KOA	knee osteoarthritis
LCL	lateral collateral ligament
LRP	like receptor-related protein
MAP	mean arterial pressure
MAPKs	mitogen-activated protein kinases
MCL	medial collateral ligament
MetS	Metabolic syndrome
MIA	monoiodoacetate
MLS	Macrophage-like Synoviocytes
MMPs	matrix metalloproteinases
MRI	magnetic resonance imaging
MRs	mineralocorticoid receptors
MSCs	mesenchymal stem cells

NF-κB	Nuclear factor-kappa B
nNOS	Neuronal nitric oxide synthase
NO	nitric oxide
NOX	Nicotinamide adenine dinucleotide phosphate oxidase
NSAIDs	nonsteroidal anti-inflammatory drugs
OA	Osteoarthritis
PCL	posterior cruciate ligament
PGE2	Prostaglandin E2
PI3K	phosphatidylinositol 3-kinase
PP	pulse pressure
PTH	Parathyroid Hormone
RAAS	renin-angiotensin-aldosterone system
RAMP1	receptor activity-modifying protein 1
RAS	Renin-angiotensin system
RNA-Seq	RNA Sequencing
ROI	regions of interest
ROS	reactive oxygen species
SASP	senescence-associated secretory phenotype
SBP	Systolic blood pressure
SHR	spontaneously hypertensive rats
SNS	sympathetic nervous system

Tb.N	trabecular number
Tb.Sp	trabecular separation
Tb.Th	trabecular thickness
TGF-β	Transforming growth factor-beta
TNF-α	tumor necrosis factor-alpha
VEGF	Vascular endothelial growth factor
VOIs	volumes of interest
VSMC	Vascular smooth muscle cell
WHO	World Health Organization
WKY	Wistar Kyoto
α7nAChR	alpha-7 nicotinic acetylcholine receptors

Chapter 1 Literature Review

1.1Osteoarthritis (OA) overview

1.1.1 Prevalence and Burden

According to data from the World Health Organization(WHO), in 2019, more than 500 million people worldwide suffered from OA, of which approximately 360 million were affected by knee joints, which are the most vulnerable joints to damage[1]. With an aging population and increasing obesity rates, the prevalence of OA has increased by 113% since 1990[2]. Data released by the National Health Commission of the People's Republic of China show that the total prevalence of osteoarthritis in China is conservatively estimated to exceed 100 million[3]. A study comprising 74,908 subjects in China found the overall prevalence of symptomatic knee OA to be 14.6%[4]. The prevalence of symptomatic knee OA increases with age, showing an almost linear growth after the age of 40. Women exhibit a higher prevalence of symptomatic knee OA compared to men. The prevalence of OA is growing very rapidly and has been listed by the WHO as the second leading cause of disability[5]. Due to the recent outbreak of the Coronavirus infectious disease (COVID-19) infectious disease, the prevalence of OA continues to increase, and the burden of OA disease is becoming more and more serious.

The overall economic burden of OA in the United States is estimated at $136.8 billion per year, this number that has more than doubled in the past decade[6]. To put this into

perspective, the annual economic cost of arthritis exceeds that of tobacco-related health effects, cancer, and diabetes. Direct medical expenses alone account for $65 billion each year[6]. In 2016, osteoarthritis ranked tenth in terms of years lived with disability in China[7]. Among the various forms of osteoarthritis, knee osteoarthritis was the most prevalent, contributing to 87% of the total years lived with disability attributed to osteoarthritis. In 2018, there were 439,324 total hip arthroplasty procedures, 245,259 total knee arthroplasty procedures, and 11,200 unicompartmental knee arthroplasty procedures performed in China[8]. In 2018, the total cost of knee replacement surgery in China was approximately over 10 billion RMB. OA is a significant economic burden and a major source of societal costs. The high number of joint replacements performed each year in every country underscores the economic strain of this disease. These costs highlight the enormous economic impact of OA, driven primarily by medical costs, lost productivity, and long-term care needs, which combine to put a strain on healthcare systems and the overall economy.

1.1.2Etiology of osteoarthritis

1.1.2.1 Age, genetic, mechanical, biochemical and environmental factors

The pathogenic mechanism of knee osteoarthritis (KOA) is complex, involving the interaction of genetic, mechanical, biochemical and environmental factors. Genetic factors play an important role in the onset of KOA. Studies have shown that KOA has familial aggregation, and several gene loci associated with KOA have been found[9]. Age is one of the main risk factors for OA. As we age, the gradual degeneration and

wear of articular cartilage increases the incidence of OA[10]. The typical age of onset of osteoarthritis is in your 40 to 50 years old. 60% of patients with osteoarthritis are women. Studies have shown that women's risk of developing OA is significantly increased after menopause, which may be related to hormonal changes[11]. Mechanical factors include overuse, injury and abnormal mechanical load of joints, which lead to mechanical damage to cartilage and cellular stress response. Congenital or acquired anatomic abnormalities of the joint, such as acetabular dysplasia or varus/valgus knee deformity, may increase joint load and wear, thereby predisposing to OA[12]. Biochemical factors involve the degradation of extracellular matrix and the release of inflammatory mediators, leading to the degeneration of articular cartilage and synovial inflammation[13]. Environmental factors such as obesity and diet are also considered to be important risk factors for KOA, which participate in the development of the disease by increasing joint load and inducing systemic inflammation.

1.1.2.2 Metabolic syndrome and osteoarthritis

OA is the most common form of arthritis, characterized by progressive cartilage degeneration, subchondral bone remodeling, and chronic pain[14]. Metabolic syndrome (MetS), defined by a cluster of metabolic abnormalities including central obesity, hypertension, hyperglycemia, and dyslipidemia, affects an estimated 25% of adults worldwide[15]. Increasing evidence suggests that these two conditions are interlinked, with MetS serving as a significant risk factor for OA beyond the mechanical burden of obesity[16, 17].

Obesity is a central component of MetS and a major risk factor for OA. Excess body weight not only increases mechanical stress on weight-bearing joints but also promotes systemic inflammation through the release of adipokines such as leptin, resistin, and adiponectin, which influence cartilage metabolism and OA progression[18]. Systemic low-grade inflammation, a hallmark of MetS, is implicated in OA pathogenesis. Elevated levels of pro-inflammatory cytokines, including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP), are associated with cartilage degradation, synovitis, and subchondral bone changes[19, 20]. Metabolic abnormalities such as insulin resistance, hyperglycemia, and dyslipidemia contribute to OA. Hyperglycemia enhances the production of advanced glycation end-products (AGEs), which may accumulate in cartilage, impair its mechanical properties, and induce oxidative stress[21, 22]. Dyslipidemia has also been linked to cartilage damage via altered lipid metabolism and increased oxidative stress.

1.1.2.3 cardiovascular disease and osteoarthritis

Cardiovascular disease (CVD) and OA are prevalent chronic conditions that significantly impact public health. Recent evidence suggests a bidirectional relationship between these conditions, highlighting shared risk factors and pathophysiological mechanisms[23, 24]. The interaction between CVD and OA has garnered increasing attention, as patients with OA often exhibit an elevated risk of developing CVD and vice versa[25].

Aging is a major risk factor for both conditions. The prevalence of OA increases after age 50, and similarly, the risk of CVD rises with advancing age[26]. Low-grade systemic inflammation is a common feature of both diseases. Elevated levels of CRP and IL-6 are observed in patients with OA and are associated with increased CVD risk[27]. OA-related pain often limits mobility, reduces physical activity levels and increasing the risk of CVD through diminished cardiovascular fitness and metabolic health[28].

1.1.3 Pathophysiology of knee osteoarthritis from clinic to basic science

1.1.3.1 Tissue level (cartilage, subchondral bone, synovitis and tissue crosstalk)

OA is a degenerative joint disease characterized by the progressive breakdown of articular cartilage, along with changes in subchondral bone and synovium. Articular cartilage is a highly specialized, avascular, aneural, and alymphatic connective tissue that provides a low-friction, load-bearing surface for joint function.

Articular cartilage:

Articular cartilage is composed of chondrocytes embedded in a dense cartilage extracellular matrix (ECM), which includes collagens (mainly type II), proteoglycans (such as aggrecan), and water. The ECM is organized into distinct zones: the superficial, middle, deep, and calcified layers, each with unique compositions and mechanical properties. Chondrocytes, the sole cellular component, are responsible for maintaining

ECM homeostasis by balancing anabolic (synthesis of matrix components) and catabolic (degradation of matrix) processes.

Cartilage undergoes several phenotypic and functional changes in OA. In OA, ECM synthesis is impaired, with reduced anabolic activity leading to insufficient production of type II collagen and aggrecan. Simultaneously, the overexpression of catabolic enzymes, such as matrix metalloproteinases (MMPs) and aggrecanases (e.g., a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-4 and ADAMTS-5), accelerates ECM degradation. Cartilage ECM undergoes extensive remodeling in OA[29]. The degradation of type II collagen and aggrecan weakens the structural integrity of cartilage. Additionally, the loss of proteoglycans reduces water retention and alters the biomechanical properties of cartilage, making it less resistant to mechanical stress. Mechanical loading plays a critical role in cartilage health. In OA, abnormal loading (e.g., due to obesity, joint malalignment, or previous injury) exacerbates microtrauma, which disrupts chondrocyte function and ECM organization. Cyclic loading at pathological levels induces mechanotransduction pathways that promote catabolic activities.

Subchondral bone

Subchondral bone is a highly specialized structure that supports the overlying articular cartilage and acts as a mechanical buffer during joint loading. It consists of two main regions: the subchondral trabecular bone, which is spongy and rich in bone marrow,

and the subchondral cortical bone, which provides strength and stability. The subchondral bone is critical in maintaining the integrity of the cartilage-bone interface, playing a role in load distribution and shock absorption during normal joint movement.

In OA, subchondral bone undergoes several structural and functional changes that exacerbate cartilage degeneration and contribute to disease progression. One of the earliest and most consistent features in OA is subchondral bone sclerosis, characterized by increased bone density and thickening of the subchondral cortical bone[30]. This increase in bone density is thought to be a compensatory response to excessive mechanical load, but it results in a loss of bone elasticity, impairing the shock-absorbing properties of the subchondral bone and contributing to cartilage degeneration. Cysts are commonly observed in OA, particularly in the later stages of the disease. They are formed by the accumulation of fluid and bone marrow, which leads to localized bone damage. Subchondral bone cysts are often associated with cartilage defects and are believed to arise due to abnormal vascularization, increased intraosseous pressure, and osteoclast activity[31]. Osteophytes, or bone spurs, are bony protrusions that develop at the joint margins in response to altered mechanical loading and inflammation. They are thought to be a result of aberrant osteoblast activity in the subchondral bone and play a role in joint instability, contributing to pain and restricted range of motion. In OA, there is an imbalance between bone resorption and formation. Increased activity of osteoclasts leads to excessive bone resorption, while osteoblasts compensate by increasing bone formation in some regions. This imbalance contributes to the formation

of sclerosis, osteophytes, and cysts, further destabilizing the joint.

Synovium

The synovium is composed of two layers: the intimal layer, which is in direct contact with the joint cavity, and the subintimal layer, which contains blood vessels, lymphatic vessels, and fibroblasts. The synovium is responsible for the production of synovial fluid, which lubricates the joint and nourishes the cartilage. In a healthy joint, the synovium maintains a delicate balance between producing sufficient synovial fluid for joint lubrication and controlling inflammatory mediators that could damage the joint tissues. However, in OA, this balance is disrupted, leading to chronic inflammation and joint dysfunction.

One of the primary features of OA is low-grade synovial inflammation, which is characterized by an increase in the number of immune cells (such as macrophages, T lymphocytes, and mast cells) and the production of pro-inflammatory cytokines[32]. This inflammation contributes to both cartilage degradation and subchondral bone remodeling. In OA, the synovial lining becomes hypertrophic and thickened due to the increased proliferation of synovial fibroblasts and macrophages[33]. This hyperplasia results in the formation of pannus, an inflamed tissue that can invade and destroy cartilage and bone. The presence of pannus in OA is particularly detrimental, as it disrupts the mechanical properties of the joint and contributes to both pain and joint instability. Synovial fluid in OA is characterized by increased viscosity and the presence

of elevated levels of pro-inflammatory cytokines, proteolytic enzymes, and catabolic mediators. The altered composition of synovial fluid impairs its ability to lubricate the joint effectively and to provide nutrients to the overlying cartilage. Additionally, synovial fluid contributes to the amplification of inflammatory processes, further exacerbating cartilage degeneration.

Tissue crosstalk

Joint tissues will crosstalk with each other, including biomechanical crosstalk, cellular molecular crosstalk, and neural crosstalk. Abnormal joint loading due to obesity, injury, or malalignment alters the mechanical environment of the joint, triggering changes in all tissues. Increased mechanical stress on cartilage induces catabolic responses, while altered bone remodeling leads to changes in joint shape and load distribution[34]. Joint tissues communicate via cytokines, growth factors, and extracellular vesicles. Synovial inflammation increases IL-6 and TNF-α levels, which diffuse into cartilage and bone, accelerating degradation and remodeling[35]. Transforming growth factor-beta (TGF-β) from subchondral bone promotes osteophyte formation and cartilage damage[36]. Vesicles from chondrocytes and synovial fibroblasts carry bioactive molecules that regulate joint homeostasis and disease progression[37]. Nociceptive signaling from subchondral bone and synovium contributes to pain, a key feature of OA. Neuroinflammatory mediators, such as substance P, are implicated in joint pain and inflammation[38].

1.1.3.2 Cellular level (chondrocytes, endothelial cells, synoviocytes, immune cells, osteoblasts and osteoclast)

Chondrocytes

Under normal conditions, chondrocytes are responsible for maintaining the integrity of articular cartilage through the balanced synthesis and degradation of ECM components. They produce collagen fibers, particularly type II collagen, and proteoglycans such as aggrecan, which form the cartilage matrix and provide structural support and resilience to compressive forces. Chondrocytes also regulate ECM turnover by secreting MMPs and aggrecanases in response to mechanical and biochemical signals. This dynamic equilibrium ensures cartilage homeostasis, allowing it to resist mechanical stress and maintain joint function.

In OA, chondrocytes often undergo a process of dedifferentiation, where they lose their cartilage-specific phenotype and adopt a fibroblastic or hypertrophic phenotype. This shift is associated with a reduction in the synthesis of ECM components, such as type II collagen and aggrecan, and an increase in the production of catabolic enzymes, including MMPs and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family members, such as ADAMTS-4 and ADAMTS-5. Dedifferentiated chondrocytes also express genes associated with inflammation and fibrosis, further exacerbating cartilage degradation[39]. Some chondrocytes in OA undergo hypertrophic differentiation, characterized by an increase in cell size and the

expression of markers typically associated with endochondral ossification, such as collagen type X and alkaline phosphatase[40]. These hypertrophic chondrocytes contribute to the calcification of the cartilage matrix, further compromising its structural integrity. The presence of calcified cartilage in OA is associated with the activation of osteoclasts and subchondral bone remodeling, promoting joint degeneration.

Synoviocytes

The synovium comprises two main types of synoviocytes.: Found in the intimal lining of the synovium, Fibroblast-like Synoviocytes (FLS) are responsible for producing synovial fluid components, such as hyaluronic acid and lubricin, which ensure proper joint lubrication and nutrient supply to cartilage. They also regulate ECM turnover in the synovium. Macrophage-like Synoviocytes (MLS) are involved in immune surveillance and clearance of debris within the joint. They secrete anti-inflammatory cytokines under normal conditions, maintaining an immunosuppressive environment.

In healthy joints, synoviocytes work in harmony to support joint function. However, in OA, these cells exhibit phenotypic and functional changes that contribute to synovial inflammation and joint degeneration. In OA, synoviocytes become activated in response to various stimuli, including mechanical stress, damage-associated molecular patterns (DAMPs) from injured cartilage, and inflammatory mediators[32]. This activation results in synovial inflammation, or synovitis, a hallmark of OA that

exacerbates cartilage degradation and bone remodeling. OA-associated FLS exhibit increased proliferation and production of pro-inflammatory cytokines, including interleukin-1 β (IL-1β), TNF- α , and IL-6. These cytokines create a catabolic environment by stimulating the production of MMPs and aggrecanases (e.g., ADAMTS-4 and ADAMTS-5), which degrade cartilage ECM. FLS undergoes hyperplasia and hypertrophy, contributing to the thickening of the synovial lining. This structural change is associated with increased production of ECM-degrading enzymes and inflammatory mediators, further perpetuating cartilage breakdown. MLS in OA synovium release chemokines such as CCL2 and CXCL8, which recruit immune cells like macrophages, T cells, and neutrophils into the synovial tissue[41]. This immune cell infiltration amplifies inflammation and contributes to joint destruction.

Immune cells

Several types of immune cells contribute to the pathogenesis of OA. Macrophages are the predominant immune cells in the synovium of OA joints. They play a central role in the initiation and perpetuation of inflammation by releasing pro-inflammatory cytokines, chemokines, and matrix-degrading enzymes. Macrophages are highly plastic, capable of assuming pro-inflammatory (M1) or anti-inflammatory (M2) phenotypes, depending on the local microenvironment. T lymphocytes, particularly CD4+ T cells, are present in the synovium of OA joints and contribute to joint inflammation and cartilage degradation[42]. T cells can promote the production of inflammatory cytokines and enhance the activity of other immune cells, such as macrophages and

synoviocytes. B cells, which are less abundant than macrophages and T cells in OA, contribute to disease progression through the production of autoantibodies and the activation of the complement system. They may also modulate the activity of T cells and macrophages. Neutrophils are often found in OA synovium during acute inflammation or exacerbations of the disease. These cells release proteolytic enzymes and reactive oxygen species (ROS), which contribute to cartilage degradation and tissue damage.

Osteoblasts and osteoclasts

Osteoblasts are the key cells responsible for bone formation. In healthy bone remodeling, osteoblasts produce ECM proteins, such as collagen type I, osteocalcin, and alkaline phosphatase, which are critical for bone mineralization and strength. In OA, osteoblast activity is dysregulated, contributing to the pathological changes in the subchondral bone. Osteoclasts are multinucleated cells responsible for bone resorption. In OA, osteoclast activity is often increased, leading to imbalanced bone remodeling and contributing to the pathological changes seen in subchondral bone and osteophyte formation.

In OA, osteoblasts in the subchondral bone are often activated and undergo aberrant differentiation, leading to excessive bone formation in some regions and impaired bone formation in others. This imbalance contributes to the formation of osteophytes (bony outgrowths) and subchondral sclerosis, which are hallmark features of OA. Osteoblasts

in the OA joint may also secrete pro-inflammatory cytokines, such as IL-1β and TNF-α, which further contribute to inflammation and the subsequent remodeling of both bone and cartilage. Subchondral bone remodeling is a critical process in OA. Osteoblasts are responsible for new bone formation at the subchondral plate, often leading to the thickening of the bone in response to mechanical loading. In OA, osteoblasts contribute to the development of subchondral bone sclerosis, which is characterized by increased bone density and hardness beneath the cartilage[43]. This sclerosis alters the biomechanical properties of the joint and exacerbates cartilage degeneration by changing the stress distribution across the articular surface. Osteoblasts are responsible for osteophyte formation in OA, which is thought to be a reparative process in response to cartilage degradation. However, excessive osteophyte formation can cause joint pain, stiffness, and loss of function. The overproduction of bone by osteoblasts at the joint margins leads to the characteristic bony outgrowths seen in OA. Osteoclast Activation and Subchondral Bone Resorption: In OA, osteoclasts are activated in response to pro-inflammatory cytokines and other mediators produced by immune cells, chondrocytes, and osteoblasts[44]. This activation leads to increased bone resorption in the subchondral bone. Osteoclasts resorb the bone matrix by secreting hydrogen ions and matrix-degrading enzymes such as cathepsin K and MMPs, leading to the thinning and destruction of the subchondral bone. This resorption contributes to the development of bone cysts, which are observed in OA and further impair joint function.

In OA, the coordinated activity of osteoblasts and osteoclasts is disrupted, leading to an imbalance between bone formation and resorption. This imbalance is a central feature in the pathogenesis of subchondral bone remodeling, cartilage degeneration, and the overall disease progression in OA. While osteoblasts contribute to the formation of subchondral bone sclerosis and osteophytes, osteoclasts drive the resorption of bone and the formation of bone marrow lesions. This dynamic leads to changes in the bone architecture, including both regions of increased bone density (sclerosis) and areas of bone loss (resorption). These alterations can disrupt the mechanical properties of the joint, resulting in increased joint stiffness, pain, and reduced function.

1.1.3.3 Molecular level (IGF-1/IGFBP pathway, Wnt/β-Catenin Signaling pathway, NF-κB Signaling Pathway, TGF-β/Smad Signaling Pathway, Oxidative Stress and ROS Pathways)

IGF-1/IGFBP pathway

Insulin-like growth factor-1 (IGF-1) and its binding proteins (IGFBPs) play pivotal roles in cartilage homeostasis and the pathophysiology of OA. The IGF-1/IGFBP pathway regulates anabolic and catabolic processes in joint tissues, impacting chondrocyte metabolism, ECM synthesis, and inflammatory responses.

IGF-1 is a critical anabolic factor involved in maintaining cartilage integrity, primarily through promoting chondrocyte proliferation and ECM synthesis[45]. IGFBPs, which

regulate IGF-1 bioavailability and activity, add a layer of complexity to this signaling pathway. Dysregulation of the IGF-1/IGFBP axis has been implicated in OA pathogenesis[46]. IGF-1 is essential for cartilage maintenance, influencing several processes. IGF-1 stimulates chondrocyte proliferation and prevents hypertrophic differentiation, preserving cartilage function[47]. IGF-1 enhances the production of collagen type II and aggrecan, key components of cartilage ECM, while inhibiting matrix-degrading enzymes like MMPs and ADAMTS[48]. IGF-1 protects chondrocytes from apoptosis induced by oxidative stress and pro-inflammatory cytokines[48]. IGFBPs modulate IGF-1 activity by either enhancing or inhibiting its interaction with the IGF-1 receptor (IGF-1R). Elevated levels of these IGFBPs in OA cartilage have been associated with impaired IGF-1 signaling and reduced cartilage repair, including IGFBP-3 and IGFBP-5[49]. IGFBP6 promotes significantly higher in vitro migration of T lymphocytes in RA patients c and is notably overexpressed in the serum and synovial tissue of RA patients[50]. Pro-inflammatory cytokines like IL-1β and TNF-α downregulate IGF-1 and upregulate IGFBPs, disrupting anabolic signaling in cartilage[51]. This shift promotes ECM breakdown and chondrocyte apoptosis. Aging, a major risk factor for OA, is associated with reduced IGF-1 expression and sensitivity. Impaired IGF-1 signaling due to increased IGFBP levels exacerbates cartilage degeneration[52].

Here's a summary table outlining the roles of Insulin-like Growth Factor Binding Proteins (IGFBPs) 1–7 in cellular senescence, including their mechanisms and tissue-

specific effects:

IGFBP	Role in Cellular Senescence	Key Mechanisms	Context Notes
IGFBP1	Limited direct evidence; potential indirect modulation via IGF-1 bioavailability.	May sequester IGF-1, indirectly reducing IGF-1-mediated anti-senescence signaling.	Role in senescence remains poorly characterized; studied more in metabolic stress.
IGFBP2	Context-dependent: anti-senescence in some cancers, pro-senescence in aging tissues.	- Inhibits IGF-1 signaling, promoting senescence in aging. - Activates PI3K/Akt to delay senescence in cancer[53].	Overexpressed in tumors to evade senescence; linked to age-related vascular dysfunction.
IGFBP3	Pro-senescence: Potent inducer in aging, oxidative stress, and cancer.	- IGF-independent activation of p53/p21 and ROS pathways[54].	Major in replicative senescence; upregulated in age-related diseases (e.g., COPD, atherosclerosis).

IGFBP	Role in Cellular Senescence	Key Mechanisms	Context Notes
		- Binds RXRα to block cell cycle progression[55]. - Suppresses telomerase activity[56].	
IGFBP4	Dual role: pro-senescence when intact; anti-senescence when cleaved by PAPP-A.	- Intact IGFBP4 sequesters IGFs, inhibiting pro-survival signaling. - Cleaved by PAPP-A releases IGFs to delay senescence[57].	Cleavage status determines function; linked to placental and vascular aging.
IGFBP5	Pro-senescence: Promotes senescence in aging	- Binds integrins to activate TGF-β/Smad3	Upregulated in fibrotic lungs, aged skin.

IGFBP	Role in Cellular Senescence	Key Mechanisms	Context Notes
	and fibrosis[58].	signaling[59]. - Inhibits IGF-1-mediated survival.	
IGFBP6	Limited data; potential pro-senescence via IGF-2 inhibition.	High affinity for IGF-2 blocks IGF-2-mediated growth/survival signals.	Role unclears; implicated in IGF-2-dependent cancers (e.g., rhabdomyosarcoma).
IGFBP7	Pro-senescence: Key induce in aging, cancer, and vascular dysfunction.	- IGF-independent activation of p53/p16INK4a-Rb pathways[60].	Overexpressed in senescent endothelial cells and age-related fibrotic tissues[61].

Wnt/β-Catenin Signaling pathway

The Wnt/ β -catenin signaling pathway plays a crucial role in maintaining joint homeostasis and is implicated in the pathogenesis of OA. Dysregulation of this pathway

contributes to cartilage degeneration, subchondral bone remodeling, and synovial inflammation. The Wnt/β-catenin signaling pathway, a key regulator of cell proliferation, differentiation, and metabolism, has been implicated in OA pathogenesis[62].

The Wnt/β-catenin pathway is a canonical signaling cascade activated by Wnt ligands binding to Frizzled receptors and co-receptors like receptor-related protein （LRP） 5/6. This interaction inhibits the β-catenin destruction complex, allowing β-catenin to accumulate and translocate to the nucleus, where it regulates the expression of target genes involved in cell fate and tissue homeostasis[63]. Excessive Wnt/β-catenin signaling increases the expression of matrix-degrading enzymes such as MMP-13 and ADAMTS-5, leading to the breakdown of type II collagen and aggrecan[64]. Simultaneously, it inhibits the anabolic activity of chondrocytes, impairing cartilage repair. Hyperactivation of Wnt signaling in osteoblasts contributes to subchondral bone thickening and osteophyte formation. These changes disrupt the mechanical balance of the joint and accelerate cartilage damage[65]. Increased Wnt activity in synovial fibroblasts promotes the production of pro-inflammatory cytokines like IL-6 and TNF-α, creating a vicious cycle of inflammation and joint destruction[66].

Nuclear factor-kappa B （NF-κB） Signaling Pathway

The NF-Kb signaling pathway is a central mediator of inflammation and catabolic processes in osteoarthritis. Its activation drives the expression of pro-inflammatory

cytokines, matrix-degrading enzymes, and apoptotic pathways, contributing to joint degeneration.

NF-κB activation in chondrocytes promotes the production of MMPs and ADAMTS, which degrade type II collagen and aggrecan, key components of cartilage ECM[67]. Additionally, NF-κB suppresses the expression of anabolic factors such as Sox9, further impairing cartilage repair. Synovial fibroblasts activated by NF-κB secrete pro-inflammatory cytokines (IL-6, TNF-α) and chemokines, exacerbating synovitis and amplifying inflammatory signaling in the joint microenvironment[68]. NF-κB signaling in osteoblasts and osteoclasts contributes to subchondral bone sclerosis and osteophyte formation. The non-canonical NF-κB pathway, in particular, plays a role in osteoclast differentiation and bone resorption[69].

TGF-β/Smad Signaling Pathway

The TGF-β/Smad signaling pathway plays a pivotal role in maintaining joint homeostasis and regulating cartilage and bone remodeling. In OA, dysregulation of this pathway contributes to both anabolic and catabolic processes, leading to complex and sometimes paradoxical effects on joint tissues.

Excessive TGF-β signaling, particularly through Smad2/3 imbalance, induces chondrocyte hypertrophy, a hallmark of OA progression[52]. High levels of TGF-β in subchondral bone promote abnormal bone remodeling and osteophyte formation,

contributing to joint dysfunction[36]. Dysregulated TGF-β signaling enhances the secretion of inflammatory mediators and matrix-degrading enzymes in synovial fibroblasts[70].

Oxidative Stress and ROS Pathways

Oxidative stress and ROS play significant roles in the pathogenesis of OA. ROS, generated by cellular metabolism and inflammatory processes, disrupt cartilage homeostasis and promote chondrocyte apoptosis, synovial inflammation, and subchondral bone remodeling. Oxidative stress, defined as an imbalance between ROS production and antioxidant defenses, is a critical factor in OA pathogenesis.

ROS promotes the expression of matrix-degrading enzymes, including MMPs and ADAMTS, which break down type II collagen and aggrecan in cartilage ECM[71]. ROS-induced DNA damage and mitochondrial dysfunction trigger chondrocyte apoptosis, a key event in OA progression. Activation of apoptotic pathways, such as p53 and caspase cascades, has been linked to oxidative stress in OA cartilage[72]. ROS amplify inflammatory responses in synovial fibroblasts by activating NF-κB and MAPK pathways, leading to the production of cytokines and chemokines[73]. Oxidative stress contributes to abnormal bone remodeling, including subchondral sclerosis and osteophyte formation, by affecting osteoblast and osteoclast activity[72].

1.1.4 Osteoarthritis diagnosis and treatment

1.1.4.1 Clinical diagnostic manifestations and radiological examinations

The diagnosis of OA mainly relies on clinical symptoms and imaging examinations. Common clinical symptoms include joint pain, stiffness, limited movement and joint deformity. Imaging examinations mainly include X-ray, magnetic resonance imaging (MRI) and ultrasound. X-ray examination is the most commonly used imaging diagnostic method, which can clearly show typical changes such as joint space narrowing, osteophyte formation and sclerosis[74]. MRI can provide more detailed soft tissue structure information, such as cartilage damage, synovitis and meniscus lesions, which is helpful for early diagnosis and assessment of the severity of the disease[75]. For isolated tissues, staining of joint sections can also determine whether the joint has OA.

1.1.4.2 Pharmacological, non-pharmacological, and surgical treatment

The treatment of OA includes Pharmacological, non-pharmacological, and surgical treatment. Medical treatments mainly include nonsteroidal anti-inflammatory drugs (NSAIDs), analgesics, and intra-articular injections of steroids or hyaluronic acid. NSAIDs are the most commonly used medications and are effective in relieving pain and inflammation[76]. For patients with severe symptoms, intra-articular injections may be chosen to provide short-term relief. Nonpharmacological treatments include physical therapy, weight loss, and the use of walkers. Physical therapy can strengthen the muscles around joints and improve joint stability and mobility. Weight loss is

particularly important for obese patients, as it can reduce the burden on joints and slow down the progression of the disease. When conservative treatments fail, surgical treatment is an effective option. Common surgical procedures include arthroscopic debridement, osteotomy, and joint replacement. Arthroplasty is an effective method to treat late-stage OA, which can significantly improve pain and function and improve patients' quality of life.

1.2 Hypertension as a major comorbidity of OA

1.2.1 Fundamental information on hypertension

1.2.1.1 Prevalence and Burden

Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg without taking antihypertensive medication. Globally, 1.28 billion adults aged 30-79 suffer from hypertension, but only 54% know they have it[77]. 58% have not received diagnosis and treatment, and about 79% of hypertension is not well controlled[77]. The survey found that the prevalence of hypertension among Chinese adults is 27.9%, accounting for about a quarter of the adult population[78]. Hypertension incurs a national economic burden ranging from approximately $131 to $198 billion annually[79]. This figure encompasses the expenses associated with healthcare services, pharmaceutical treatments for managing hypertension, and the productivity losses attributed to premature mortality[79].

1.2.1.2 Pathophysiology and etiological factors of hypertension

The pathophysiology of hypertension is complex and multifactorial, involving various genetic, environmental, and physiological factors. Genetic factors play an important role in the development of hypertension. Genome-wide association studies (GWAS) have identified many genetic loci associated with blood pressure regulation[80]. Genetic variants associated with the renin-angiotensin-aldosterone system (RAAS), the sympathetic nervous system (SNS), and renal function are particularly influential. RAAS is a key regulator of blood pressure and fluid balance. Dysregulation of this system can lead to hypertension. Increased renin release in the kidney triggers the conversion of angiotensinogen to angiotensin I, which is then converted to angiotensin II by angiotensin-converting enzyme (ACE). Angiotensin II is a potent vasoconstrictor that stimulates aldosterone secretion, leading to sodium retention and increased blood volume[81]. An overactive SNS increases heart rate, cardiac output, and peripheral vascular resistance, leading to hypertension. Chronic SNS stimulation can also induce changes in vascular structure, further exacerbating hypertension[82]. Endothelial cells lining the blood vessels play a crucial role in maintaining vascular tone and homeostasis. Dysfunctional endothelial cells, characterized by decreased nitric oxide (NO) bioavailability and increased oxidative stress, lead to vasoconstriction, inflammation, and vascular remodeling, which can cause hypertension[83]. The kidneys regulate blood pressure by controlling fluid and electrolyte balance. Impaired renal function due to genetic or acquired factors can lead to sodium and water retention, increased blood volume, and hypertension[84]. In addition, renal vascular resistance may be altered,

affecting blood pressure regulation.

Studies have shown that hypertension is closely related to endothelial dysfunction, which is not only an important factor in the occurrence of hypertension, but also one of the key factors in the occurrence of its complications[85]. Vascular endothelial cells secrete NO, endothelin and other active substances to regulate vascular contraction and relaxation, blood flow and vascular remodeling, thereby maintaining the normal function of blood vessels.

The characteristics of endothelial dysfunction mainly include the following aspects:

1. Decreased production of NO: NO synthesized by endothelial cells is a key molecule for regulating vascular tension. Its reduced production will lead to vasoconstriction and increased blood pressure[86].

2. Increased production of endothelin-1 (ET-1): ET-1 is a potent vasoconstrictor factor. Its increased production will cause vasoconstriction and further increase vascular resistance[87].

3. Decreased endothelium-dependent vasodilation response: The role of endothelial cells in regulating vasodilation is weakened, resulting in reduced vascular responsiveness to vasodilation signals[88].

4. Increased oxidative stress: High levels of ROS can damage endothelial cells, weaken their function, and reduce the bioavailability of NO[85].

5. Enhanced inflammatory response: Under pathological conditions such as

hypertension, endothelial cells will release more proinflammatory cytokines, further damaging endothelial function[89].

6. Imbalance between endothelial cell proliferation and apoptosis: The normal renewal and repair process of endothelial cells is impaired, leading to the destruction of endothelial barrier function.

Endothelial dysfunction caused by hypertension is mainly achieved through the following mechanisms: 1. Oxidative stress: The increased shear force of the vascular wall caused by hypertension promotes the increase of ROS, thereby reducing the bioavailability of NO in endothelial cells and leading to enhanced vasoconstriction[90]. 2.Inflammatory response: Hypertension can induce vascular inflammation and endothelial cell damage by activating the NF-κB pathway and increasing the expression of proinflammatory cytokines such as TNF-α and IL-6[91]. 3.Endothelial cell dysfunction: Hypertension can lead to an imbalance between endothelial cell proliferation and apoptosis, affecting the endothelial repair ability and impairing endothelial function[90].

Endothelial dysfunction is not only a result of hypertension, but also an important driving factor in its development. Endothelial dysfunction can lead to increased vascular resistance and blood pressure, further aggravating hypertension. In addition, endothelial dysfunction can also promote the occurrence of atherosclerosis, myocardial infarction and stroke, increasing the risk of cardiovascular events in hypertensive

patients.

1.2.1.3 Therapeutic Approaches for Hypertension

Drug therapy is the core of hypertension management, and its main goal is to lower blood pressure to the target level to reduce the occurrence of cardiovascular events. Commonly used drug categories include:

1. Diuretics: such as thiazide diuretics, have been shown to have significant effects in lowering blood pressure and improving cardiovascular prognosis[92].

2. ACE inhibitors: such as enalapril, can effectively inhibit ACE, lower blood pressure, and have a protective effect on the heart and kidneys [93].

As the first oral ACE inhibitor, Captopril has become one of the important drugs for the treatment of hypertension since its introduction in the 1970s[94]. Captopril lowers blood pressure by inhibiting ACE. ACE is an enzyme that converts angiotensin I (Ang I) into angiotensin II (Ang II), which has a strong vasoconstrictor effect. Ang II activates its receptors, causing vascular smooth muscle contraction, thereby raising blood pressure. Captopril inhibits the activity of ACE, reduces the production of Ang II, causes vasodilation, and lowers blood pressure. In addition to reducing the production of Ang II, Captopril also lowers blood pressure by reducing the secretion of aldosterone. Aldosterone is a hormone secreted by the adrenal cortex that acts primarily on the kidneys to promote the reabsorption of sodium and water, increase blood volume, and raise blood pressure[95]. Captopril indirectly inhibits the secretion of aldosterone

by reducing the level of Ang II, thereby reducing blood volume and lowering blood pressure. Studies have shown that Captopril can also lower blood pressure by reducing the activity of the sympathetic nervous system[96]. The sympathetic nervous system plays an important role in the occurrence and development of hypertension. Its overactivity can lead to vasoconstriction and increased cardiac output, thereby increasing blood pressure[97]. Captopril reduces the production of Ang II and the activity of the sympathetic nervous system, thereby helping to lower blood pressure.

3. Calcium channel antagonists: such as amlodipine, by blocking calcium ions from entering vascular smooth muscle cells, dilating blood vessels and lowering blood pressure [98].

4. β-blockers: such as metoprolol, can slow down heart rate, reduce cardiac output, and thus lower blood pressure[81]

.

1.2.2 Epidemiology evidence

One experiment compared the early industrial era with the post-industrial era, and after controlling for age and Body mass index (BMI), the prevalence of OA increased from 8% to 16%[99]. The results of the study suggest that increases in life expectancy and BMI are insufficient to explain the roughly doubling of the prevalence of knee osteoarthritis in the United States since the mid-20th century, so there are other important factors that contribute to the worsening of osteoarthritis.

Metabolic syndrome is a common risk factor for hypertension and osteoarthritis. Metabolic syndrome includes obesity, insulin resistance, hyperlipidemia, etc. These factors promote the occurrence of hypertension and osteoarthritis through various pathways. An experiment involving 151 people showed that the severity of osteoarthritis was significantly positively correlated with the presence of high blood pressure, high blood sugar, and high blood lipids. Similarly, osteoarthritis patients were more likely to have abnormal blood sugar, blood pressure, and blood lipids[99].

Hypertension and osteoarthritis are two common chronic diseases that often coexist in the elderly. A large number of studies have shown that these two diseases often coexist in the same patient, a phenomenon known as comorbidity. Higher systolic blood pressure and pulse pressure were found to be associated with increased incidence of radiographic knee OA in a US cohort with 1930 individuals[100]. In a total of 26 studies involving 97,960 participants, the overall odds of developing OA significantly increased in hypertensive patients compared to normotensive subjects. There is a strong relationship between high blood pressure and damage to the bone and joint structures of the knee, not just chronic joint pain, especially in women[101]. Preliminary findings of a study of 21 female patients (aged 52-82 years) undergoing total knee replacement surgery also revealed that systolic blood pressure, pulse pressure, and heart rate were associated with microstructural changes in subchondral bone mineral density (BMD) and advanced knee OA[102].

1.2.3 Vasculature in joint disease

Due to the high incidence of complications of hypertension and OA, researchers have focused their attention on vascular dysfunction in the etiology of OA in recent years. Systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), mean arterial pressure (MAP), and heart rate (HR) are standard hemodynamic indices[103]. According to the Osteoarthritis Initiative, high systolic blood pressure is associated with an increased radiographic risk of knee OA[100]. Although a large number of studies have been devoted to studying the relationship between hypertension and OA, the vascular etiology of OA remains unclear.

1.2.3.1 Vessels in subchondral bone

Cartilage and subchondral bone combine to produce a functional osteochondral unit that primarily functions as a shock absorber and load distributor[104]. In this unit, the subchondral bone is a layer underlying the hyaline cartilage[105]. Non-calcified cartilage, cartilage, and subchondral bone form a sandwich structure divided by two lines. One is tidemark, which separates calcified and non-calcified cartilage; another is cement line, which separates the subchondral bone and calcified cartilage. The subchondral bone has two anatomical components: subchondral bone plate and subchondral bone trabeculae[106]. As a porous cortical plate, the subchondral bone plate is crossed by numerous blood vessels and nerve fibers, providing the primary nutritional support for cartilage, including glucose, oxygen, and water[106, 107]. Subchondral bone trabeculae are highly vascularized and significant function in stress absorption, structural support, and bone remodeling[105]. During the early stage of OA,

the subchondral bone plate becomes more porous and thinner, and subchondral trabeculae deteriorate, while subchondral bone sclerosis and reduced bone marrow spacing were observed in the late stage of OA[106]. Subchondral bone marrow lesions (BMLs) are common MRI features of OA and occur prior to cartilage degeneration. It is also called subchondral bone edema or cyst-like lesions because it has a hyperintense MRI signal similar to the edema-like signal[108]. Although the underlying mechanism remains unclear, more evidence suggests that vascular aging and endothelial dysfunction may play an important role[109, 110]. Compared to zones without BMLs, zones of human OA knee samples with BMLs have a higher density of vascular channels near calcified cartilage and more extended vascular channels near bone marrow. In addition, the tideline is penetrated by more vascular channels, implying a vascular contribution to BMLs[110]. To investigate the relationship between hypertension and OA, our team observed changes in subchondral bone in SHR and the deoxycorticosterone acetate (DOCA) hypertensive rat model, respectively. In the SHR model, the subchondral cystic lesions near the cruciate ligament entheses were more evident than in controls. However, in the DOCA model, fewer and thinner subchondral bones were observed in the lateral and medial regions of the tibial plateau[111]. These results proved that hypertension significantly influences subchondral bone.

Besides, many vascular associate cytokines have a strong association with OA. Neuronal nitric oxide synthase (nNOS), inducible nitric oxide synthase (iNOS), and endothelial nitric oxide synthase (eNOS) are three enzymes that produce nitric oxide (NO)[112]. NO regulates vasodilation and thus controls blood pressure. Although the most important for vascular tone is eNOS, nNOS and iNOS contribute to vascular function[113]. NO is also an essential mediator in subchondral bone alteration. Through

immunohistochemical technique, a high level of iNOS was observed in both the articular cartilage and subchondral bone of the monoiodoacetate （MIA） model[114]. Oral administration of avocado soybean unsaponifiables can attenuate the expression of iNOS in subchondral bone and mitigate the severity of OA [114].

The subchondral bone of the knee joint in the MIA rat model was severely resorbed and replaced by fibrous tissue. However, after the use of ACE inhibitor captopril, a RAS inhibiting agent, fibrosis and the number of osteoclasts around the subchondral bone decrease[115].

ET1 is a potent endogenous vasoconstrictor secreted by the endothelial cell. Obesity, hypertension, and diabetes may also contribute to a high level of ET-1[116]. Our team found that increased ET1 expression in subchondral bone was accompanied by a rise in plasma ET1 levels in the destabilization of medial meniscus (DMM) model, indicating that ET1 is positively linked with the severity of OA[117]. After ETAR inhibitor BQ123 or ETBR inhibitor BQ788 treatment, the osteophytes formation is reserved in the DMM model, suggesting blocking endothelin receptors may be a potential therapy to rescue OA changes of subchondral trabecular bone[117].

VEGF is a cytokine that induces the migration and proliferation of endothelial cells and increases vascular permeability[118]. It also induces angiogenesis, mediates catabolic processes, and recruits inflammatory cells (macrophages, B, and T cells) to the disease site, further aggravating the OA process[119, 120]. Biopsy investigation of subchondral bone from patients with advanced OA found that individuals with bone marrow lesions had greater levels of VEGF, ET1, and ANG II than those without bone marrow lesions,

suggesting that bone marrow lesions induce increased vascularity in the subchondral bone of patients with OA[121]. During the subchondral bone degradation, nascent subchondral H-type vessels invade cartilage by secreting more VEGF to activate the mTORC1 pathway exacerbating the structural changes and promoting the progression of OA[122].

1.2.3.2 Synovial angiogenesis

The synovium, another way to supply cartilage with nutrients, is a barrier between the internal joint structures and the surrounding musculoskeletal tissues. It comprises two layers: intima and subintima. The intima contains two synoviocytes, type A (macrophage or monocyte cell lines) and type B (fibroblast). The M1-to-M2 transition of synovial macrophages in joints can influence the chondrogenic differentiation of mesenchymal stem cells (MSCs), leading to OA[123]. Fibroblast-like cells are essential in supplying lubricating chemicals such as hyaluronic acid, the main component of synovial fluid, to the joint cavity and nearby cartilage to preserve articular mobility[124]. The subintima comprises a complex vascular network of blood and lymphatic vessels and different connective tissue (fibrous, fatty, or areolar) connecting to the joint capsule's outer layer[104, 125]. Synovial cells lack a basal lamina and the cellular connections that allow synovial fluid to interchange with blood or lymphatic vessels[104].

The synovium self-regulates through homeostatic mechanisms in healthy people, whereas inflammation occurs in OA patients. As a primary pathogenic alteration of OA, synovial inflammation has long been recognized as a primary cause of joint pain and is involved in OA onset and progression of OA[126, 127]. Synovitis is a clinical term for synovial inflammation, which can be detected by ultrasound in more than 50% of

patients with early OA[104]. In the early stage of OA, synovitis is typically characterized by synovial hyperplasia, stromal vascularization, and inflammatory cell infiltration, which causes the production and release of various cytokines and chemokines, resulting in more severe synovitis[119, 128-130]. Clinically, numerous vascularity, villi, and fibrin deposits can be seen on the synovium during arthroscopy in patients with OA[131].

Because the synovium has a rich vascular system, the vascular cytokine changes associated with hypertension may promote the process of osteoarthritis through the vascular system. High levels of iNOS were seen in the synovial fluid of OA patients[131]. The level of iNOS in the synovial fluid has become an indicator for the evaluation of OA. Prostaglandin E2 (PGE2) contributes to the development of synovitis and causes hyperalgesia by enhancing the production of the pro-inflammatory molecule NO by raising the expression of iNOS[131]. In an animal study, transthyretin deposition worsens the severity of OA, as evidenced by increased synovitis and a substantial increase in iNOS expression in the synovium[132].

ANG II is another RAS active peptide involved in microvascular remodeling[133]. It is shown that ANG2 is elevated in hypertension, and ANG II receptor blockers (ARBs) have been used as first-line agents in treating hypertension[134]. Recent studies found that ANG II is involved in the migration of fibroblast-like synoviocytes[115]. The agonism of the ANG II type 2 receptor efficiently decreases the proliferation and migration of fibroblast-like synoviocytes in RA[135]. Similarly, intra-articular injection of ANG II in the rat knee caused hyperalgesia, joint swelling, and increased leukocytes in the synovial joint. In contrast, in an OA model, injection of the angiotensin-

converting enzyme inhibitor (ACEI) captopril decreased synovial leukocyte recruitment and had a trend in reducing hyperalgesia[115]. In addition, a microarray-based analysis found that angiotensinogen (AGT) was an upregulated gene both in the early and late stages of the knee OA synovial member sample and played a crucial role in the development and progression of knee OA, which means that AGT might be a potential biomarker for gene therapy for OA[136].

There have been many types of research about the VEGF level in OA synovium in recent years. In human synovial fibroblast samples, VEGF expression and angiogenesis levels were higher in patients with OA than in healthy people[137]. High glucose levels also increase VEGF secretion by generating ROS, resulting in increased angiogenesis, tissue damage, and inflammation[138]. In addition, VEGF content was significantly higher in the synovium and synovial fluid of patients with post-traumatic osteoarthritis, negatively correlated with the level of miR-519d-3p[139]. Another clinical investigation gathered synovial membranes from 102 knee OA patients who had total knee arthroplasty and discovered that VEGF-positive cells were detected in the lining of the synovial membrane and that VEGF expression levels were positively connected with the visual analog pain scale[140]. In addition, EG-VEGF, FGF4, and PIGF were discovered to be differently expressed in synovial fluid from 24 OA patients and 24 healthy people, suggesting that they may accelerate the progression of OA[141]. Synovial cell proliferation and the preponderance of tiny immature vessels are hallmarks of primary hip OA. More VEGF can be observed in immunohistochemical labeling of hip OA synovium, suggesting that VEGF-dependent angiogenesis may cause hip OA[142]. The anterior cruciate ligament transaction (ACLT) model, DMM model, and MIA model are classic OA models. A recent study discovered that the

angiogenesis markers CD31, VEGF, and TGF-b were elevated in the synovial tissue of all three models than in the controls, promoting synovial fibrosis and positively correlated with pain sensitivity. Among them, synovial fibrosis was most typical in the DMM model on day 14 but most typical in the ACLT model on day 28[143]. Increased VEGF expression in chondrocytes and synovium cells is connected with the catabolic process[144]. Therefore, intra-articular anti-VEGF antibodies, oral administration of VEGFR2 kinase inhibitor vandetanib, and conditional knockdown of VEGF have been proven to suppress OA progression[144].

1.2.3.3 Vascular invasion at cartilage

Cartilage is an elastic tissue without blood vessels and nerves on the surface of joints. Cartilage acts as support and protection. Articular cartilage is composed of sparsely distributed highly specialized chondrocytes and matrix [145]. Chondrocytes are composed of three types of molecules: collagen, glycoproteins and noncollagenous proteins. The matrix maintains the chondrocyte phenotype and protects the cells from damage caused by joint use [146]. Chondrocytes go through a complex differentiation process throughout their lives. Mesenchymal precursor cells differentiate into prechondrocytes, which then form early chondrocytes. Then chondrocytes progress to prehypertrophic, hypertrophic and end-stage, and finally to apoptosis. Bone-forming cells invade the lumen to expand primary ossification centers. Chondrocyte terminal differentiation represents another major transition in fate and differentiation, as cells transform into an osteoblast-like phenotype and eventually undergo apoptosis [147]. Terminally differentiated hypertrophic chondrocytes are an efficient source of osteoblasts [148].

There are no blood vessels in cartilage. Nutrient supply to cartilage comes from vascular supply in other surrounding tissues, such as synovium and subchondral bone. The nutrients and metabolism required by articular cartilage are obtained by diffusion exchange through synovium. In adults, the subchondral plate separates the articular cartilage matrix from the subchondral vascular space. The cartilage matrix can limit the exchanged material by size, charge, and molecular configuration[149].

Degeneration of articular cartilage is a cause of joint pain and dysfunction and is part of the osteoarthritis syndrome [150]. The degeneration and repair of normal cartilage matrix is a balanced process. Chondrocytes respond to cytokines and physicochemical stimuli to maintain joint homeostasis. When the equilibrium is disrupted, cartilage matrix degradation is greater than synthesis, resulting in the release of matrix fragments into the synovium. The phagocytosis of cartilage matrix degradants by synovial macrophages in turn leads to the local synthesis of more proteases and pro-inflammatory cytokines. Cytokines stimulate cartilage to synthesize more proteases, which further break down more cartilage matrix. After a vicious cycle, resulting in osteoarthritis[151]. When the articular cartilage is worn out in a large area, the protective function of the cartilage for the joint is greatly reduced. Without the protection of articular cartilage, during joint movement and loading, bone-to-bone wears directly, which is also the cause of pain.

Blood vessels feed cartilage through the synovium and subchondral bone. Loss of a nutrient source in either of these pathways results in cartilage deformation and functional degradation[152]. The subchondral region is highly vascularized. Its terminal vascular portion is in direct contact with the deepest cartilage layer. Nutrients

provided by these blood vessels including glucose, oxygen and water make up more than 50% of the cartilage. Injury and persistent mechanical overload of blood vessels, accompanied by insufficient healing, may eventually lead to avascular necrosis, osteochondritis dissecans, or degenerative disease[153]. Because blocked or damaged venous drainage reduces blood flow, the supply of nutrients and oxygen is reduced, and the excretion of waste products is affected. In osteoarthritis, venous stasis deactivates bone cells. Highly vascular subchondral areas are also affected, resulting in reduced nutrition of the overlying cartilage. At the same time, necrotic bone tissue may also lead to structural changes in the subchondral bone, thereby affecting the structural support of the cartilage[154].

Many growth factors secreted by blood vessels also regulate cartilage development. Regulation of vascular growth factor is also one of the methods for the treatment of osteoarthritis. For example, VEGF increases the secretion of MMP-1, MMP-3, and especially MMP-13. Furthermore, VEGF induced IL-1β, IL-6, TNF-α and nitric oxide expression and stimulated the proliferation of immortalized chondrocytes[155]. These findings suggest that VEGF is an autocrine stimulator of immortalized chondrocytes involved in the destructive process of osteoarthritis[156]. Conditional knockout of VEGF attenuates induced OA. Oral VEGFR2 kinase inhibitor vendetanib attenuates OA progression[144]. Studies have shown that early growth response-1 (Egr-1) induces cartilage angiogenesis through the NT-1/DCC-VEGF pathway, which may be associated with abnormal OA angiogenesis[157]. ET-1 was originally thought to be a potent vasoconstrictor. Our group's studies have shown that endothelin-1 induces chondrocyte senescence and cartilage damage through endothelin receptor B in a mouse model of post-traumatic osteoarthritis[117].

1.2.3.4 Vessels with ligaments and muscles

The joint capsule of the knee joint is loose and weak, and the stability of the joint mainly depends on the ligaments and muscles. There are four main ligaments in the knee joint: lateral collateral ligament (LCL), medial collateral ligament (MCL), anterior cruciate ligament (ACL), and posterior cruciate ligament (PCL). The LCL and the MCL are located on the lateral and medial sides of the knee, respectively, and mainly provide lateral stability of the knee. The ACL prevents excessive anterior displacement of the calf bone relative to the femur. The PCL prevents excessive posterior displacement of the calf bone relative to the femur.

Anterior cruciate ligament degeneration is very common in knees with cartilage defects, possibly even before cartilage changes[158]. ACL sensory loss may lead to joint dysfunction and subsequent changes in OA[159]. Sustained high levels of the cytokines IL-6 and IL-8 and the chemokines RANTES, IP-10, MMP1, MMP3 and HSPA1A involved in the inflammatory process sometime after an anterior cruciate ligament injury suggest that they may play a role in maintaining the inflammatory environment Disease effect, leading to post-traumatic OA[160]. The ligament receives very little blood supply and is partly nourished by synovial fluid in addition to its normal blood supply[161]. How dysvascularization leads to degeneration of intra-articular ligaments in OA is unclear, but some degenerated ACLs show increased microvascularity[162].

Skeletal muscle structure and function are associated with the development and progression of knee OA disease[163]. Muscle cells play a role in regulating cartilage matrix production. Chondrocytes co-cultured in muscle cell conditioned medium significantly enhanced the expression of collagen II and IX[164]. Muscle cells can

reduce chondrocyte responses to IL-1β and TNFα, thereby enhancing resistance to proinflammatory cytokine-induced cartilage destruction[165]. One study found that plasma levels of the myocytokine brain-derived neurotrophic factor (BDNF) were higher in OA patients than in healthy controls. In OA patients, synovial fluid BDNF levels were 6-fold lower than plasma levels, and plasma BDNF levels were significantly associated with pain levels[166]. Some cytokines are released from skeletal muscle into skeletal muscle blood vessels to participate in circulation during exercise[167].

1.2.4 Shared molecular pathomechanisms between systemic hypertension and local osteoarthritis

1.2.3.1 Renin-angiotensin system (RAS) pathway

RAS is a crucial regulator of blood pressure and fluid homeostasis, playing a central role in the pathogenesis of hypertension. Studies suggest a potential link mediated by systemic inflammation, oxidative stress, and metabolic dysregulation, with the RAS serving as a common pathway[168-174]. RAS components play a significant role in the pathogenesis of OA, and their inhibitors have been widely utilized in OA treatment, targeting not only inflammation but also angiogenesis associated with the disease[175].

Ang II induces vasoconstriction, endothelial dysfunction, and oxidative stress through AT1R, promoting hypertension[176]. Ang II stimulates the production of MMPs and ADAMTS, degrading cartilage ECM[177]. Ang II promotes bone erosion in osteoarthritis[178]. Ang II-induced ROS generation through NADPH oxidase (NOX)

contributes to vascular and joint damage by exacerbating oxidative stress and cellular senescence[72]. Both conditions are characterized by chronic low-grade inflammation driven by Ang II, which upregulates NF-κB signaling and inflammatory mediators in vascular and joint tissues[179].

1.2.3.2 Oxidative Stress pathways

Both hypertension and OA are associated with mitochondrial dysfunction, leading to excessive ROS production and impaired energy metabolism in vascular endothelial cells and chondrocytes, respectively[180, 181]. ROS-driven activation of NF-κB and MAPK signaling promotes systemic and local inflammation in both conditions, creating a feedback loop that exacerbates tissue damage[72]. Oxidative stress stimulates abnormal angiogenesis in subchondral bone and synovium in OA and contributes to vascular remodeling in hypertension through Vascular endothelial growth factor （VEGF） and hypoxia-inducible factor-1α (HIF-1α) signaling pathways[182, 183].

1.2.3.3 IGF-1/IGFBP pathway

IGF-1 and its binding proteins play essential roles in cellular growth, differentiation, and metabolism. Dysregulation of the IGF-1/IGFBP pathway contributes to the pathogenesis of both hypertension and OA, highlighting shared mechanisms such as vascular remodeling, inflammation, and ECM turnover.

IGF-1 promotes vascular smooth muscle cell (VSMC) proliferation and endothelial cell migration, contributing to arterial remodeling and stiffness[184]. Dysregulated IGFBPs can exacerbate this process by altering IGF-1 activity and promoting vascular calcification. IGF-1 regulates angiogenesis in subchondral bone and synovial tissues, a hallmark of OA progression. Overexpression of IGFBP-3 and IGFBP-5 disrupts cartilage homeostasis and promotes aberrant angiogenesis[185]. IGF-1 enhances ECM synthesis by promoting collagen and proteoglycan production while inhibiting MMPs. However, IGFBPs can counteract these effects, leading to ECM degradation and structural changes in arteries and joints[47].

1.2.3.4 Inflammation pathway

Hypertension and osteoarthritis share inflammatory pathways, such as the activation of NF-κB and MAPK signaling, contribute to systemic and local inflammation, oxidative stress, and ECM degradation.

NF-κB activation in vascular endothelial cells and smooth muscle cells promotes the expression of pro-inflammatory cytokines (e.g., IL-6, TNF-α) and adhesion molecules, contributing to vascular remodeling and stiffness[186]. NF-κB activation in chondrocytes and synovial fibroblasts drives the production of catabolic enzymes (MMPs, ADAMTS) and inflammatory mediators, exacerbating cartilage degradation and synovial inflammation[187]. Pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6 are elevated. These cytokines exacerbate vascular inflammation by recruiting

immune cells, inducing endothelial dysfunction, and increasing oxidative stress[188]. Cytokines enhance the expression of MMPs and ADAMTS in cartilage and amplify synovial inflammation, accelerating OA progression[189].

1.2.3.5 Senescence pathway

Chondrocyte senescence

Chondrocyte senescence is increasingly recognized as a pivotal factor in the pathogenesis of OA[190]. Chondrocytes are the only resident cells in articular cartilage, responsible for maintaining the ECM homeostasis through the balanced synthesis and degradation of matrix components such as collagen type II and aggrecan. However, aging and joint injury can induce a state of irreversible cell cycle arrest in chondrocytes, referred to as chondrocyte senescence[52]. Senescent chondrocytes exhibit altered phenotypes, including reduced responsiveness to growth factors, impaired anabolic activity, and increased expression of catabolic enzymes, such as MMPs and ADAMTS [40, 190]. This imbalance between anabolic and catabolic processes leads to ECM breakdown, contributing to cartilage destruction and OA progression.

.

Chondrocyte senescence is driven by several molecular mechanisms, including oxidative stress, telomere shortening, and chronic inflammatory signaling. Oxidative stress, primarily mediated by increased ROS production, has been identified as a major inducer of DNA damage and mitochondrial dysfunction, promoting chondrocyte senescence. In addition, age-related telomere attrition results in the activation of DNA

damage response (DDR) pathways, leading to senescence via upregulation of p53/p21 and $p16^{INK4a}$ signaling cascades[191]. These pathways result in cell cycle arrest and contribute to the secretion of pro-inflammatory cytokines and proteases, a characteristic feature of the senescence-associated secretory phenotype (SASP).

Given the central role of chondrocyte senescence in OA, therapeutic strategies targeting senescent cells, known as senolytics, have emerged as a promising avenue for OA treatment. By selectively eliminating senescent chondrocytes or inhibiting their SASP, these therapies aim to restore cartilage homeostasis and prevent further degeneration. Other approaches, such as antioxidant therapy and modulation of key senescence pathways (e.g., p53/p21, $p16^{INK4a}$), are also being explored for their potential to delay or reverse chondrocyte senescence in OA. For example, Studies using senolytic compounds, such as ABT-263 (Navitoclax) and combining Dasatinib and Quercetin, have shown that eliminating senescent chondrocytes from OA-affected joints can reduce inflammation, improve ECM integrity, and enhance cartilage repair[192, 193]. Additionally, these interventions can decrease pain and improve joint function, suggesting that targeting senescence could be a viable strategy to alter the course of OA.

Senolytic drugs, designed to selectively eliminate senescent cells, represent a promising new therapeutic avenue for OA. By targeting the key signaling pathways that maintain the survival of senescent cells, senolytics can alleviate the inflammatory burden and restore tissue homeostasis. It works by selectively eliminating senescent cells through

targeting the pathways that promote their survival. Senescent cells depend on specific anti-apoptotic networks to resist cell death, and senolytics are designed to inhibit these networks, thereby inducing apoptosis selectively in senescent cells. Below, we explore several key mechanisms by which senolytic drugs function in the management of OA:

1. Targeting the BCL-2 Family Proteins

The BCL-2 family proteins, including BCL-2, BCL-xL, and BCL-w, are key regulators of apoptosis that are often overexpressed in senescent cells to prevent programmed cell death[194]. Senolytic drugs such as ABT-263 (Navitoclax) and ABT-737 target these anti-apoptotic proteins, disrupting their function and triggering apoptosis in senescent cells[192, 195]. By inhibiting BCL-2 family proteins, these senolytics can selectively eliminate senescent chondrocytes and synoviocytes, reducing the inflammatory burden and halting cartilage degradation in OA.

2. Inhibition of PI3K/AKT Pathway

The phosphatidylinositol 3-kinase (PI3K)/AKT pathway is another survival pathway that is upregulated in senescent cells[196, 197]. This pathway promotes cell survival and inhibits apoptosis. Senolytic compounds like Quercetin, a naturally occurring flavonoid, inhibit the PI3K/AKT pathway, thereby sensitizing senescent cells to apoptotic signals[198]. In OA models, Quercetin has been shown to reduce the number of senescent cells in cartilage and synovium, leading to reduced inflammation and improved joint structure[199].

3. Forkhead Box O4 (FOXO4)-p53 Interaction Disruption

Another mechanism by which senolytic drugs operate is through disrupting the interaction between the transcription factor FOXO4 and p53, a key regulator of cellular senescence[200]. FOXO4 helps maintain the viability of senescent cells by inhibiting p53-mediated apoptosis. The senolytic peptide FOXO4-DRI (disrupting peptide) has been shown to selectively induce apoptosis in senescent cells by disrupting the FOXO4-p53 interaction, thereby promoting p53-driven cell death[201]. This approach has demonstrated efficacy in reducing the number of senescent cells in tissues, suggesting potential applicability in OA management.

4. Inducing Mitochondrial Dysfunction

Mitochondrial dysfunction is a hallmark of senescent cells, characterized by elevated production of ROS[202]. Senolytic drugs such as Dasatinib induce mitochondrial dysfunction specifically in senescent cells, leading to increased oxidative stress and cell death[203]. By targeting the mitochondrial vulnerabilities of senescent chondrocytes, these drugs can reduce the presence of SASP-expressing cells in OA joints, thereby alleviating cartilage breakdown and inflammation.

5. JAK-STAT Pathway Inhibition

The Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway is frequently activated in senescent cells and contributes to the production of

SASP factors[204]. Inhibition of the JAK-STAT pathway using drugs like Ruxolitinib can reduce SASP expression, thereby diminishing the pro-inflammatory environment in OA joints[205]. Although Ruxolitinib is more senomorphic than senolytic, it can synergize with senolytic drugs to enhance overall efficacy in reducing the negative impact of senescent cells on joint tissues.

The selective elimination of senescent cells offers a promising strategy for the disease-modifying treatment of OA. Senolytic therapies address the root causes of tissue degeneration by reducing the pro-inflammatory and catabolic signals released by senescent cells. Preclinical studies have demonstrated that senolytic treatment in OA models not only reduces the number of senescent cells but also results in decreased inflammation, reduced cartilage damage, and improved joint function. Importantly, senolytics have the potential to enhance tissue regeneration by creating a more favorable environment for the remaining healthy chondrocytes. Furthermore, senolytic drugs may also have synergistic effects when used in combination with other OA therapies, such as anti-inflammatory agents, viscosupplementation, or physical therapy. By targeting the senescent cell population, senolytics may enhance the efficacy of these treatments and provide more comprehensive benefits to patients.

Endothelial senescence in hypertension

Endothelial senescence can be triggered by several stressors, including oxidative stress, chronic inflammation, and telomere shortening, all of which are prevalent in

hypertensive individuals[206]. The SASP, characterized by the release of pro-inflammatory cytokines, chemokines, and proteases, further exacerbates vascular inflammation and dysfunction, promoting arterial stiffness and increased peripheral resistance[207]. Oxidative stress, driven by excessive ROS, not only induces DNA damage and mitochondrial dysfunction in endothelial cells but also perpetuates the inflammatory environment, thereby creating a vicious cycle that fosters endothelial senescence and vascular damage[206, 208].

Mechanistically, pathways involving p53/p21 and $p16^{INK4a}$ have been identified as major regulators of endothelial senescence[209]. Activation of these pathways leads to cell cycle arrest and facilitates the expression of inflammatory mediators, contributing to vascular endothelial dysfunction. Additionally, emerging evidence indicates that the RAS may have a direct role in promoting endothelial senescence. Angiotensin II, a key mediator of RAS, induces oxidative stress, inflammation, and subsequent senescence of endothelial cells, thereby linking hypertension to vascular aging.

The impact of endothelial senescence in hypertension is not confined to impaired vasodilation. It also influences endothelial permeability, promotes leukocyte adhesion, and alters vascular remodeling, further contributing to end-organ damage in hypertensive patients. Targeting endothelial senescence is thus a promising strategy for alleviating hypertension and its associated vascular complications. Various therapeutic approaches, including senolytic agents, antioxidants, and inhibitors of the RAS, are

currently under investigation for their potential to delay or reverse endothelial senescence, thereby restoring vascular homeostasis and reducing hypertension-related morbidity.

1.2.4 Antihypertensive medicine and osteoarthritis

In recent years, some studies have found that antihypertensive drugs may have a certain impact on the pathogenesis and symptom management of osteoarthritis.

Beta-blockers reduce cardiac output and renin secretion by blocking cardiac beta-receptors. An experimental study of 873 patients with hypertension and symptomatic hip or knee OA who were taking antihypertensive medications as prescribed, with joint pain assessed, found that patients who used beta-adrenergic blockers to lower blood pressure had lower pain scores and a lower prevalence of joint pain[210]. Another study of 223,436 people, half of whom used beta-blockers and the other half did not, found that beta-blocker prescriptions were associated with a reduced cumulative risk of knee and hip pain and knee OA consultations[211]. However, in another study of 1,168 participants, beta-blockers were not found to reduce the severity of knee pain in participants with symptomatic knee OA[212]. Therefore, it is still controversial whether beta-blockers are effective in relieving pain in patients with osteoarthritis, but most evidence still points to the fact that they can reduce pain signal transmission and improve the symptoms of patients with osteoarthritis. Its specific mechanism is still unclear.

Potassium-sparing diuretics and aldosterone antagonists were causally associated with OA in 62,497 European patients with OA[213]. Studies have shown that aldosterone promotes inflammation, and inflammation is involved in the progression of osteoarthritis[214]. Aldosterone antagonists inhibit the production of proinflammatory cytokines and have a certain anti-inflammatory effect. Therefore, aldosterone antagonists are potentially effective in the treatment of OA.

A study using a sample of 62,497 European osteoarthritis patients and 12 antihypertensive drugs found a significant causal relationship between calcium channel blockers and all OA, including osteoarthritis[213]. Another study of 4,796 subjects found that among those taking different high blood pressure medications, the average pain scores of the group taking calcium channel blockers were significantly higher than those of the other groups[215].

The RAS has been reported to play a role in OA, including its involvement in regulatory mechanisms associated with OA progression. The antihypertensive drug Captopril, a widely used ACE inhibitor, has been shown to mitigate OA-induced joint damage in rat models. Oral administration of Captopril was found to locally suppress the RAS, thereby alleviating chondrocyte abnormalities, such as hypertrophy caused by OA-related joint damage[216]. In addition, several other RAS inhibitors have been shown to reduce inflammation and influence angiogenesis within OA, suggesting their

potential application in OA treatment[175].

In summary, most of the current research on antihypertensive drugs in the context of OA suggests their primary effects are related to anti-inflammatory actions and symptomatic relief, such as pain reduction in symptomatic OA. However, studies focused on the effects of these drugs on joint structure and cartilage integrity remain relatively limited.

1.3 Research gap and objectives

Current evidence suggests that individuals with hypertension are at an increased risk of developing OA. However, the mechanisms through which hypertension influences joint health remain poorly understood. Specifically, it is unclear how hypertension contributes to joint damage and whether specific molecular or cellular pathways are involved in this process. A critical research gap exists in identifying the potential mechanisms by which hypertension affects joints, including its influence on cartilage degradation, subchondral bone remodeling, and synovial inflammation. Moreover, for patients already diagnosed with OA, it remains unknown whether hypertension exacerbates disease progression or, conversely, if OA could intensify hypertensive pathology.

Our hypothesis posits that hypertension exacerbates joint damage regardless of OA status, with the senescence pathway emerging as a possible underlying mechanism.

Understanding the bidirectional relationship between hypertension and OA could provide novel insights into shared pathophysiological pathways and identify therapeutic targets aimed at mitigating joint and vascular damage simultaneously. Future research should aim to explore these connections through clinical studies and mechanistic investigations to address these critical gaps in knowledge.

Chapter 2 Blood pressure drug Captopril reduces senescent cells in joints and mitigates cartilage degradation in rats with drug-induced hypertension

2.1 Introduction

Hypertension can be broadly classified into two main categories based on its underlying cause:

1. Primary (Essential) Hypertension:

   Primary hypertension accounts for approximately 90-95% of all hypertension cases and has no identifiable cause[217]. It is considered a multifactorial condition influenced by a combination of genetic predisposition and environmental factors, such as obesity, sedentary lifestyle, high salt intake, and stress. The exact pathophysiological mechanisms are complex and involve alterations in the RAAS, sympathetic nervous system activity, and vascular endothelial dysfunction.

2. Secondary Hypertension:

   Secondary hypertension is less common, accounting for 5-10% of cases, and results from an identifiable underlying condition. Common causes include kidney disease (e.g., chronic kidney disease, renal artery stenosis), endocrine disorders (e.g., hyperaldosteronism, pheochromocytoma, Cushing's syndrome), and medication-induced hypertension (e.g., use of corticosteroids, oral contraceptives). Addressing the underlying condition is often essential for effective management of secondary hypertension.

DOCA salt-induced hypertension is a well-established experimental model used to study the pathophysiology of hypertension and its associated cardiovascular complications.

Mechanism of DOCA-Salt-Induced Hypertension：

1. Mineralocorticoid Receptor Activation

The administration of DOCA, a synthetic analog of the mineralocorticoid deoxycorticosterone, leads to persistent activation of mineralocorticoid receptors (MRs) in various tissues, including the kidney, heart, and vasculature[218]. Mineralocorticoid receptors, when activated by DOCA, promote sodium and water retention, leading to an expansion of blood volume, which is a key initial factor in the development of hypertension in this model[219]. Unlike aldosterone, DOCA does not require the stimulation of the RAAS, and therefore, the DOCA-salt model is characterized by low renin and low aldosterone levels.

Mineralocorticoid receptors are expressed in renal tubular epithelial cells, where they regulate sodium reabsorption[220]. Activation of these receptors by DOCA enhances the expression and activity of epithelial sodium channels (ENaC) and sodium-potassium ATPase pumps, resulting in increased reabsorption of sodium in the distal nephron. This sodium retention leads to water retention, expansion of extracellular fluid volume, and a subsequent increase in blood pressure[221].

2. Renal Sodium Retention and Volume Expansion

In addition to increased sodium reabsorption, DOCA has been shown to downregulate the expression of natriuretic peptides, further impairing the kidney's ability to excrete excess sodium[222]. The impaired pressure-natriuresis relationship in DOCA-treated rats contributes to the persistence of elevated blood pressure despite the expansion of blood volume, thereby indicating a failure of the normal physiological compensatory mechanisms.

3. Sympathetic Nervous System Activation

The DOCA-salt model also involves activation of the SNS. Mineralocorticoid excess has been associated with increased sympathetic outflow, which contributes to elevated vascular tone and increased peripheral resistance[223]. The combination of volume expansion and heightened SNS activity leads to both increased cardiac output and elevated systemic vascular resistance, creating a dual mechanism for sustained hypertension[224].

Studies have shown that DOCA treatment can increase norepinephrine release and promote vasoconstriction[225]. This sympathoexcitation is partly mediated by MR activation in the central nervous system and in the vasculature, which sensitizes the sympathetic ganglia, leading to increased adrenergic tone and enhanced vascular contractility.

4. Oxidative Stress and Endothelial Dysfunction

Oxidative stress plays a pivotal role in the pathogenesis of DOCA-salt-induced hypertension. Excessive activation of MRs leads to an increase in the production of ROS, which contributes to endothelial dysfunction and vascular damage[226]. In DOCA-salt-treated rats, increased levels of NADPH oxidase activity have been observed, which results in enhanced ROS generation in the vasculature[227]. The elevated ROS levels reduce the bioavailability of NO, an essential vasodilator, thereby impairing endothelium-dependent vasodilation and promoting vasoconstriction.

Oxidative stress also activates various redox-sensitive signaling pathways, including mitogen-activated protein kinases (MAPKs) and NF-κB, which contribute to vascular inflammation, fibrosis, and remodeling[228]. This oxidative-inflammatory cascade exacerbates vascular stiffness and increases peripheral resistance, further perpetuating the hypertensive state.

5. Vascular Remodeling and Inflammation

Vascular remodeling is a hallmark of the DOCA-salt model of hypertension[229]`. Chronic MR activation leads to structural changes in the vasculature, including increased collagen deposition, smooth muscle cell proliferation, and media thickening, which contribute to increased vascular stiffness. These changes result in elevated systemic vascular resistance and reduced arterial compliance, which are key features of

hypertension.

Inflammation is another crucial component of DOCA-salt-induced hypertension. The activation of MR in vascular and immune cells triggers the release of pro-inflammatory cytokines, such as IL-6 and TNF-α, which promote vascular inflammation and fibrosis. Inflammatory cell infiltration, particularly by macrophages, has been observed in the vasculature of DOCA-salt-treated rats, contributing to endothelial dysfunction and vascular remodeling. This chronic inflammatory state further exacerbates the hypertensive phenotype by impairing vascular reactivity and enhancing stiffness.

A study found that DOCA salt-induced hypertensive rats induced somatic cellular senescence[230]. Therefore, here, we also use the DOCA-induced hypertension model as one of the hypertension models we studied to observe the changes in the knee joint caused by hypertension.

2.2 Materials and Methods

Animal

Animal Subjects Ethics Sub-Committee (ASESC) of the Hong Kong Polytechnic University approved all the experimental procedures (19-20/36-ABCT-R-GRF). We refer to the blood pressure data of the DOCA group and its control group by Bae et al.[231] and use the statistical tool statulator[232] to calculate that the average value of

the control group and the test group has a two-sided significance level difference of 5%. Each group must contain at least 5 samples. To prevent attrition, we included 8 SD rats in each group. Control group, DOCA group (hypertension group) and Captopril treatment group (hypertension treatment group), a total of 3 groups, 24 animals in total. Rats were purchased from the Concentrated Animal Facility (CAF) of the Hong Kong Polytechnic University and housed individually in a controlled room at 21 ± 2°C and 55% ± 10% relative humidity with a 12:12 h light-dark cycle. They are equipped with autoclaved bedding and cages. Rats were maintained in this facility for at least two weeks before use in this study.

Hypertension was induced for a total of 14 weeks. Subcutaneous injections of 20 mg/kg of DOCA were given twice a week, while daily drinking water containing 1.0% NaCl and 0.2% KCl was administered in DOCA group and Captopril treatment group. Rats in the control group were injected with an equal volume of normal saline twice a week and drank water without 1.0% NaCl and 0.2% KCl. At the ninth week, the Captopril treatment group received an additional daily oral dose of 50 mg/kg Captopril. Blood pressure was measured every two weeks throughout the process. Fecal samples were collected at the end of 14 weeks and all rats were sacrificed. Collect rat tissue and organ samples such as aorta, leg, etc., pending further histological analysis.

Measurement blood pressure

Blood pressure was measured by the tail-cuff method using a BP-2000 blood pressure

analysis system (Visitech System, Inc., Apex, NC, USA). Before measurement, the rats were placed under a 37°C constant temperature plate and holder for 10 minutes to stabilize their blood pressure. Measurement Take ten readings and calculate the average of all measurements. To minimize stress-induced changes in blood pressure, all measurements were performed by the same person in the same calm environment. A two-week training period was set before the actual trial time to allow the rats to adapt to the procedure.

Histology

After taking rat samples, they were fixed with 4% paraformaldehyde for 24 hours, transferred to 70% alcohol, and then decalcified with 10% ethylenediaminetetraacetic acid. Two months after decalcification, the tissue was treated with alcohol-xylene-paraffin and the samples were embedded in wax. Cut the samples into 5 μm sections using a microtome. We use immunohistochemical staining to detect specific proteins in the sample. After dewaxing and antigen retrieval, we used horse serum for blocking. Afterwards, primary antibodies were incubated with samples overnight at 4°C. The primary antibodies used were p16 (1:500; Abcam, ab54210), p53 (1:200; Abcam, ab131442) and MMP13 antibodies (1:500; Abcam, ab39012). For 3,3′-diaminobenzidine (DAB) staining, we stained the target antigen using the Vectastain ABC kit and DAB peroxidase substrate kit (Vector Labs, USA). Then counterstain with hematoxylin. We performed safranin O staining of the tibia using safranin O/fast green. After staining, cells positive for p16 and p53 had brown nuclei. All images were taken

using a Nikon Eclipse 80i microscope (Nikon, Japan).

Micro-CT

As an anesthetic, 100 mg/ml ketamine and 20 mg/ml xylazine were mixed at a liquid volume of 2:1, and the rats were anesthetized by intraperitoneal injection. The legs and joint cavities were scanned using a Micro-CT system. The scanning X-ray voltage and filter were 88 kV and 1.0mm respectively. The scanning width was 35mm and the size was 18μm. One image was scanned every 0.4° rotation. The collected data were reconstructed and analyzed in the coronal plane using the software DataViewer (version 1.4.4.0, SKYSCAN). Use CTAn software to select the same volumes of interest (VOIs) and the same regions of interest (ROI) for each sample. For Subchondral bone trabeculae, we used the disappearing ends of the two menisci as the identification point, and selected 50 slices upwards and 100 slices downwards, for a total of 150 slices for analysis. The selected gray level threshold is uniformly 90-225. Bone volume fraction (BV/TV), trabecular separation (Tb.Sp), trabecular number (Tb.N), and trabecular thickness (Tb.Th) were calculated for each sample.

Data analysis

All data were presented as mean ± S.E.M. one-way ANOVA was performed for staining intensity and percentage of positively stained cells between different group. Respective post-hoc tests were carried when overall significance was detected between groups. The level of significance was set at $p < 0.05$. Analyses and graphs were

generated using Prism 9 (GraphPad).

2.3 Figures and Results

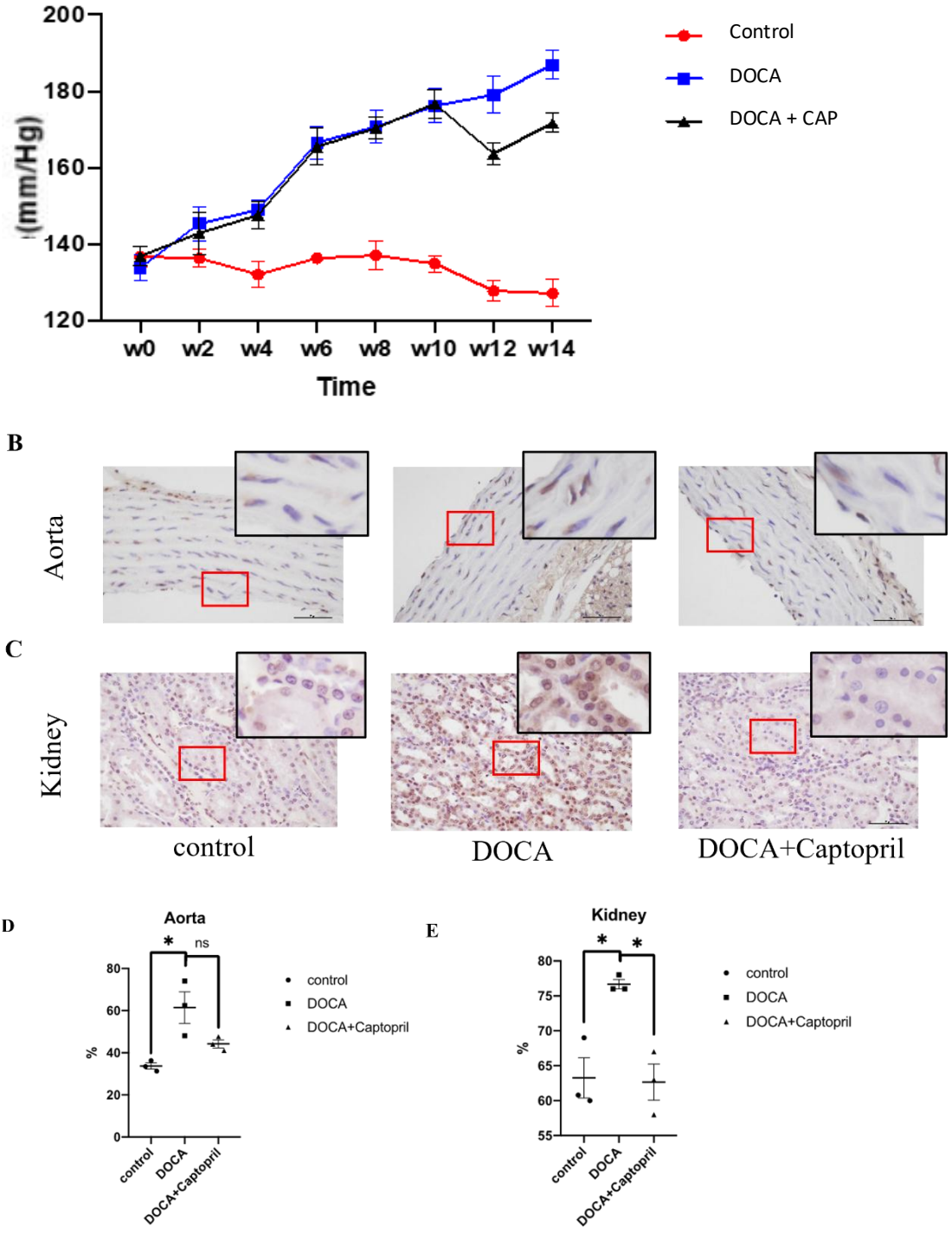
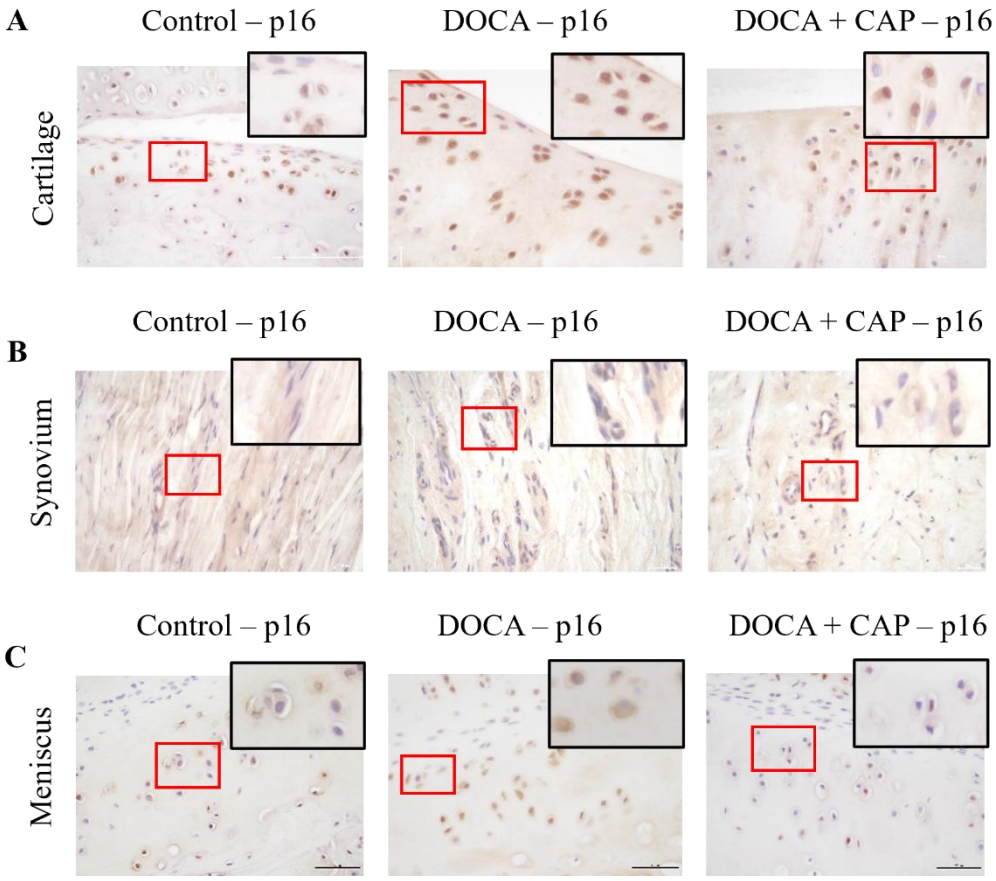


Figure 1. 1 DOCA-induced hypertensive rats exhibited elevated blood pressure

and endothelial cell senescence. Captopril treatment effectively reduced both blood pressure and cellular senescence. (A) Blood pressure measured every 2 weeks from week 0 to week 14 of the Control, DOCA, and DOCA + Captopril groups. The treatment of DOCA starts from w0. In week 9, DOCA + Captopril groups began to feed captopril. The results of $p16^{INK4a}$ staining in aorta (B) and kidney (C) with red boxes highlighting the $p16^{INK4a}$ -positive cells. (D) and (E) show the percentage of $p16^{INK4a}$ -positive cells in aorta and kidney respectively. [All data were presented as mean ± S.E.M. One-way ANOVA was performed for staining intensity and percentage of positively stained cells between different group. * $p<0.05$; ** $p<0.005$; *** $p<0.001$].



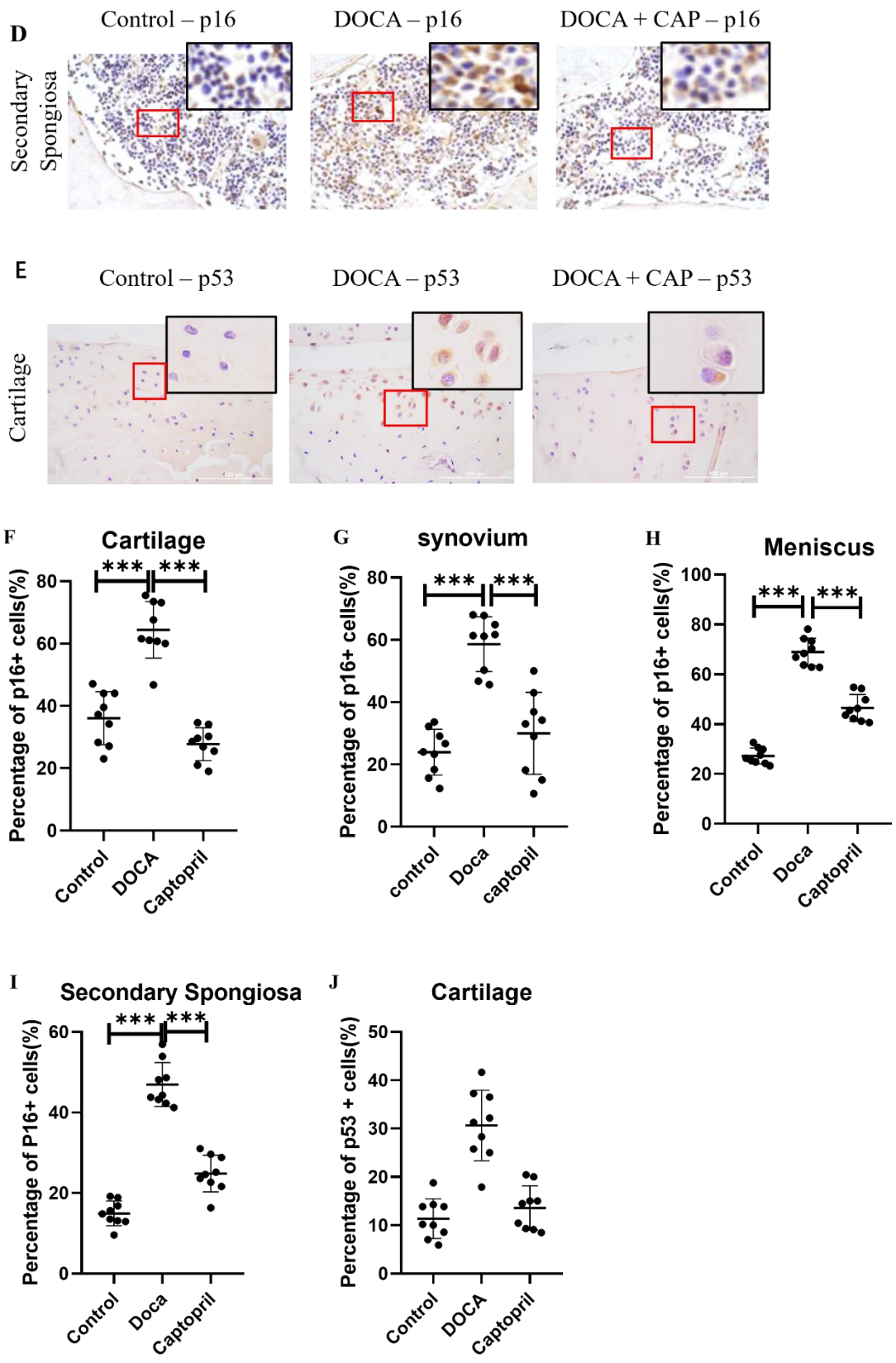
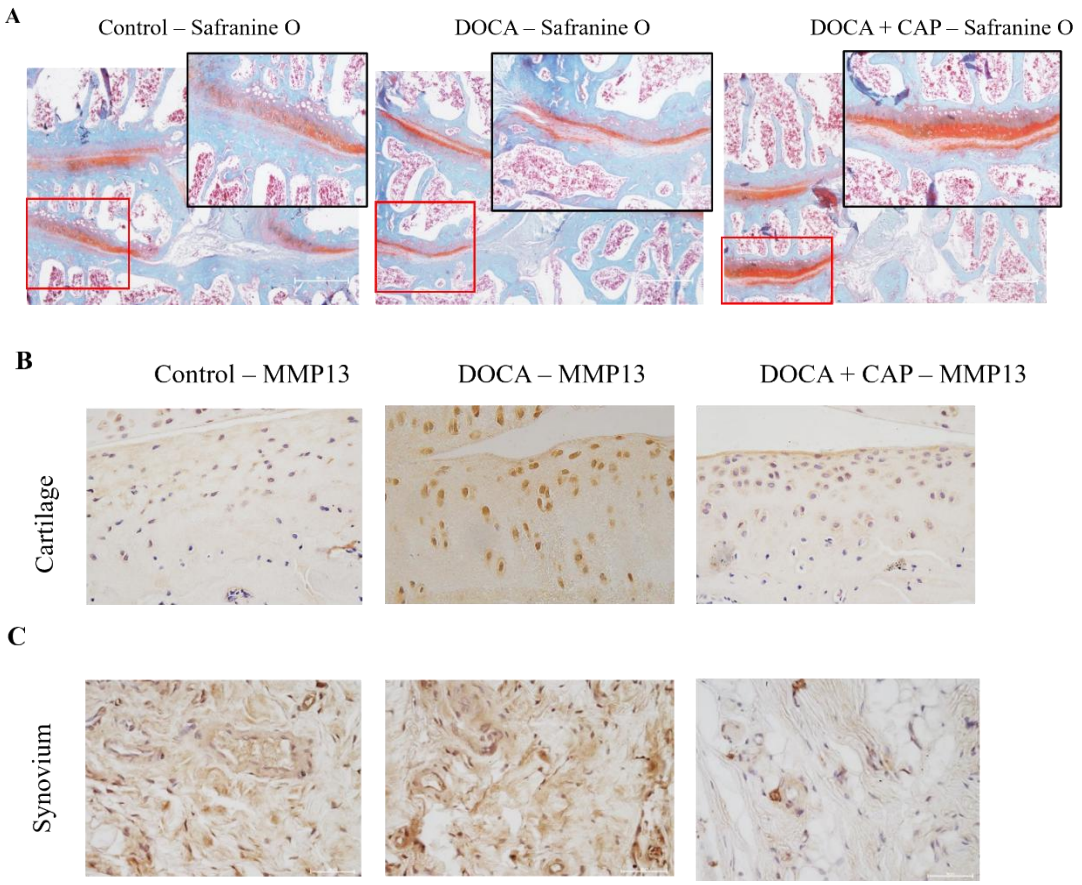


Figure 1. 2. Hypertensive rats showed increased senescent cells in joints, while

captopril reduced their accumulation. The $p16^{INK4a}$ staining results of articular cartilage (A), synovium (B), meniscus (C) and secondary spongiosa(D) were shown with the red boxes indicating the $p16^{INK4a}$ - positive regions. The percentage of $p16^{INK4a}$ -positive cells in the (F) cartilage, (G) synovium, (H) meniscus and secondary spongiosa(I) in control, DOCA, and captopril-treated group were plotted. The p53 staining of the cartilage (E) and the percentage of p53 -positive cells (J) of the Control, DOCA, and DOCA + Captopril groups respectively. [All data were presented as mean ± S.E.M. One-way ANOVA was performed for staining intensity and percentage of positively stained cells between different group. * $p<0.05$; ** $p<0.005$; *** $p<0.001$].



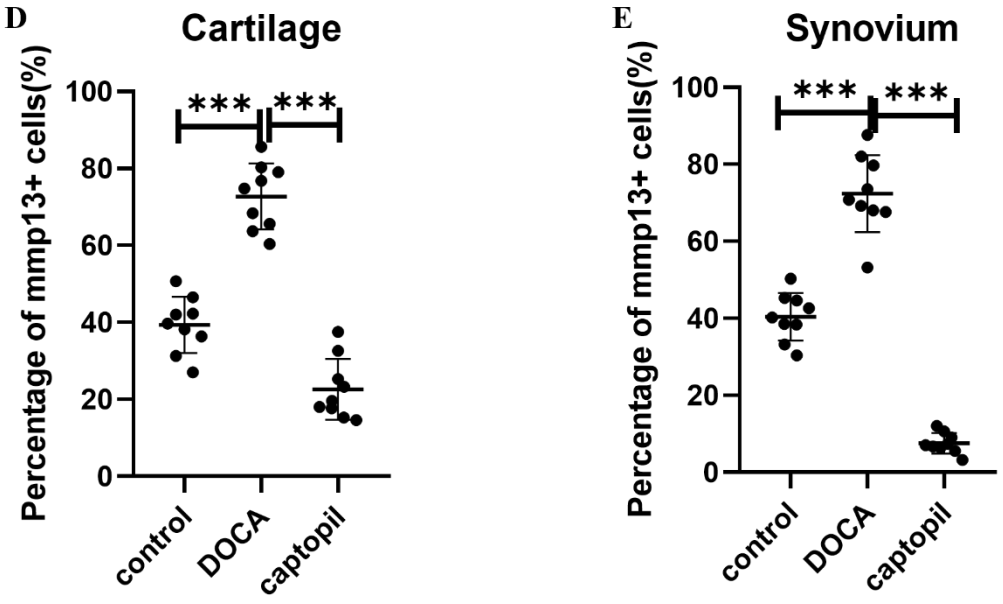


Figure 1. 3. Hypertensive rats exhibited increased cartilage degradation and reduced proteoglycan levels, which were rescued by captopril treatment. (A) The results of Safranin O staining in cartilage in joints of the Control, DOCA, and DOCA + Captopril group, with the red box highlighting the regions exhibited key differences of the staining intensity. The MMP13 staining of cartilage (B-D) and synovium (C-E) of the Control, DOCA, and DOCA + Captopril groups respectively. [All data were presented as mean ± S.E.M. One-way ANOVA was performed for staining intensity and percentage of positively stained cells between different group. * $p<0.05$; ** $p<0.005$; *** $p<0.001$].

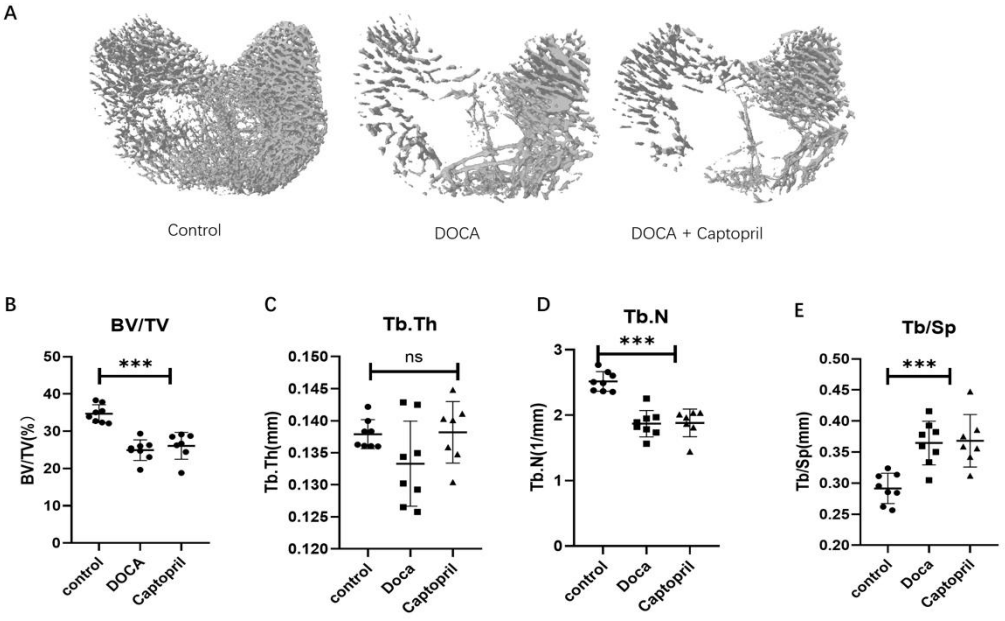


Figure 1. 4. Hypertensive rats exhibited bone loss, which was not rescued by captopril treatment. (A) The 3D bone structure of secondary spongiosa under micro-CT with the (B) BV/TV, (C) Tb.N, (D) Tb.Sp, and (E) Tb.Th calculated for the Control, DOCA, and DOCA+Captopril groups respectively. [All data were presented as mean ± S.E.M. One-way ANOVA was performed for staining intensity and percentage of positively stained cells between different group. * $p<0.05$; ** $p<0.005$; *** $p<0.001$]

Doca-induced hypertensive rats show accumulation of senescent cells in blood vessels, captopril can relieve it

After injecting DOCA into SD rats, the blood pressure obviously showed an upward trend. After further treatment with captopril, blood pressure decreased (Figure1.1A). Aorta and Kidney of hypertensive rats showed a large accumulation of senescent cells compared with normal rats. However, after treatment with captopril, senescent cells

were significantly reduced and almost returned to normal levels (Figure1.1B-1.1E). The senescence marker p16 accumulated increase significantly in cells of cartilage, synovium, meniscus and bone in secondary Spongiosa in hypertensive rats. After captopril treatment, the number of senescent cells significantly decreased, and some even returned to the level of normal rats (Figure1.2A-D, 1.2G-J). In order to verify that it is indeed senescence, we used p53, an senescence marker, to do the immunostaining for cartilage. The results shown by the same p53 are the same as those of p16(Figure1.2E,1.2J). Both p16 and p53 are involved in cell cycle arrest and therefore have been widely used as senescence markers. Accumulation of senescent cells was often reported in age-related diseases, including OA[233].

Hypertensive rats exhibited increased cartilage degradation and reduced proteoglycan levels, which were rescued by captopril treatment

We then performed safranin O staining on articular cartilage, and the results showed that hypertensive rats experienced loss of proteoglycans, while the cartilage layer became thinner, and the cartilage surface became uneven. When treated with captopril, cartilage proteoglycan loss and cartilage thinning were improved (Figure 1.3A). At the same time, the protease MMP13, which is mainly involved in cartilage degradation, is detected. It was found that the content of MMP13 increased significantly in the cartilage and synovium of hypertensive rats. After captopril treatment, the content of MMP13 decreased (Figure1.3B-1.3E).

Hypertensive rats exhibited bone loss, which was not rescued by captopril treatment

We use micro-CT to detect the content of Subchondral bone trabeculae. We found that hypertensive rats experienced obvious bone loss, both bone volume fraction and trabecular bone number decreased significantly, and the distance between bones, increased. But what is different from others is that after treatment with captopril, the bone content does not return to normal levels like other factors but still maintains the state of bone loss (Figure1.4A-1.4E).

2.4 Conclusion and discussion

In our study utilizing a DOCA-induced hypertensive rat model, we observed the emergence of senescence phenotypes in systemic vasculature, alongside an increased incidence of cellular senescence within various joint tissues, including articular cartilage, synovium, and subchondral bone. The cartilage exhibited proteoglycan loss, thinning of the cartilage layer, and elevated levels of cartilage degradation markers. Treatment with the antihypertensive drug captopril attenuated both vascular and joint senescence, resulting in proteoglycan and cartilage restoration and a reduction in cartilage degradation markers. However, unlike other tissues, hypertensive rats showed a decrease in subchondral bone mass, and this reduction was not reversed by captopril treatment. My lab mate Lok Chun Chan study finds that captopril not only mitigates cellular aging in cartilage but also correlates with changes in the gut microbiome, suggesting a possible gut-joint axis influencing joint health and cartilage preservation

under hypertensive conditions. Combining our two findings, we published these results as co-first authors in cells.

Using drug-induced hypertension models, while valuable for studying specific mechanisms, presents certain limitations. One key concern is the potential direct effects of the drug itself on joints, independent of the blood pressure increase. This introduces a confounding factor, as it becomes unclear whether joint changes are caused by hypertension or pharmacological intervention. Although no current studies suggest a direct impact of DOCA on joints, over 90% of hypertension cases are classified as primary hypertension, with secondary causes comprising less than 10%. Therefore, relying solely on drug-induced models is insufficient. To address this, our next step will involve employing a primary hypertension model for further validation.

Chapter 3 Antihypertensive drug Captopril reduces senescent cells in joints and mitigates cartilage degradation in spontaneously hypertensive rats

3.1 Introduction

Essential hypertension, which accounts for more than 90% of hypertension cases in humans, is a multifactorial disorder influenced by genetics, environment, and lifestyle factors. The SHR model was first developed in the 1960s by Okamoto and Aoki through selective inbreeding of Wistar rats with elevated blood pressure. Since then, SHRs have become the gold standard for investigating the genetic and physiological mechanisms underlying primary hypertension and its complications, including cardiovascular diseases, kidney dysfunction, and metabolic syndrome.

SHR was generated by breeding rats with naturally occurring high blood pressure over several generations. By the fourth generation, the offspring exhibited a stable hypertensive phenotype, making SHRs a unique genetic model for studying the progression of essential hypertension. By 8 to 12 weeks of age, SHRs begin to develop sustained high blood pressure, reaching systolic levels of approximately 180-200 mmHg in adulthood. In severe cases, blood pressure can reach over 200 mmHg. This mirrors the gradual onset of hypertension in humans and provides a relevant time frame for studying disease progression.

Key features of SHRs include:

1. Cardiovascular Phenotypes: SHRs exhibited left ventricular hypertrophy, increased

arterial stiffness, and endothelial dysfunction, which are common complications of human hypertension. Vascular remodeling and increased peripheral resistance contribute to the maintenance of high blood pressure in these rats.

2. Renal Dysfunction: The kidney plays a crucial role in SHR hypertension, with studies demonstrating impaired sodium handling, glomerular hypertrophy, and increased renal vascular resistance. These renal abnormalities are critical in sustaining long-term hypertension.

3. Sympathetic Nervous System Activation: SHRs are characterized by elevated SNS activity, which contributes to the hypertensive phenotype through increased heart rate, cardiac output, and vasoconstriction. Enhanced sympathetic drive is also implicated in the development of left ventricular hypertrophy and vascular resistance.

Several mechanisms contribute to the hypertensive state in SHR. Genetic Factors are one of the most important reasons. SHRs have been extensively used for genetic studies to identify candidate genes associated with hypertension. Genetic analyses have revealed alterations in genes related to the RAS, sympathetic regulation, and sodium transport. At the same time, RAS dysfunction is also one of the reasons. Increased activity of the Ang II pathway contributes to vasoconstriction, sodium retention, and aldosterone production, further elevating blood pressure. Ang II also promotes

oxidative stress and inflammation in vascular tissues. SHRs exhibit elevated levels of ROS and markers of inflammation, both of which play key roles in endothelial dysfunction and vascular remodeling. Chronic oxidative stress contributes to endothelial NO depletion, impairing vasodilation and promoting vascular stiffness. Reduced bioavailability of NO, resulting from oxidative stress, leads to impaired vasodilation and increased vascular tone. This endothelial dysfunction exacerbates hypertension and contributes to the progression of cardiovascular diseases in SHRs.

The SHR model has helped to improve our understanding of hypertension and its related diseases. Some current applications include:

1. Cardiovascular Studies: SHRs are widely used to investigate cardiac hypertrophy, heart failure, and vascular remodeling, providing insights into the progression from hypertension to end-organ damage. Their use has shed light on mechanisms such as myocardial fibrosis and diastolic dysfunction.

2. Renal Pathophysiology: SHRs provide a valuable platform for studying renal adaptations to hypertension, including glomerulosclerosis, nephrosclerosis, and altered sodium handling. The model is frequently employed to explore therapeutic interventions that target renal function in hypertensive patients.

3. Pharmacological Testing: SHRs are extensively used for preclinical testing of antihypertensive drugs, including RAS inhibitors, beta-blockers, calcium channel

blockers, and novel therapeutic agents. The model allows for the evaluation of blood pressure-lowering effects, as well as long-term outcomes on heart, kidney, and vascular function.

We used SHR rats as our hypertension model and Wistar Kyoto (WKY) rats as a control group to verify our hypothesis

3.2 Materials and Methods

Animal

ASESC of the Hong Kong Polytechnic University approved all the experimental procedures (20-21/103-BME-R-GRF). Sample size was determined using the online statistical tool Statulator[234], based on preliminary experiments of blood pressure measurement. To observe a difference between WKY and SHR (from 160.0 in WKY to 204.5 in SHR in females; 165.7 in WKY to 221.5 in SHR in males; common standard deviation 12.8), this study requires a sample size of 3 in each group to achieve 80% power at a 5% significance level ($p < 0.05$). To allow for ineligibility, we selected a sample size of 6 in each group. Total 6 Wistar Kyoto (WKY) rats (6 male) and 18 stroke-prone Spontaneously hypertensive (SP-SHR) rats (12 male and 6 female) were deployed for subsequent assessment at different time points. Rats were randomly selected. Captopril group was treated with captopril (50 mg/kg) by oral gavage every day since 5 months old. Blood pressure was measured by the tail-cuff method every 2

weeks using BP-2000 Blood Pressure Analysis System (Visitech System, Inc., Apex, NC, USA). Knee joints and aorta were collected for histology.

Ultrasound and Photoacoustic imaging

Photoacoustic (PA) imaging utilised near-infrared lasers to exploit the light absorption properties of endogenous haemoglobin in blood vessels, measuring signals at two wavelengths: 750 nm (for deoxygenated haemoglobin) and 850 nm (for oxygenated haemoglobin). The red signal represents a ratio of these two measurements. PA imaging has been applied to noninvasively measure joint tissue oxygen content in vivo without the need for any contrast agents[235].

Animals were anesthetised with 3% isoflurane and imaged with Vevo 2100 high-frequency micro-imaging system (VisualSonics, Toronto, Ontario, Canada). The entire knee joint was scanned in 3D by linear transposition of the transducer perpendicular to a single sagittal plane for 2D imaging. A triangular region of interest (ROI) was manually drawn in the frame of the best represented triangle region defined by the patellar tendon, proximal tibia, and distal femur. During 3D data analysis, the same ROI was automatically isolated in all 2D slices by a custom-designed script on Matlab (Vessel Analysis v1.4). Vascular volume (%) was quantified by counting the number of colored pixels in the ROI at a resolution of 0.010 mm × 0.010 mm on each PD and PA slice, then multiplying by the slice thickness and then dividing by the total number of voxels in the volume of interest (VOI). Similarly, mean sO2 (%) was converted from

the mean PA signal intensity of the voxels in the VOI. LZ250 detectors were used for knee joint imaging. For PA mode, an ultrasound transducer emitted a pulse with a centre frequency of 21 MHz, PA Gain 35dB, Depth 9mm, Width 14.04mm, Wavelength 750/850 nm. The probe was placed parallel to the joint until a triangular area of knee joint formed by patella tendon, distal femur and proximal tibia was identified. LZ400 detectors were used for carotid artery. For B mode, an ultrasound transducer emitted a pulse with a center frequency of 30MHz, Gain 28dB, Depth 12mm, width 13.36mm. As a surrogate marker of arterial stiffness, pulse wave velocity quantification was typically performed at the carotid artery.

Micro-CT

Consistent with the method described in Chapter 2.2, Micro-CT was used to calculate BV/TV, Tb.Sp, Tb.N, and Tb.Th for each sample.

Histology

Same as chapter2.2 histology method. Primary antibodies used were p16 (1:500, Abcam, ab54210), 4HNE (1: 1000, Invitrogen, MA5-27570), CD31 (1:500, Abcam, ab182981), MMP13 (1:500; Abcam, ab39012), Masson's trichrome kit (ab150686, Abcam), alpha smooth muscle Actin (1:1500; Abcam, ab124964). All images were taken with Nikon Eclipse 80i microscope (Nikon, Japan) and analysed with ImageJ. Grading osteoarthritic cartilage damage was based on the assessment system proposed by Waldstein's group[236].

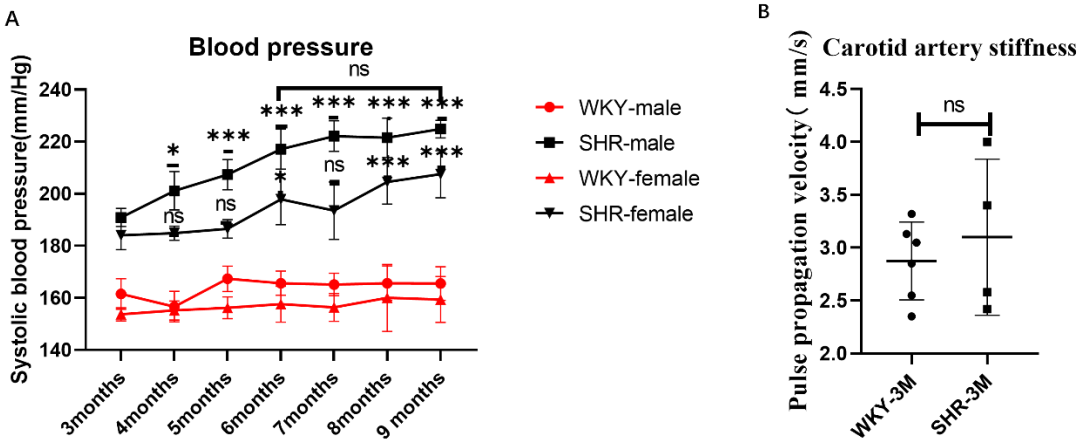
RNA sequencing

Aorta and cartilage from 5days and 3-month-old WKY and SHR were isolated for primary cells (n=3 per group). RNA Sequencing (RNA-Seq) Services provided by BGI company. Zhang Lanlan helped to analysis the RNA-Seq data.

Statistical Analysis

All data were presented as mean ± S.E.M. Two-tailed Student's t-test (unpaired) was performed for staining intensity and percentage of positively stained cells between WKY and SHR, or SHR and captopril-treated group. The comparison of tissue oxygen saturation between WKY and SHR joints of different ages was performed using one-way analysis of variance (ANOVA). Respective post-hoc tests were carried when overall significance was detected between groups. The level of significance was set at $p < 0.05$. Analyses and graphs were generated using Prism 9 (GraphPad).

3.3 Figures and Results



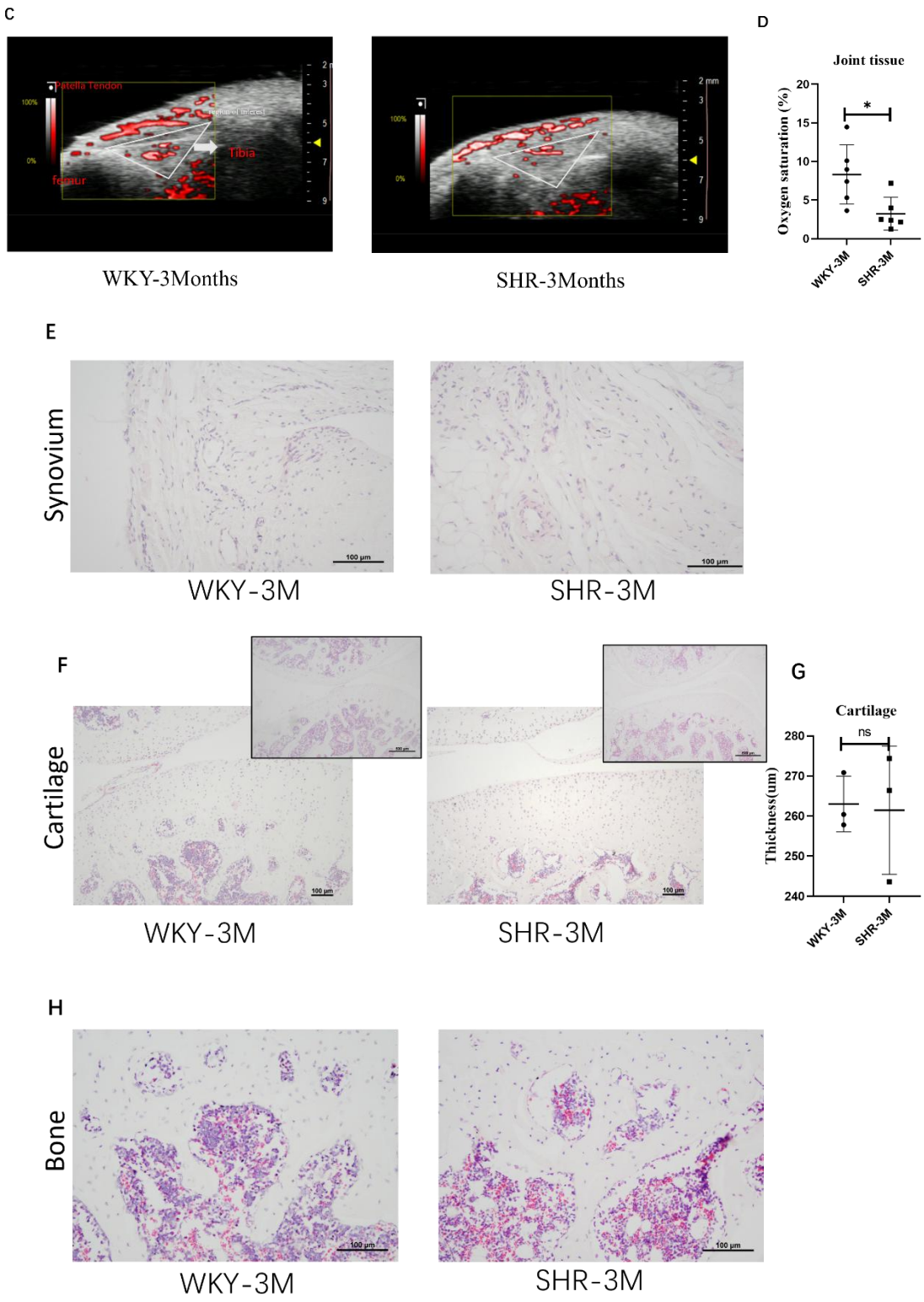


Figure 2. 1. Endothelial dysfunction in SHR developed by 3 months of age without knee joint microstructure changes. (A) Systolic blood pressure measured by tail-cuff

method in male WKY and SHR from 3 to 9 months. (N=6) (B) Carotid artery stiffness in 3-month-old WKY and SHR. (N=6, male) (C-D) Photoacoustic images and measurement of oxygen saturation in joint tissue in 3-month-old WKY and SHR. (N=6, male) (E）H&E staining of joint synovium in WKY and SHR at 3 months. (F-G) H&E staining and quantification of joint cartilage in WKY and SHR at 3 months. (N=3, female) (H) H&E staining of joint bone in WKY and SHR at 3 months. [All data were presented as mean ± S.E.M. Two-tailed Student's t-test （unpaired） was performed between WKY and SHR. * $p<0.05$; ** $p<0.005$; *** $p<0.001$]

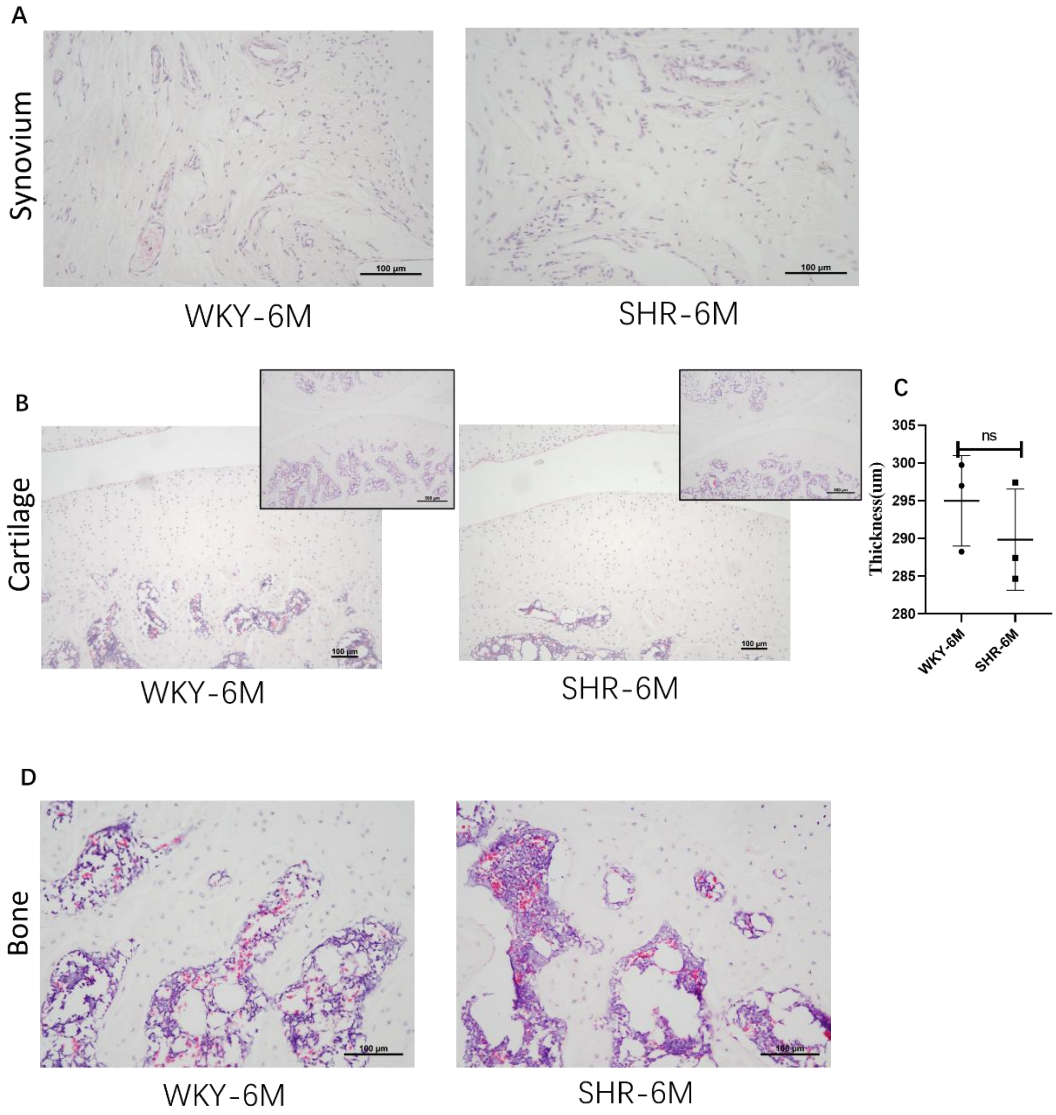
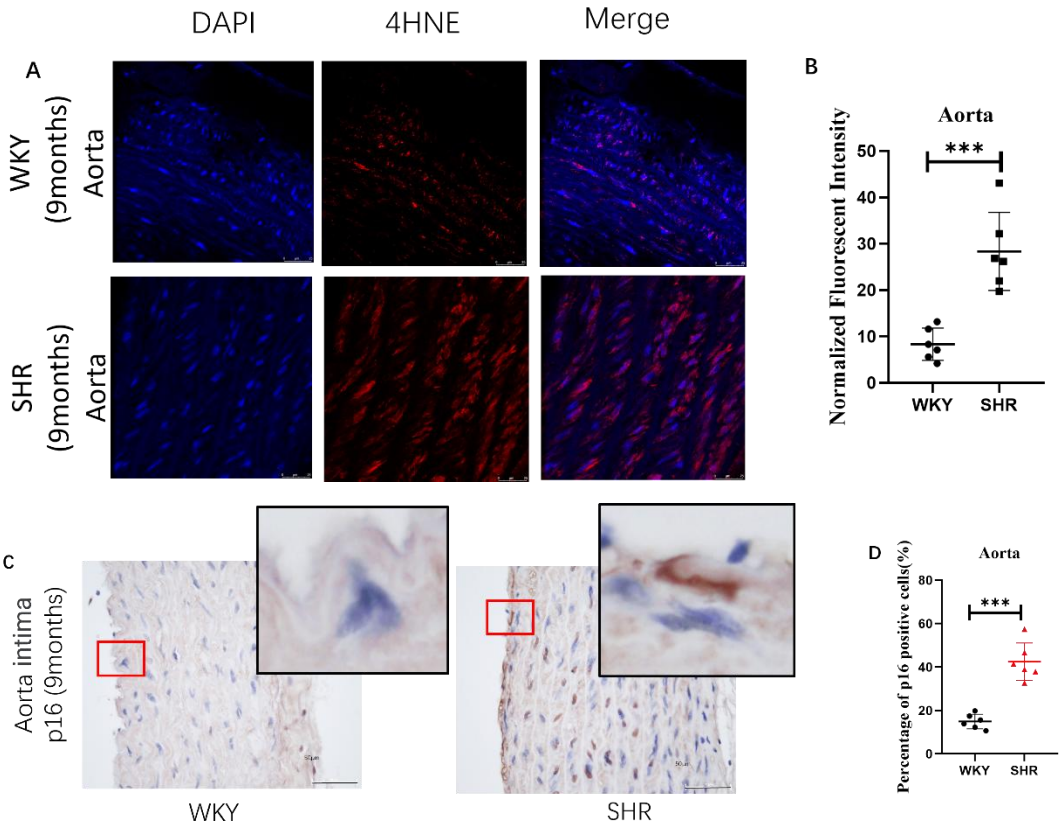
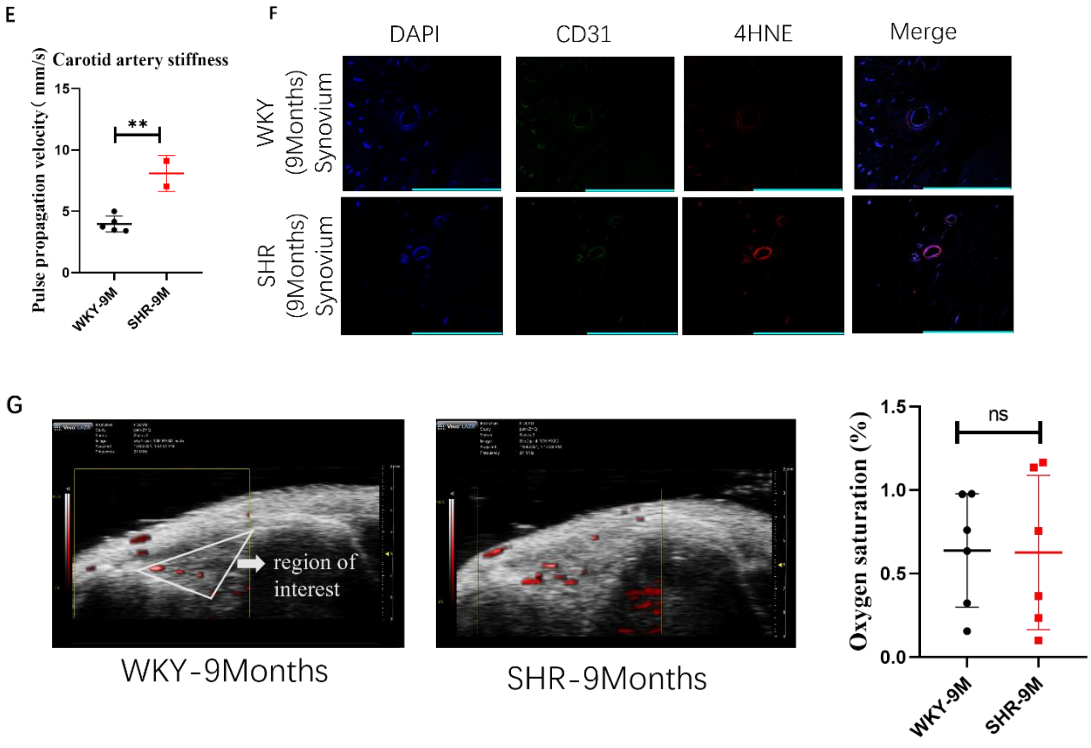
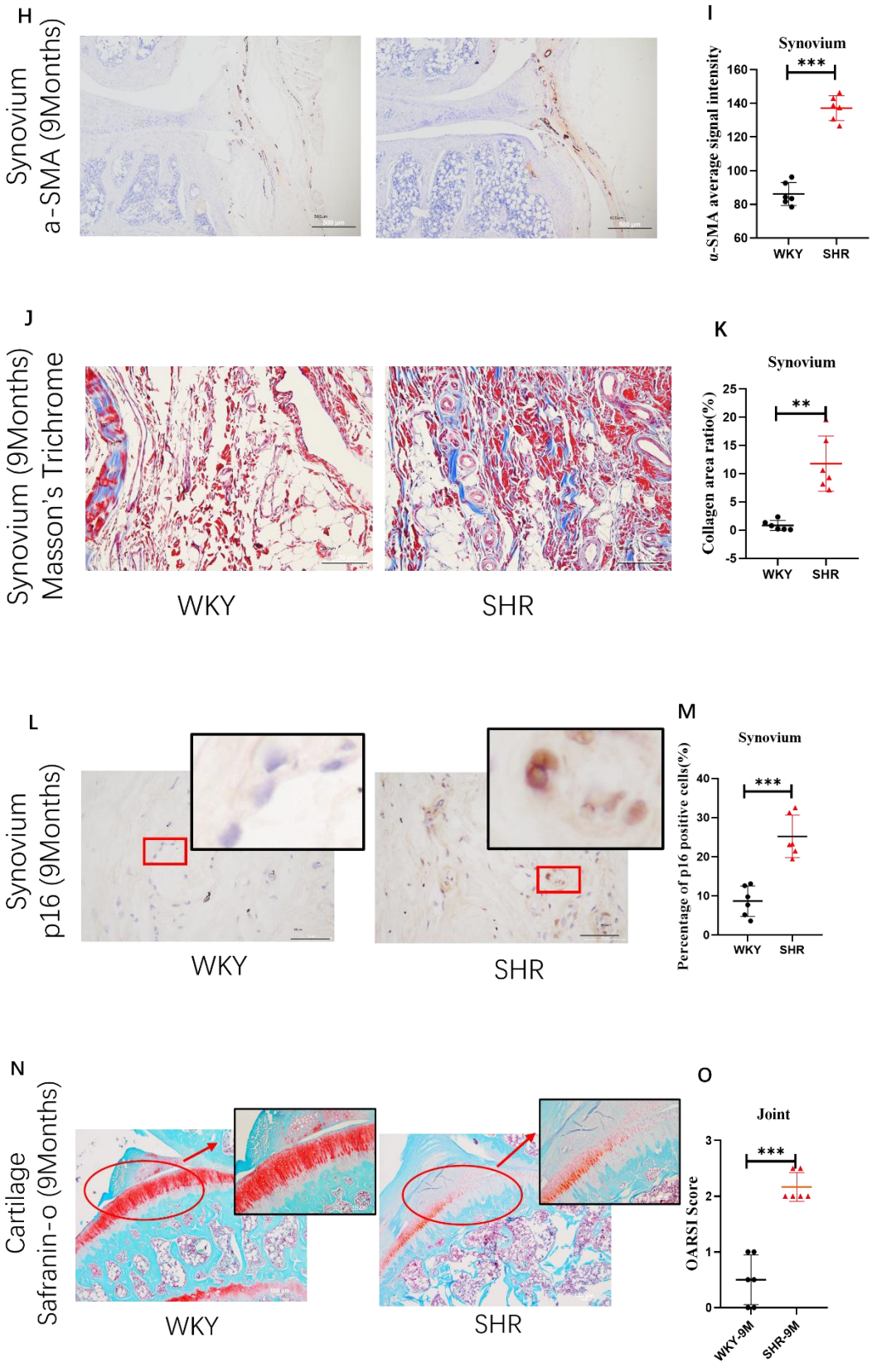


Figure 2. 2. No significant changes were detected in knee joint microstructure of WKY and SHR at 6 months. (A）H&E staining of joint synovium in WKY and SHR at 3 and 6 months. (B-C) H&E staining and quantification of joint cartilage in WKY and SHR at 3 and 6 months. (N=3, male) (D) H&E staining of joint bone in WKY and SHR at 3 and 6 months [All data were presented as mean ± S.E.M. Two-tailed Student's t-test (unpaired) was performed between WKY and SHR.* $p<0.05$; ** $p<0.005$; *** $p<0.001$]


DAPI
4HNE
Merge
A
WKY (9months) Aorta
SHR (9months) Aorta
B
Aorta
***
Normalized Fluorescent Intensity
50
40
30
20
10
0
WKY
SHR
C
Aorta intima p16 (9months)
WKY
SHR
D
Aorta
***
Percentage of p16 positive cells(%)
80
60
40
20
0
WKY
SHR


E
Carotid artery stiffness
**
Pulse propagation velocity (mm/s)
15
10
5
0
WKY-9M
SHR-9M
F
DAPI
CD31
4HNE
Merge
WKY (9Months) Synovium
SHR (9Months) Synovium

G
region of interest
WKY-9Months
SHR-9Months
ns
Oxygen saturation (%)
1.5
1.0
0.5
0.0
WKY-9M
SHR-9M


H
Synovium
a-SMA (9Months)
I
Synovium
***
α-SMA average signal intensity
160
140
120
100
80
60
WKY
SHR
J
Synovium (9Months)
Masson's Trichrome
WKY
SHR
K
Synovium
**
Collagen area ratio(%)
25
20
15
10
5
0
-5
WKY
SHR
L
Synovium
p16 (9Months)
WKY
SHR
M
Synovium
***
Percentage of p16 positive cells(%)
40
30
20
10
0
WKY
SHR
N
Cartilage
Safranin-o (9Months)
WKY
SHR
O
Joint
***
OARSI Score
3
2
1
0
WKY-9M
SHR-9M

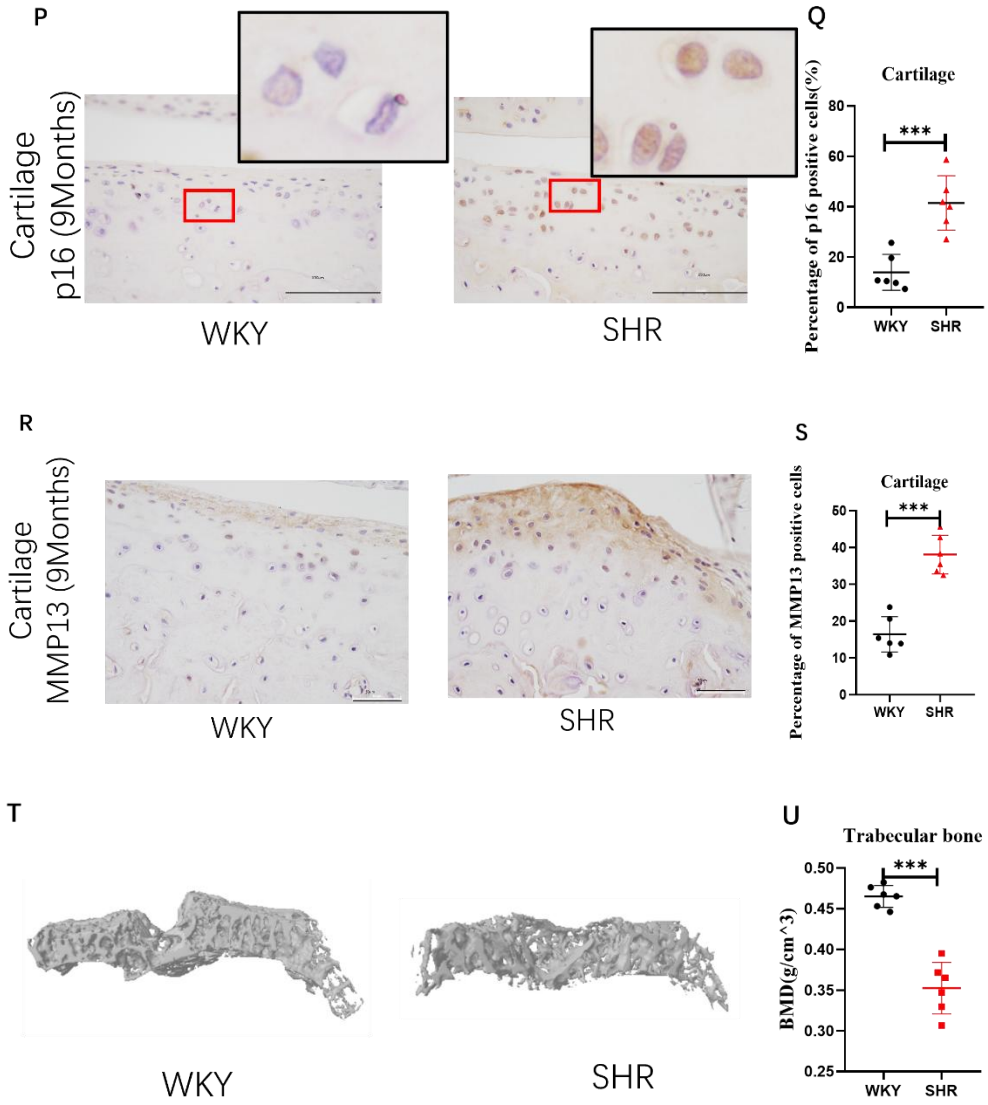
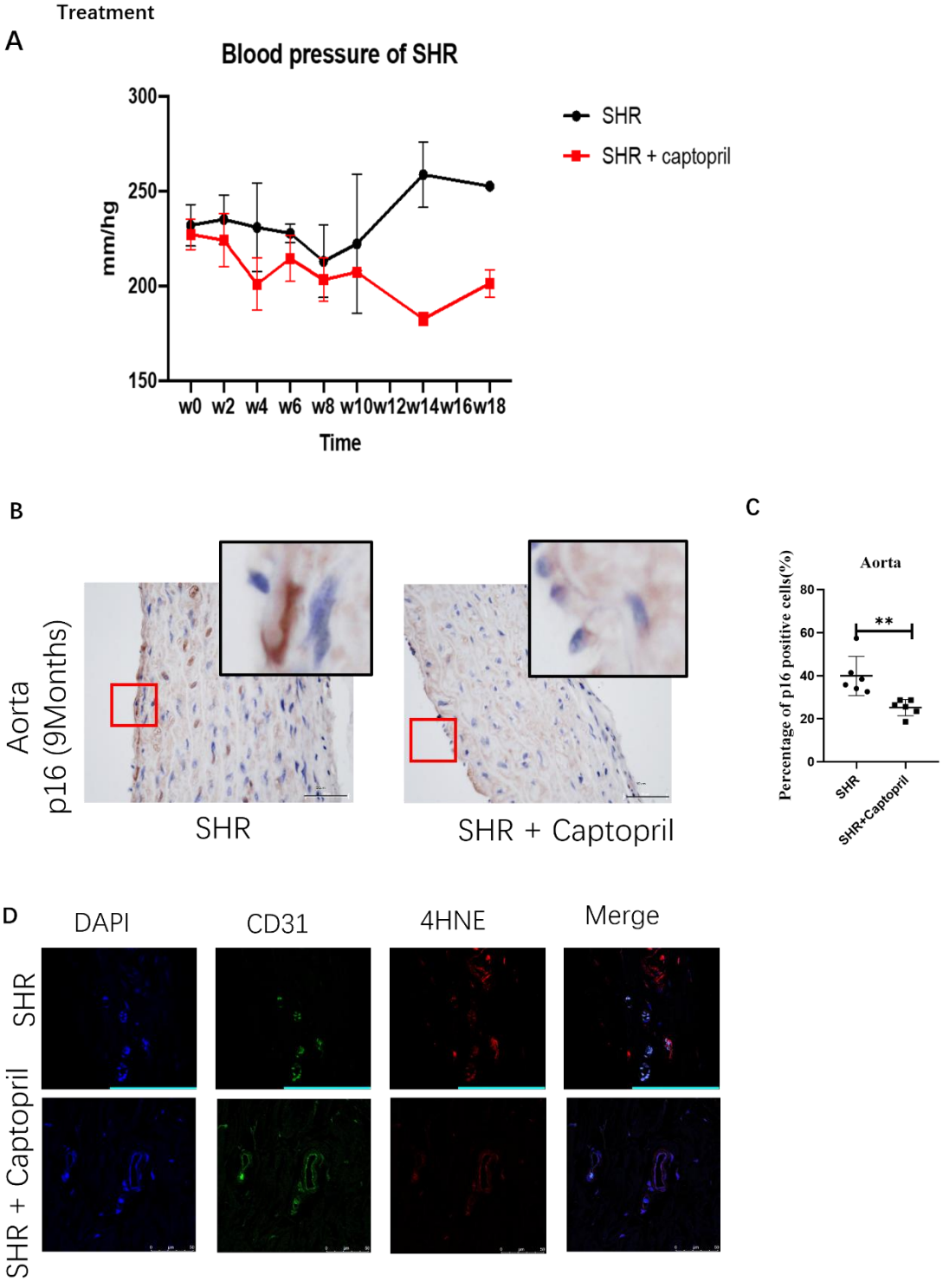
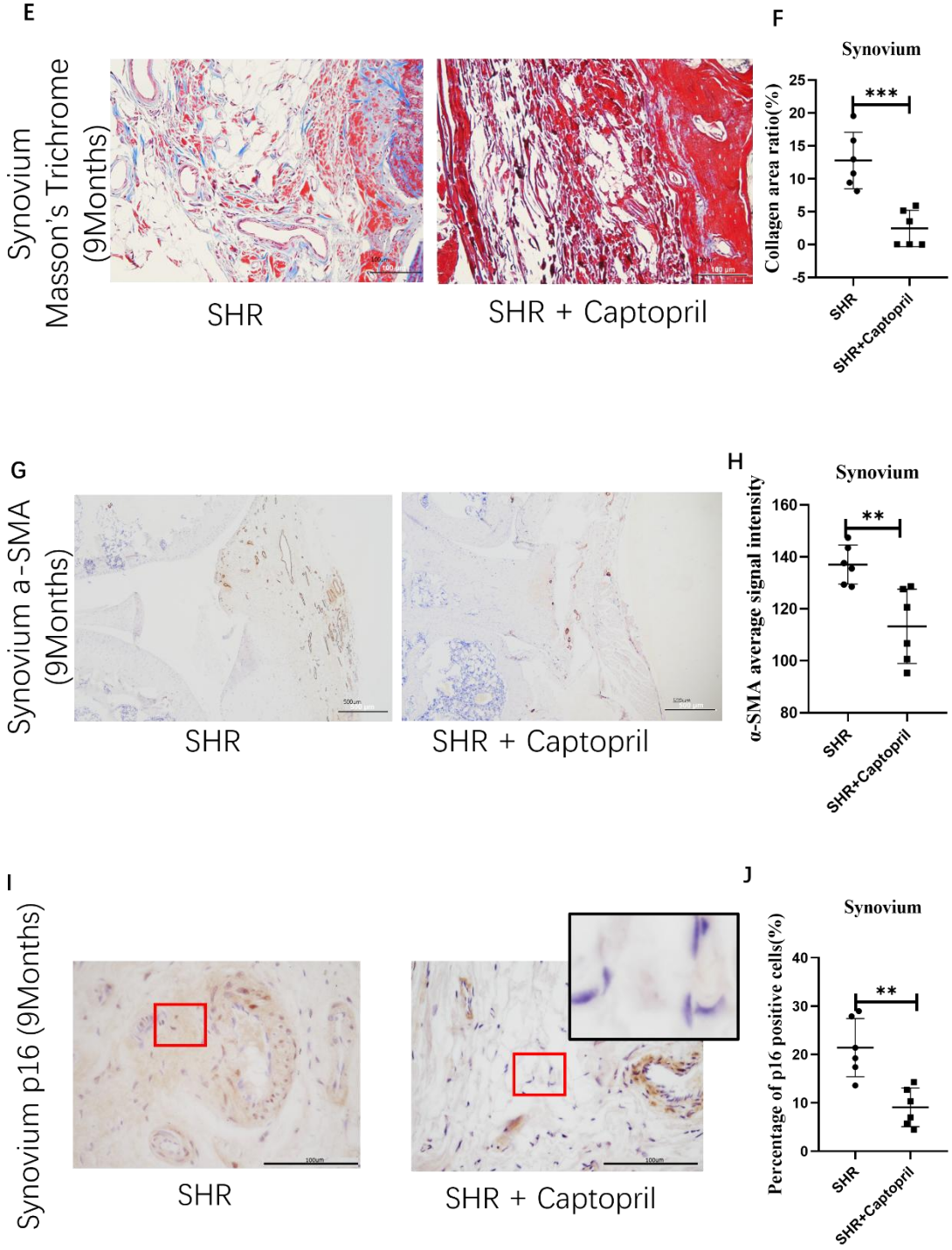
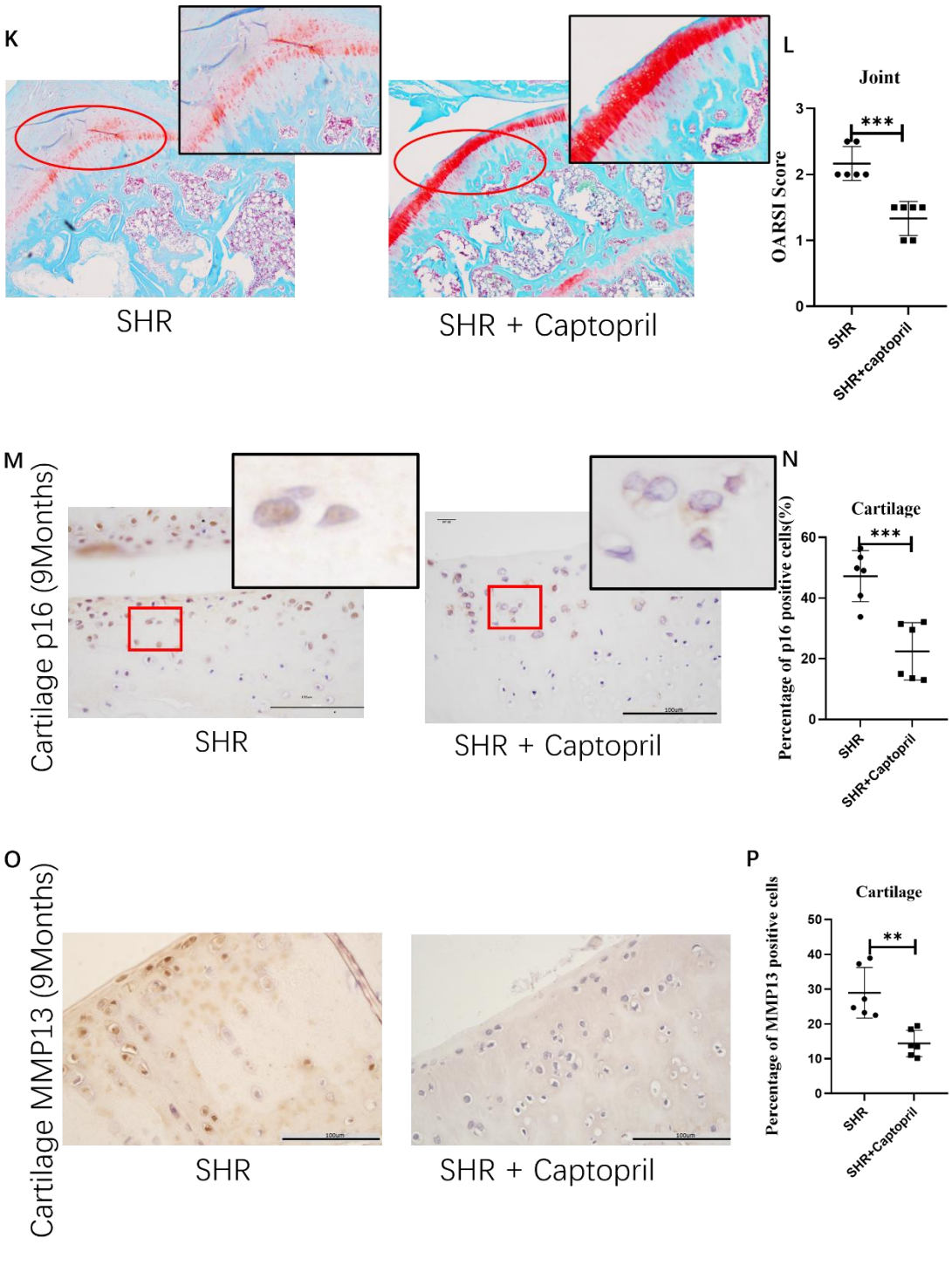


Figure 2. 3. Joint structural and functional deterioration in SHR at 9-month-old. (A-B) Representative immunofluorescent staining and quantification of 4HNE in aorta in 9-month-old rats. (N=6, male) (C-D) Representative images and quantification of p16ink4a staining aorta in 9-month-old animals. Scale bar 50um. (N=6, male) (E) Carotid artery stiffness in 9-month-old WKY and SHR. (F) Representative immunofluorescent staining of 4HNE and CD31 in synovial tissue in 9-month-old WKY and SHR. (N=6, male) (G) Photoacoustic images and measurement of oxygen saturation in joint tissue in 9-month-old WKY and SHR. (N=6, male) (H-K)

Representative images and quantification of alpha smooth muscle actin (a-SMA) and Masson’s Trichrome (blue) staining in synovium of 9-month-old WKY and SHR. (N=6, male) (L-M)Immunohistochemistry staining and quantification of p16ink4a in synovium of 9-month-old WKY and SHR. (N=6, male) (N) Representative images of safranin-O/Fast Green staining for 9-month-old WKY and SHR joints. (N=6, male) (O) OARSI score grading system was adopted from {OARSI osteoarthritis cartilage histopathology assessment system: A biomechanical evaluation in the human knee - Waldstein - 2016 - Journal of Orthopaedic Research - Wiley Online Library} (N=6, male) (P-Q) Immunohistochemistry staining and quantification of p16ink4a in cartilage of 9-month-old WKY and SHR. (N=6, male) (R-S) Immunohistochemistry staining and quantification of MMP13 in cartilage of 9-month-oldWKY and SHR. (N=6, male) (T-U) Representative images and quantification of Micro-CT result for subchondral bone of WKY and SHR joint for 9-month-old WKY and SHR. (N=6, male) [All data were presented as mean ± S.E.M. Two-tailed Student's t-test (unpaired) was performed between WKY and SHR. * $p<0.05$; ** $p<0.005$; *** $p<0.001$]


Treatment
A
Blood pressure of SHR
300
250
200
150
mm/hg
w0 w2 w4 w6 w8 w10w12w14w16w18
Time
SHR
SHR + captopril
B
Aorta
p16 (9Months)
SHR
SHR + Captopril
C
Aorta
Percentage of p16 positive cells(%)
80
60
40
20
0
**
SHR
SHR+Captopril
D
DAPI
CD31
4HNE
Merge
SHR
SHR + Captopril


E
Synovium
Masson's Trichrome
(9Months)
SHR
SHR + Captopril
F
Synovium
Collagen area ratio(%)
***
SHR
SHR+Captopril
G
Synovium a-SMA
(9Months)
SHR
SHR + Captopril
H
Synovium
α-SMA average signal intensity
**
SHR
SHR+Captopril
I
Synovium p16 (9Months)
SHR
SHR + Captopril
J
Synovium
Percentage of p16 positive cells(%)
**
SHR
SHR+Captopril


K
SHR
SHR + Captopril
L
Joint
OARSI Score
***
SHR
SHR+captopril
M
Cartilage p16 (9Months)
SHR
SHR + Captopril
N
Cartilage
Percentage of p16 positive cells(%)
***
SHR
SHR+Captopril
O
Cartilage MMP13 (9Months)
SHR
SHR + Captopril
P
Cartilage
Percentage of MMP13 positive cells
**
SHR
SHR+Captopril

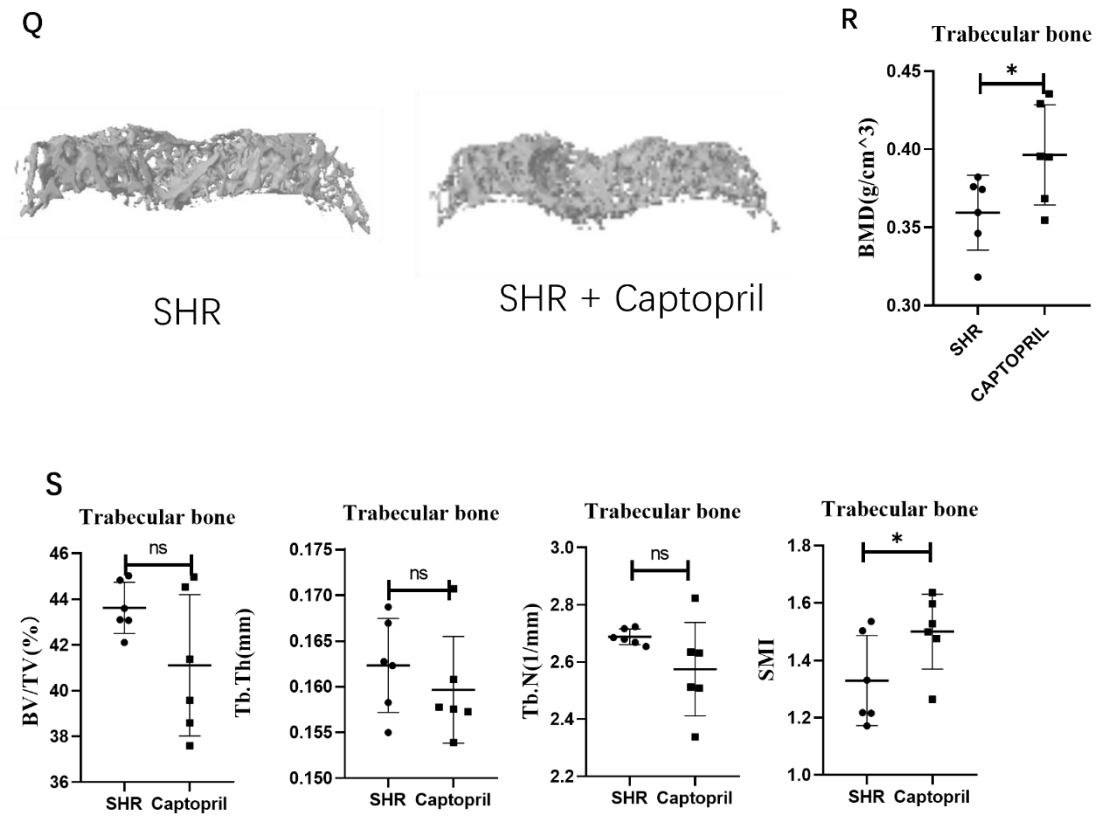


Figure 2. 4. Captopril attenuated joint structural and functional deterioration in SHR at 9-month-old. (A)Systolic blood pressure in SHR and SHR after captopril treatment. (B-C) Immunohistochemistry staining and quantification of p16ink4a in aorta of captopril treated and untreated 9-month-old SHR. (N=6, 3 male and 3 female) (D) Representative immunofluorescent staining of 4HNE and CD31 of synovial vasculature in 9-month-old SHR and SHR after captopril treatment. (E-H) Representative images and quantification of alpha smooth muscle actin (a-SMA) and Masson’ s Trichrome staining in synovium of 9-month-old SHR and SHR after captopril treatment. (N=6, 3 male and 3 female) (I-J) Immunohistochemistry staining and quantification of p16ink4a in synovium of captopril treated and untreated 9-month-old SHR. (N=6, 3 male and 3 female) (K) Representative images of safranin-O/Fast Green staining for 9-month-old joints of SHR and SHR after captopril treatment. (N=6,

3 male and 3 female) (L) OARSI score for treated and untreated 9-month-old SHR and SHR after captopril treatment. (N=6, 3 male and 3 female) (M-N) Immunohistochemistry staining and quantification of p16ink4a in cartilage of captopril treated and untreated 9-month-old SHR. (N=6, 3 male and 3 female) (O-P) Immunohistochemistry staining and quantification of MMp13 in cartilage of captopril treated and untreated 9-month-old SHR. Scale bar 50um. (N=6, 3 male and 3 female) (Q-S) Representative images and quantification of Micro-CT result for subchondral bone of joint for 9-month-old SHR and SHR after captopril treatment. (N=6, N=6, 3 male and 3 female)[All data were presented as mean ± S.E.M. Two-tailed Student's t-test (unpaired) was performed for staining intensity and percentage of positively stained cells between SHR and captopril-treated group. * $p<0.05$; ** $p<0.005$; *** $p<0.001$]

Endothelial dysfunction in SHR developed by 3 months of age without knee joint microstructure changes.

Higher blood pressure was observed in SHR compared to WKY rats at as early as 3 months old in both male and female (Figure 2.1A). Male SHR demonstrated a significant increase starting from four months until nine months （224.83±1.38 mm/Hg） when comparing to the timepoint at three months （190.92 ±1.42 mm/Hg）. The blood pressure stabilized and remained relatively constant from six to nine months comparing to six month （214.17 ±3.16 mm/Hg）. In female SHR, blood pressure also increased in six months （197.83±3.97 mm/Hg） comparing to the timepoint at three months (184±2.22 mm/Hg), continuing to rise until six months （197.83 ± 3.97 mm/Hg）, after which it stabilized and remained relatively constant up to nine months (207.5±3.69 mm/Hg). Carotid artery stiffness in 3-month-old WKY and SHR did not yet show significant differences [0.225, 95%CI (-0.5746 to 1.025)] (Figure 2.1B). In addition, SHR also showed local vascular changes in joints at 3 months rather than WKY rats. Joint oxygen saturation was significantly lower in young SHR (3.254 ± 0.867%) compared to WKY (8.325 ± 1.561%) (Figure 2.1C-D). However, there were no significant histological changes observed in SHR joints when compared with WKY. H&E staining at 3 and 6 months revealed similar cartilage, synovium, and bone microarchitecture in both strains (Figure 2.1E-H, Figure 2.2A-D).

Systemic blood vessels dysfunction with joint structural and functional deterioration in SHR at 9-month-old.

4HNE was higher in SHR aorta compared with WKY (Figure 2.3A-B). Senescent endothelial cell accumulation was found in the aorta of 9-month-old SHR rats [27.52, 95%CI (19.1 to 35.94)] (Figure 2.3C-D). Unlike at three months, the vascular stiffness of SHR (3.966±0.290mm/s) at nine months old was significantly higher than that of WKY (8.09 ± 1.030mm/s) (Figure2.3E). Consistent with our findings in aortic endothelial cells, there was a higher expression of oxidative stress maker 4HNE in SHR synovial vasculature, co-localized with CD31, a marker of blood vessels (Figure 2.3F). SHR joints displayed OA-like changes from 9 months. With increasing age, both SHR and WKY exhibited reduction in joint oxygen saturation, and no statistically significant difference was observed between the two groups(p=0.9606) (Figure 2.3G). The synovium of 9-month-old SHR appeared to be more fibrotic than age-matched WKY rats, as indicated by alpha smooth muscle actin (α-SMA) and Masson's Trichrome staining （blue）(Figure 2.3H-K). There were also more p16INK4a-positive senescent cells in synovium of SHR than WKY (Figure 2.3L-M). In the articular cartilage, SHR showed lower proteoglycan content and higher OARSI score compared with WKY [1.667, 95%CI (1.197 to 2.136)] (Figure 2.3N-O). At cellular level, accumulation of p16INK4a-positive cells was observed at the articular cartilage in SHR [27.6, 95%CI (16.91 to 38.28)] (Figure 2.3P-Q). This was accompanied by a higher level of MMP13 in the extracellular matrix of SHR chondrocytes [21.67, 95%CI (15.17 to 28.16)] (Figure 2.3R-S). Bone loss in subchondral bone was also observed in SHR compared with WKY (Figure 2.3T-U).

Captopril attenuated joint structural and functional deterioration in SHR at 9-month-old.

Captopril is an FDA-approved antihypertensive drug frequently prescribed to patients. To validate the effect of captopril in vivo, SHR was administered with captopril at 50 mg/kg. The treated group showed significant reductions in blood pressure (Figure 2.4A). Meanwhile, aortic senescence was alleviated after captopril treatment [-14.7, 95%CI (-23.68 to -5.723)] (Figure 2.4B-C). This result suggested that captopril improved vascular health both functionally and phenotypically. Immunostaining with 4HNE and CD31 also revealed reduced vascular oxidative stress in SHR following treatment with captopril (Figure 2.4D). Synovium of captopril-treated SHR showed lower staining intensity of fibrotic content and α-SMA, indicating lessened fibrosis (Figure 2.4E-H). A reduction in senescent cell accumulation was observed in the synovial tissue (Figure 2.4K-L). Following captopril treatment, the proteoglycan content in cartilage were significantly higher and OARSI score decreased compared to the untreated group [-0.8333, 95%CI (-1.165 to -0.5012)] (Figure 2.4K-L). The clearance of senescent cells was not only limited in vasculature, but also noticeable in cartilage (Figure 2.4M-N). Expression of MMP13 in articular cartilage was significantly attenuated [-14.56, 95%CI (-22.03 to -7.089)] (Figure 2.4O-P). Interestingly, Captopril was able to rescue the BMD of the subchondral bone [0.03694, 95%CI (0.001 to 0.07335)] (Figure 2.4Q-R). However, BV/TV, Tb.Th, and Tb.N were not restored. (Figure 2.4S). This data suggested captopril also exerted chondroprotective effect apart from its antihypertensive function.

3.4 Conclusion and discussion

Similar to the previous DOCA-induced hypertension model, the spontaneous hypertension rat model also showed systemic vascular aging, and the stiffness of the blood vessels became harder. Protein deposition and fibrosis were also found in the blood vessels in the joints, indicating that local blood vessels also had dysfunction. At the same time, the oxygen content in the joints also decreased. The number of senescent cells in the articular cartilage and synovium increased, the cartilage degradation markers increased, and the cartilage proteoglycan decreased, and the cartilage layer became thinner. After captopril treatment, blood pressure of SHR decreased, and senescent cells in both blood vessels and joints were effectively reduced. Protein deposition and fibrosis of vascular synovial membranes were reduced. Similarly, captopril could not restore bone loss in subchondral bone caused by hypertension.

There have been many articles reporting on why high blood pressure causes bone loss. Osteoporosis has been shown to be associated with the risk of hypertension[237]. One study for 3676 women which average age at 73 years old suggests that hypertension in elderly white women is associated with an increased rate of bone-mineral loss, indicating a potential link between high blood pressure and osteoporosis in this population[238]. Another study included 2,039 participants and found that patients with hypertension had a high rate of osteoporosis[239]. At the same time, the proportion of patients with hypertension was higher in people with osteoporosis than in people without osteoporosis.

Several mechanisms have been proposed to explain the association between hypertension and bone loss, which involve both direct and indirect pathways:

1. Calcium Metabolism and Parathyroid Hormone (PTH) Dysregulation

Hypertension is associated with alterations in calcium metabolism, which can negatively affect bone health. High blood pressure has been linked to increased renal calcium excretion, potentially leading to negative calcium balance. As calcium is a critical component of bone, chronic depletion can contribute to reduced bone mineral density. Furthermore, increased calcium loss may stimulate the release of PTH, which, in turn, enhances bone resorption to maintain serum calcium levels. Chronic elevations in PTH can exacerbate bone loss over time, leading to a higher risk of osteoporosis.

2. Oxidative Stress and Inflammation

Hypertension is characterized by increased oxidative stress and inflammation, both of which play critical roles in bone metabolism. Elevated levels of ROS and pro-inflammatory cytokines, such as IL-6 and TNF-α, can stimulate osteoclast differentiation and activity, leading to increased bone resorption. This pro-inflammatory environment is detrimental to osteoblasts, the cells responsible for bone formation, thus tipping the balance toward net bone loss. In hypertensive patients, chronic inflammation may, therefore, accelerate age-related bone loss and contribute to the development of osteoporosis.

3. Angiotensin II and Bone Remodeling

The RAS, particularly Ang II, plays a central role in the regulation of blood pressure and fluid balance. Recent studies have demonstrated that Ang II can also influence bone remodeling. Ang II has been shown to promote osteoclastogenesis, the process by which osteoclasts (bone-resorbing cells) are formed, by increasing the expression of receptor activator of nuclear factor-kappa B ligand (RANKL), a key mediator of bone resorption. Additionally, Ang II-induced oxidative stress further enhances osteoclast activity, leading to increased bone degradation. This dual role of Ang II in promoting both hypertension and bone loss underscores the interconnected nature of these conditions.

4. Vascular Dysfunction and Bone Microcirculation

Hypertension is often accompanied by vascular dysfunction, which may impair bone microcirculation. Adequate blood flow is essential for the delivery of nutrients and oxygen to bone tissue, and reduced perfusion can impair osteoblast function, limiting bone formation. Moreover, vascular calcification, a common feature of hypertension, has been associated with reduced bone mineralization, suggesting that vascular and skeletal systems may be linked through shared pathological mechanisms.

In addition to the above reasons, our laboratory also conducted transcriptomic analysis of cartilage explants on SHR and WKY. We found that the number of mast cells in the

hypertrophic layer of SHR decreased. We speculated that this was because the number of chondrocytes heading towards aging increased, so the number of cells turning to hypertrophic fate decreased. Studies have shown that hypertrophic chondrocytes produce osteoblasts and osteocytes during bone development[240, 241]. Therefore, we speculate that this may also be one of the reasons for the decrease in bone mass. However, after captopril treatment, blood pressure was restored, but bone loss did not recover. This may be due to the influence of other pathways, and more experiments are needed to verify it.

Although SHRs are an invaluable tool in hypertension research, they do have limitations. The SHR model primarily mimics genetic hypertension, which may not fully represent the complexity of essential human hypertension influenced by environmental and lifestyle factors. Additionally, the SHR model may not fully capture the effects of comorbidities such as obesity and diabetes, which are common in human hypertensive populations. It is known that hypertension can cause premature aging of endothelial function and accelerate vascular deterioration[242, 243]. previous articles have found accelerated bone maturation in children with primary hypertension[244]. In other words, primary hypertension accelerates joint maturation and degeneration.

Chapter 4 Dysfunction of blood vessels leads to dysfunction of cartilage

4.1 Introduction

We have previously observed senescence and dysfunction of endothelial cells and chondrocytes using two models of hypertension. Therefore, we speculated whether the senescence and dysfunction of endothelial cells affected the aging and dysfunction of chondrocytes. To verify our hypothesis, my labmate Karen used human endothelial cells HUVECs and human chondrocytes c28/i2. We conducted in vitro cell experiments to verify and explore the potential mechanism. At the same time, I am also exploring the early vascular and joint changes of SHR in animal models.

4.2 Materials and Methods

Histology

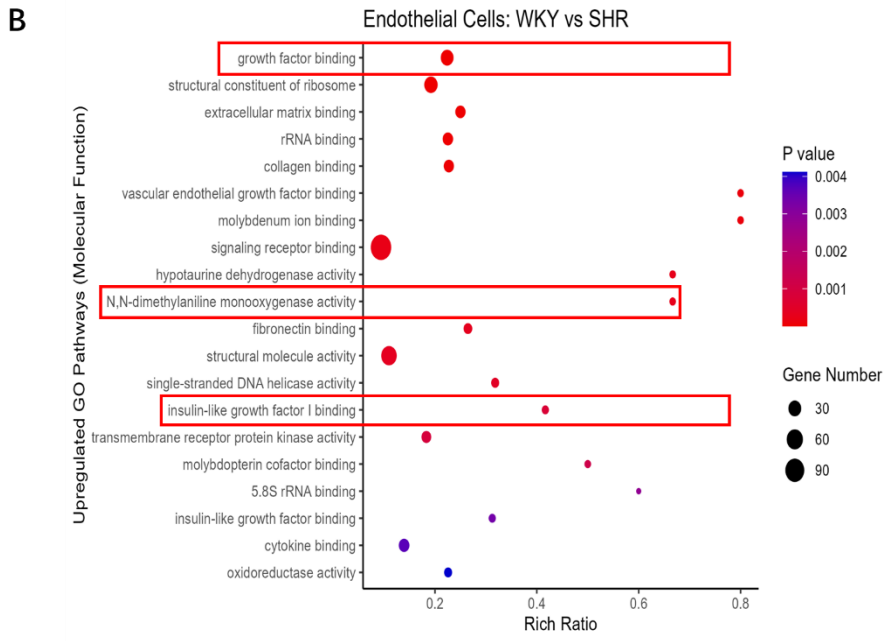
Same as chapter2.2 histology method. Primary antibodies used were 4HNE (1: 1000, Invitrogen, MA5-27570), CD31 (1:500, Abcam, ab182981), MMP13 (1:500; Abcam, ab39012). For immunofluorescent staining, secondary antibodies conjugated with Alexa Fluor™ 488 (1:500, Thermo Fisher, A11008) or Alexa Fluor™ 594 (1:500, Thermo Fisher, A11005) were used.

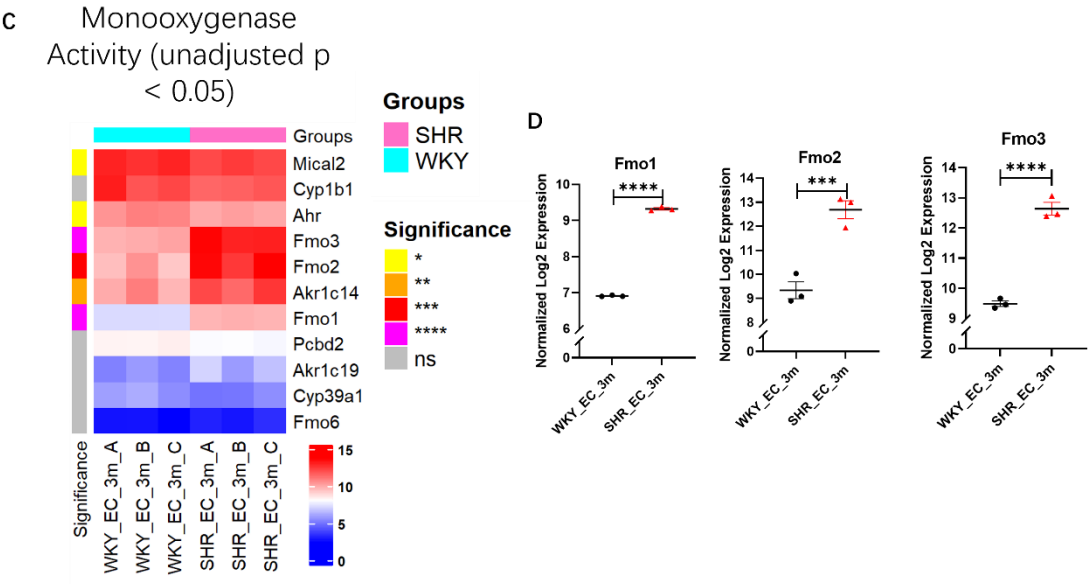
RNA sequencing

Total RNA was extracted from primary endothelial cells from the aorta of 3-month-old WKY and SHR (n=3 per group) with E.Z.N.A.® Total RNA Kit (Omega Biotek)

according to manufacturer's protocols. Library preparation and sequencing were outsourced to BGI Genomics. Briefly, samples were sequenced using DNBSEQ platform with a paired end read length of 100 bp (PE100). Reads of low quality, with adaptor sequences, or high levels of N base were filtered with SOAPnuke (v1.5.6) [248]. Bowtie2 (v2.4.4) was employed to align the clean reads to the reference genes in genome GCF_000001895.5_Rnor_6.0 [249]. Gene expression was quantified using RSEM (v1.2.28) [250]. Normalization and differential analysis of gene expression were performed with DESeq2 (v1.42.1) and corrected using the Benjamini-Hochberg procedure [251]. Gene ontology (GO) enrichment analysis was performed on differentially expressed genes with adjusted p-value less than 0.05 using GOstats (v2.68.0) [252]. The most prominent pathways in molecular function were compared between WKY and SHR in endothelial cells. Images were generated with ggplot2 (v3.5.0) and ComplexHeatmap (v2.15.4) in RStudio.

4.3 Figures and Results


B
Endothelial Cells: WKY vs SHR
growth factor binding
structural constituent of ribosome
extracellular matrix binding
rRNA binding
collagen binding
vascular endothelial growth factor binding
molybdenum ion binding
signaling receptor binding
hypotaurine dehydrogenase activity
N,N-dimethylaniline monooxygenase activity
fibronectin binding
structural molecule activity
single-stranded DNA helicase activity
insulin-like growth factor I binding
transmembrane receptor protein kinase activity
molybdopterin cofactor binding
5.8S rRNA binding
insulin-like growth factor binding
cytokine binding
oxidoreductase activity
Upregulated GO Pathways (Molecular Function)
0.2
0.4
0.6
0.8
Rich Ratio
P value
0.004
0.003
0.002
0.001
Gene Number
30
60
90


c
Monooxygenase Activity (unadjusted p < 0.05)
Groups
Mical2
Cyp1b1
Ahr
Fmo3
Fmo2
Akr1c14
Fmo1
Pcbd2
Akr1c19
Cyp39a1
Fmo6
Significance
WKY_EC_3m_A
WKY_EC_3m_B
WKY_EC_3m_C
SHR_EC_3m_A
SHR_EC_3m_B
SHR_EC_3m_C
15
10
5
0
Groups
SHR
WKY
Significance
*
**
***
****
ns
D
Fmo1
Fmo2
Fmo3
****
***
****
Normalized Log2 Expression
WKY_EC_3m
SHR_EC_3m

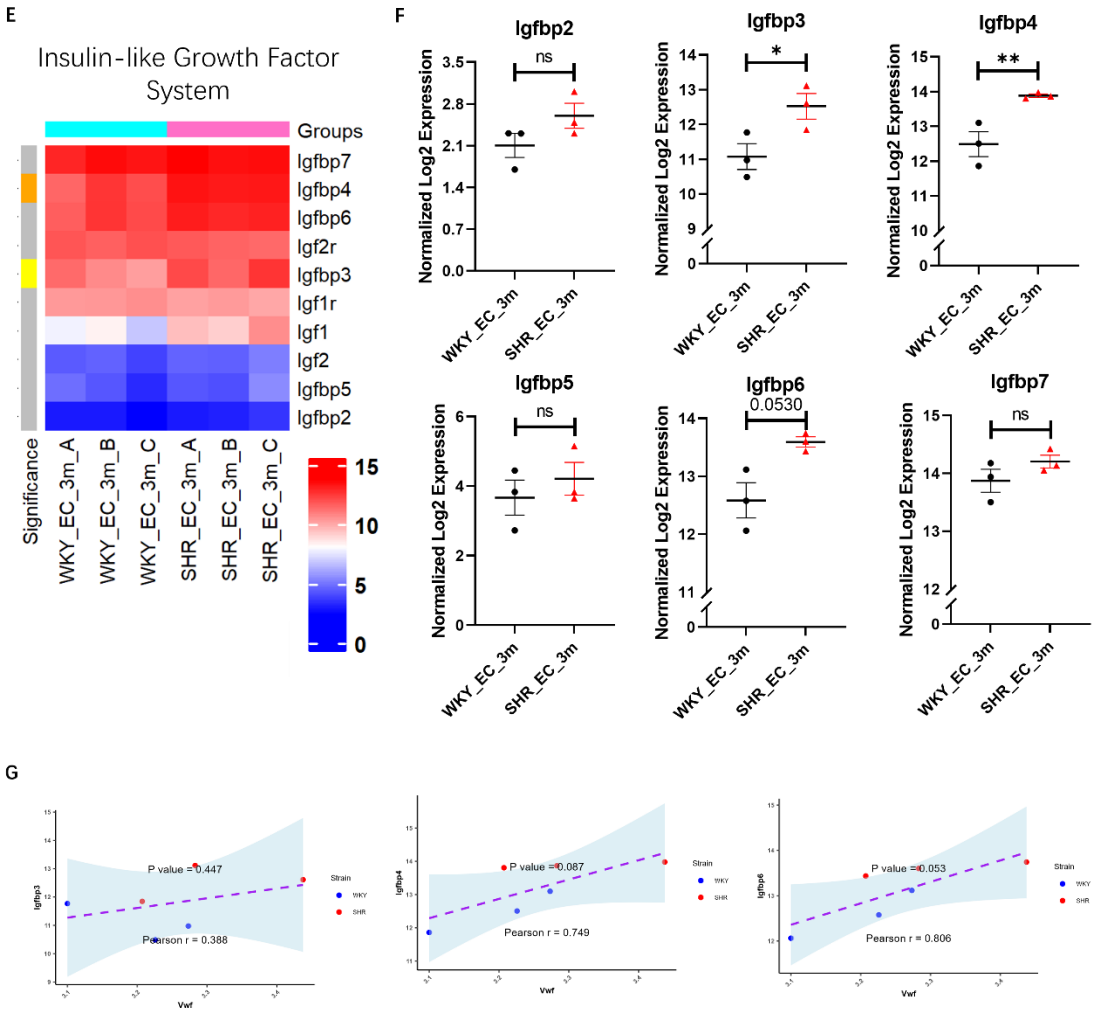


Figure 3. 1. Elevated IGF binding proteins in vasculature of 3-month-old SHR compared to WKY. (A) Schematic for isolation of primary endothelial cells for RNA-Seq. (B) Top upregulated Gene Ontology terms pertaining to molecular function in SHR compared with WKY. (n=3) (C-D) Heatmap of genes involved in monooxygenase activity and the normalized expression of selected genes, Fmo1, Fmo2 and Fmo3. (E-F) Heatmap of genes in insulin-like growth factor system and the normalized expression of selected genes, Igfbp6. (G) IGFBP3, IGFBP4 and IGFBP6 in correlation with Vwf. Gene expression was normalized using R package DESeq2' s median of ratios. Adjusted p value was shown for selected genes of interest. Likelihood-ratio test included in DESeq2 was used to compute the adjusted p value.

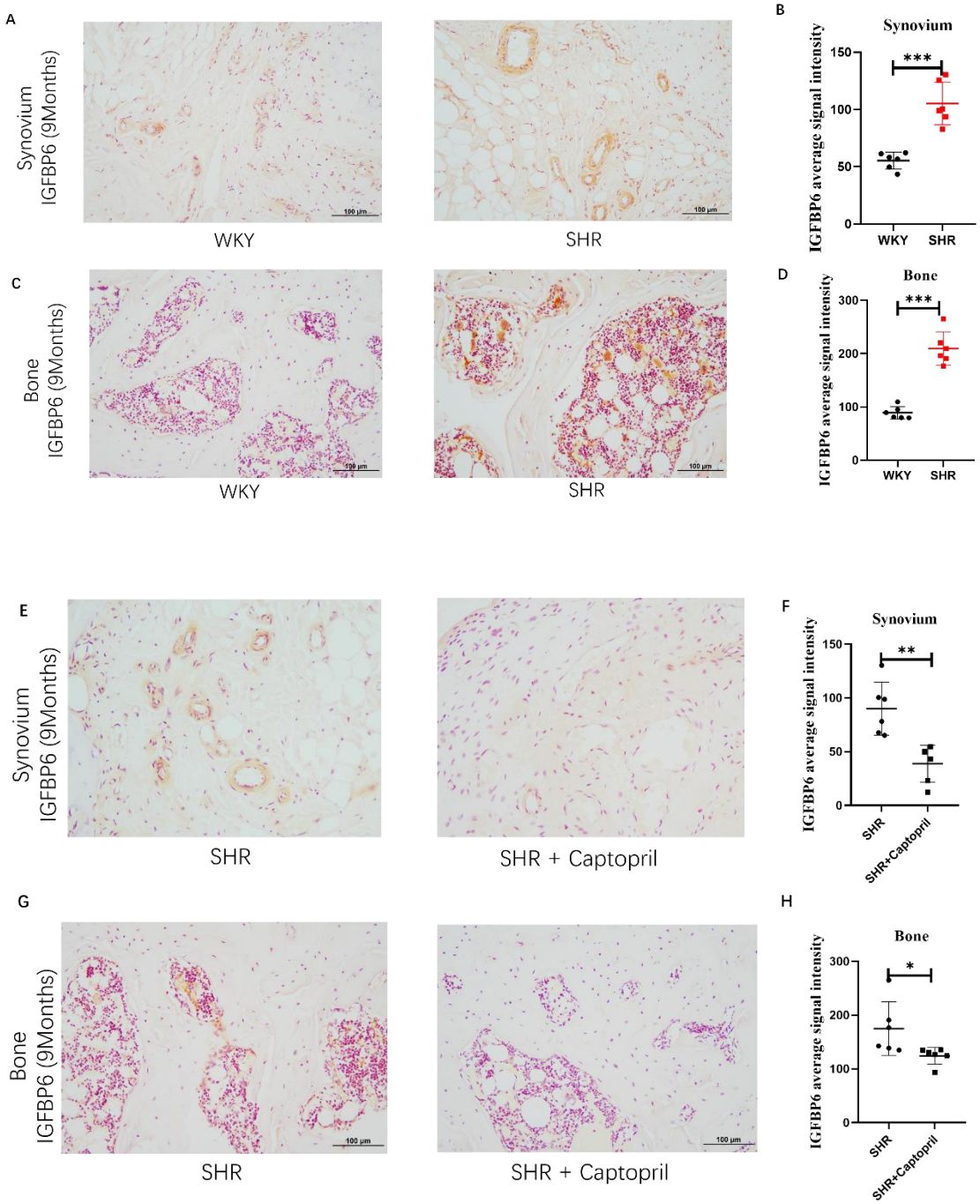
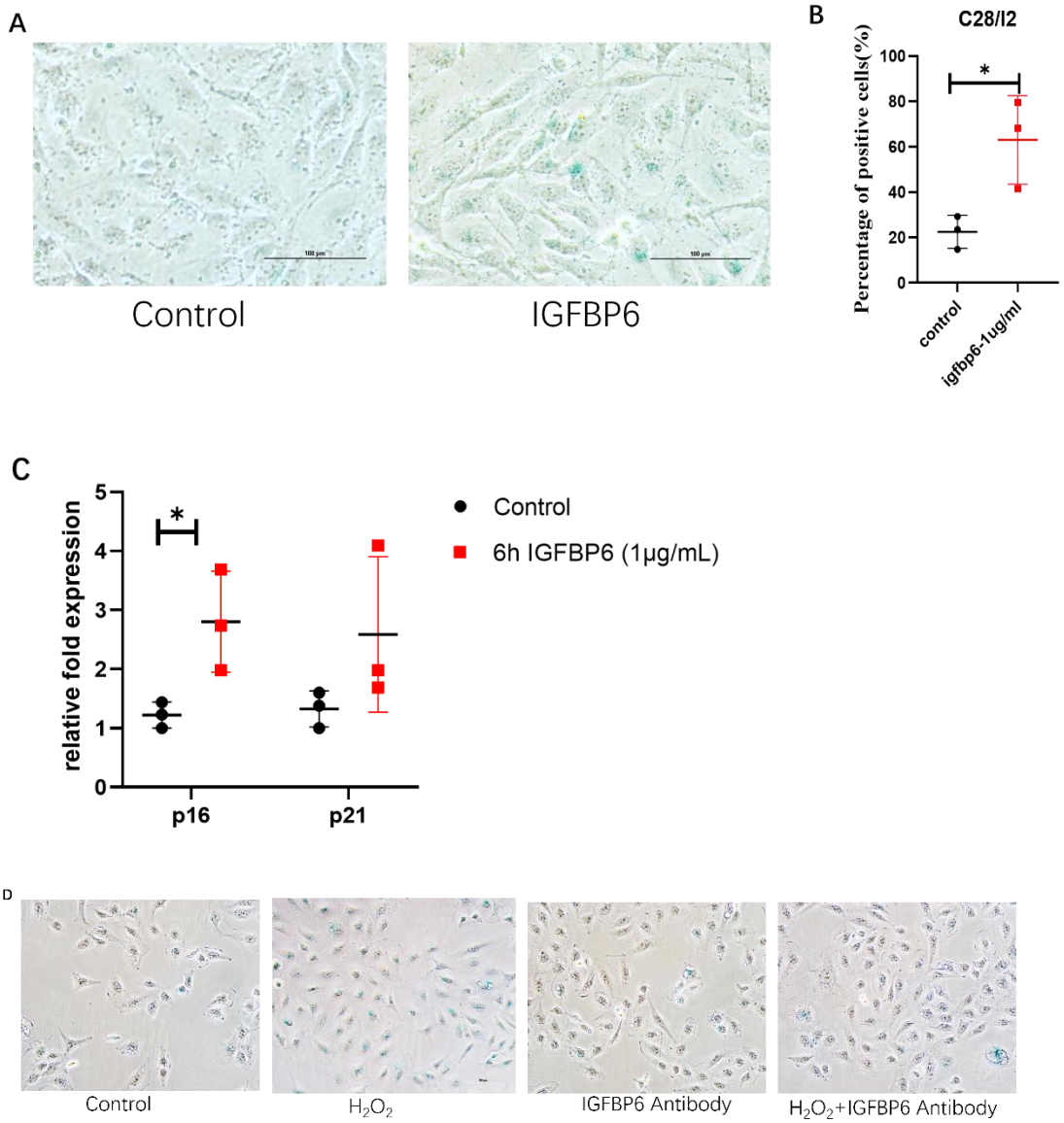


Figure 3. 2. IGFBP6 plays a role in crosstalk between endothelial cell and chondrocyte in rat model. (A-D) Immunohistochemistry staining and quantification of IGFBP6 in synovium and bone of 9-month-old WKY and SHR. (N=6, male) (E-H)

Immunohistochemistry staining and quantification of IGFBP6 in synovium and bone of captopril treated and untreated 9-month-old SHR. (N=6, 3 male and 3 female) [All data were presented as mean ± S.E.M. Two-tailed Student's t-test (unpaired) was performed for staining intensity and percentage of positively stained cells between SHR and captopril-treated groups. * p<0.05; ** p<0.005; *** p<0.001]



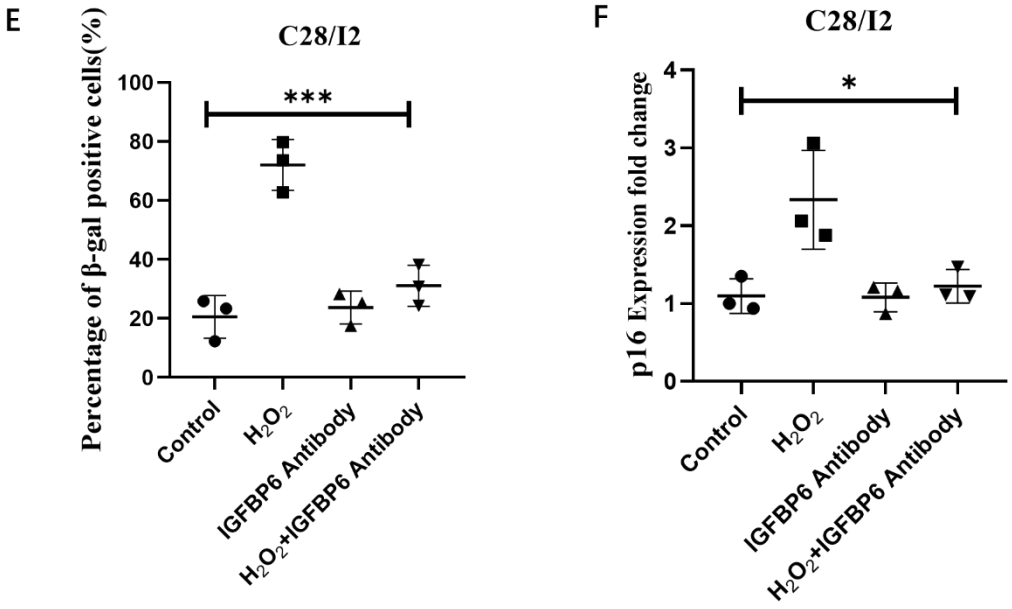


Figure 3. 3. The IGFBP6 plays a role in crosstalk between blood vessels and cartilage. (A-B) β -Gal staining in C28/I2 cell with or without IGFBP6. (n=3）（C） Relative mRNA level of p16INK4a and p21 in C28 cell with or without IGFBP6. (n=3) (D-E) β-Gal staining in C28/I2 cell for control, H2O2, IGFBP6antibody and H2O2+IGFBP6 antibody group. (n=3) (H) Relative mRNA level of p16INK4a for control, H2O2, IGFBP6 antibody and H2O2+IGFBP6 antibody group(n=3). All data are shown as means ± SEM, and each data point represents an individual experiment. One-way ANOVA with Tukey's multiple-comparisons test was employed for statistical analysis to compare between each experimental group and control group. * $p<0.05$; ** $p<0.005$; *** $p<0.001$; **** $p<0$.

Elevated IGF binding proteins in vasculature of 3-month-old SHR compared to WKY

To further decipher cellular changes in vasculature of SHR, primary endothelial cells

were isolated from the aorta of the 3-month-old animals (Figure 3.1A). To describe the differences in endothelial function, pathway enrichment analysis was performed using the gene ontology (GO) terms for molecular function. Enrichment in growth factor binding, oxidoreductase activities, and IGF signalling-associated gene were noted (Figure 3.1B). Expression of Fmo1, Fmo2, and Fmo3 was significantly higher in SHR endothelial cells ($p < 0.001$) (Figure 3.1C-D). Fmo1, one of the top upregulated genes in SHR endothelial cells, is involved in H2O2 accumulation induced by DNA damage. Mammalian flavin-containing monooxygenase (FMOs) are a source of H2O2 production. Elevated oxidative stress was thus implied in 3-month-old SHR rats. IGF-binding proteins, Igfbp3 ($p=0.0373$), Igfbp4 ($p=0.0053$) and Igfbp6 ($p=0.053$) were also elevated in SHR endothelial cells compared to WKY counterparts, while there was no significant difference between two strains in terms of Igfbp2($p=0.3905$), Igfbp5($p=0.9856$), Igfbp7($p=0.6237$) (Figure 3.1E-F). We performed correlation analyses between IGFBP3, IGFBP4, IGFBP6 and von Willebrand factor (VWF), and found that IGFBP4 and IGFBP6 showed stronger correlations (Figure 3.1G). Insulin-like growth factor (IGF) signalling was implicit in cellular senescence and oxidative injury and contributed to the pathogenesis of hypertension. Roles of IGFBP family have been discussed in OA. While prior studies focused on IGFBP3 (anti-inflammatory and pro-apoptotic) and IGFBP-7 (pro-apoptotic) in OA. The role of IGFBP4 in aging is controversial. Some studies have shown that it has pro-senescence and anti-senescence property. There are relatively few studies related to IGFBP6, so we chose IGFBP6 as the research object.

IGFBP6 plays a role in crosstalk between endothelial cell and chondrocyte in rat model

There were more IGFBP6 in synovium of SHR than WKY (Figure 3.2A-B). The expression level of IGFBP6 in the bone tissue of SHR was significantly higher compared to that in WKY (Figure 3.2C-D). The expression level of IGFBP6 in synovium was lower in the captopril-treated group compared to the untreated group (Figure 3.2E-F). The expression level of IGFBP6 in bone tissue was lower in the captopril-treated group compared to the untreated group (Figure 3.2G-H).

Endothelial senescence induced senescence in chondrocytes in cell model

Based on our observation in animal models that the oxidative stress level of SHR blood vessels increased significantly in the early stage, Karen, a classmate in our laboratory, used hydrogen peroxide (H2O2) to induce an increase in the oxidative stress of human endothelial cells HUVECs and observed the senescence of endothelial cells at the same time. So, the culture medium of these endothelial cells was extracted and mixed with normal chondrocyte culture medium in a ratio of 1:1 (H2O2 decomposes when exposed to light, and there is no H2O2 in the mixed culture medium), and human chondrocytes C28/I2 were cultured in the mixed culture medium. The results showed that chondrocytes cultured in the mixed culture medium also showed senescence. However, HUVECs cultured after H2O2+captopril treatment did not show senescence, and chondrocytes cultured in the mixed culture medium did not show senescence. To prove that captopril does not directly act on chondrocytes, we directly treated chondrocytes

with H2O2, and chondrocytes showed senescence. When H2O2+captopril treatment was directly added to chondrocytes, chondrocytes still showed senescence. This shows that captopril cannot directly rescue the senescence of chondrocytes. In order to analyze which components in endothelial cells cause chondrocyte senescence, we collected the culture medium of endothelial cells treated with H2O2 for proteomics analysis. Dr. Zhu lin helped us complete the results of this part. The results of the screening showed that IGFBP6 showed significant differences between WKY and SHR in both proteomics analysis. IGFBP6 was much higher in SHR than in WKY.

IGFBP6 plays a role in crosstalk between endothelial cell and chondrocyte in human cell model

Both transcriptomic and secretomic analysis indicated that endothelial cells expressed a higher level of IGFBP6. After stimulating C28/I2 cells with 1μg/ml IGFBP6 protein, cells had increased SA-β-gal activities and elevated expression of p16INK4a mRNA (Figure 3.3A-C). We collected the culture medium of endothelial cells with the addition of H2O2 and IGFBP6 antibody to culture chondrocytes. The results showed that compared with the case where only H2O2 was added, the addition of IGFBP6 antibody can effectively reduce the generation of senescent cells (Figure 3.3D-F). This implied that IGFBP6 may play a role in crosstalk between endothelia cell and chondrocyte senescence.

4.4 Conclusion and discussion

In this chapter, we found that secretions from senescent endothelial cells can lead to chondrocyte senescence, and we determined that IGFBP6 plays a role in this process. Oxidative stress and the IGFBP family are critical mediators of endothelium-cartilage communication. In SHR, increased oxidative stress was observed in both the aorta and synovial vasculature of the knee joint. This aligns with our previous lab findings where vascular dysfunction and hypoxia preceded joint damage in an OA mouse model[253]. To mimic vascular dysfunction in vitro, we used H2O2-induced senescence in endothelial cells. Proteomic and transcriptomic analyses highlighted the IGFBP family's significance, particularly IGFBP6, which showed elevated expression in both endothelial and joint tissues under oxidative stress.

H_2O_2 is widely used in research as an inducer of oxidative stress and cellular senescence. It acts as a ROS that can readily penetrate cell membranes, initiating a cascade of intracellular oxidative reactions. H_2O_2 exposure leads to the accumulation of ROS within cells, causing oxidative damage to cellular components, including lipids, proteins, and DNA[254].

This oxidative stress triggers the DNA damage response and activates signaling pathways associated with cellular aging, such as the p53-p21 and p16-Rb pathways[255]. Persistent activation of these pathways results in irreversible cell cycle arrest, a key characteristic of cellular senescence. Senescent cells then adopt SASP,

releasing pro-inflammatory cytokines, growth factors, and proteases, which can exacerbate tissue inflammation and degeneration.

Thus, H_2O_2-induced oxidative stress serves as a model for studying the mechanisms underlying cellular aging and senescence, as well as for exploring potential interventions to counteract oxidative damage and delay the onset of senescence-related cellular dysfunction.

IGFBP6 plays a crucial role in modulating the availability and activity of IGFs, which are key regulators of cell proliferation, differentiation, and survival in various tissues, including cartilage. Recent studies suggest that IGFBP6 is implicated in joint homeostasis and pathology, particularly in the context of OA and other joint-related disorders[50, 256, 257]. Joint health depends on a delicate balance between anabolic and catabolic processes within the cartilage, synovium, and subchondral bone. IGFs, particularly IGF-1, are known to promote cartilage repair and anabolic processes, playing a protective role in joint health[45]. However, the activity of IGFs is tightly regulated by a family of binding proteins, IGFBPs. Among these, IGFBP6 stands out due to its unique ability to selectively bind IGF-2 with high affinity[258], potentially modulating IGF signaling in joint tissues.

Cartilage is a specialized tissue that relies on ECM for mechanical strength and resilience. IGF-1 is a critical anabolic factor in cartilage, promoting ECM synthesis and

inhibiting catabolic enzymes[45]. IGFBP6, through its regulation of IGF-2, indirectly affects IGF-1 activity in cartilage by influencing the availability of IGF-2 for binding to the IGF1R[258]. This regulation has downstream effects on chondrocyte proliferation, differentiation, and ECM production.

In osteoarthritis, the balance between cartilage breakdown and repair is disrupted. Studies have shown that IGFBP6 is upregulated in osteoarthritic[50], suggesting a compensatory mechanism or a role in disease progression. Specifically, IGFBP6's inhibitory effects on IGF signaling could limit cartilage repair mechanisms in OA, exacerbating joint degeneration. On the other hand, IGFBP6 may also play a protective role in limiting pathological IGF-2 signaling in the synovium, where excessive IGF activity can contribute to fibrosis and inflammation.

Oxidative stress is a key driver of joint degeneration, particularly in aging and osteoarthritis. ROS produced by chondrocytes and synovial cells can lead to ECM degradation, chondrocyte apoptosis, and synovial inflammation. IGFBP6 has been shown to interact with oxidative stress pathways, particularly through its ability to modulate IGF signaling under oxidative conditions[259]. In vitro studies have demonstrated that IGFBP6 expression is upregulated in response to oxidative stress in endothelial cells and synovial tissues[50], suggesting a role in the oxidative stress response in joints.

Moreover, IGFBP6 may influence oxidative stress indirectly by modulating IGF-2 activity, which has been linked to cellular survival pathways. Under conditions of oxidative damage, IGFBP6 could act to fine-tune IGF-mediated survival signals[260], thus influencing the fate of chondrocytes and synovial cells in OA. This dual role in both oxidative stress and IGF signaling positions IGFBP6 as a key player in joint health, particularly in the context of aging and chronic inflammatory diseases like OA.

Given the complex role of IGFBP6 in joint tissues, it has emerged as a potential therapeutic target for osteoarthritis. Modulating IGFBP6 activity could offer a means to restore the anabolic-catabolic balance in cartilage, enhancing IGF-mediated repair mechanisms while preventing excessive IGF activity in the synovium. Targeting IGFBP6 could also mitigate the effects of oxidative stress on joint tissues, providing a dual benefit in the context of OA management. One potential therapeutic strategy involves the use of IGFBP6 inhibitors or neutralizing antibodies to enhance IGF signaling in cartilage. By blocking the inhibitory effects of IGFBP6 on IGF-2, it may be possible to promote chondrocyte proliferation, ECM synthesis, and overall joint repair.

Another avenue of research involves the use of gene therapy to modulate IGFBP6 expression in joint tissues. Gene therapy could be used to either upregulate or downregulate IGFBP6 expression depending on the specific needs of the tissue—promoting cartilage repair while limiting synovial fibrosis. Preclinical studies in animal

models of OA are needed to further explore these therapeutic possibilities and to establish the safety and efficacy of targeting IGFBP6 in human joint diseases.

In addition, we also observed that in the early stage, the content of cartilage degradation factor MMP family in SHR articular cartilage was lower than that in WKY, which seemed that the state of SHR cartilage was better than that of WKY in the early stage. On the contrary, in the late stage, the aging and degradation of SHR joints were more serious than those of WKY. We speculated whether it was premature puberty and premature aging caused by hypertension. It is known that hypertension can cause premature aging of endothelial function and accelerate vascular deterioration[242, 243]. Previous articles have found accelerated bone maturation in children with primary hypertension[244]. So, we speculate that primary hypertension accelerates joint maturation and degeneration, but this needs more research to confirm.

Chapter 5 Hypertension further aggravate already existing osteoarthritis

5.1 Introduction

Previously, we discussed that hypertension causes aging and degeneration in the joints of non-OA rats. Thus, for the joints of rats with OA, will hypertension further aggravate the damage to the joints? And will OA in turn aggravate hypertension? Therefore, in this chapter, we induced OA in WKY and SHR rats by ACLT surgery.

5.2 Materials and Methods

Animal

ASESC of the Hong Kong Polytechnic University approved all the experimental procedures (20-21/103-BME-R-GRF). 12 Male WKY rats and 12 male SHR rats of ages 6-month-old were used. We randomly selected 6 WKY and 6 SHR from 12 WKY and 12 SHR respectively for ACLT surgery, and the rest were regarded as the sham group, and only the skin was incised and sutured. All rats were divided into four groups, WKY control group, SHR control group, WKY ACLT group, SHR ACLT group, 6 rats in each group. All rats were sacrificed after two months. Knee joints, aorta and other organs and tissues were collected for histology.

Micro-CT

Following the method in Chapter 2.2, Micro-CT was utilized to measure BV/TV, Tb.Sp, Tb.N, and Tb.Th for each sample.

Histology

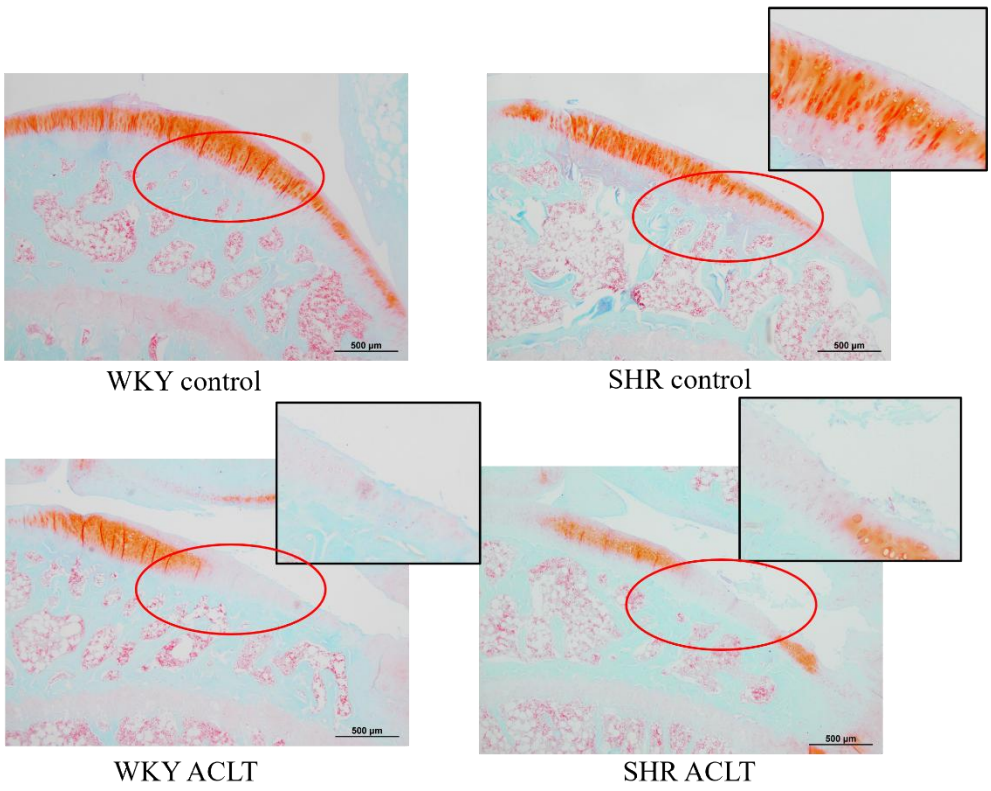
Following the method in Chapter 2.2, p16, Mmp13, Hematoxylin and Eosin staining and Safranin-O/Fast Green staining was performed

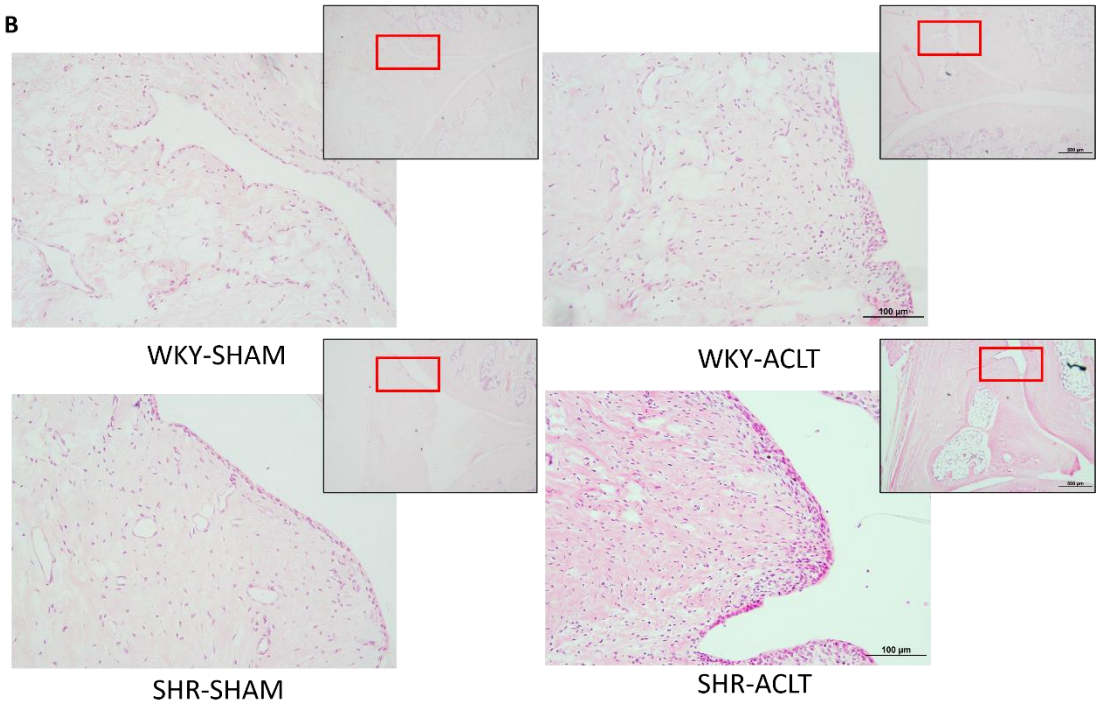
Ultrasound and Photoacoustic imaging

Using the method described in Chapter 2.2, we use ultrasound probe LZ400 to test carotid artery stiffness was assessed in SHR and WKY rats.

5.3 Figures and Results

A





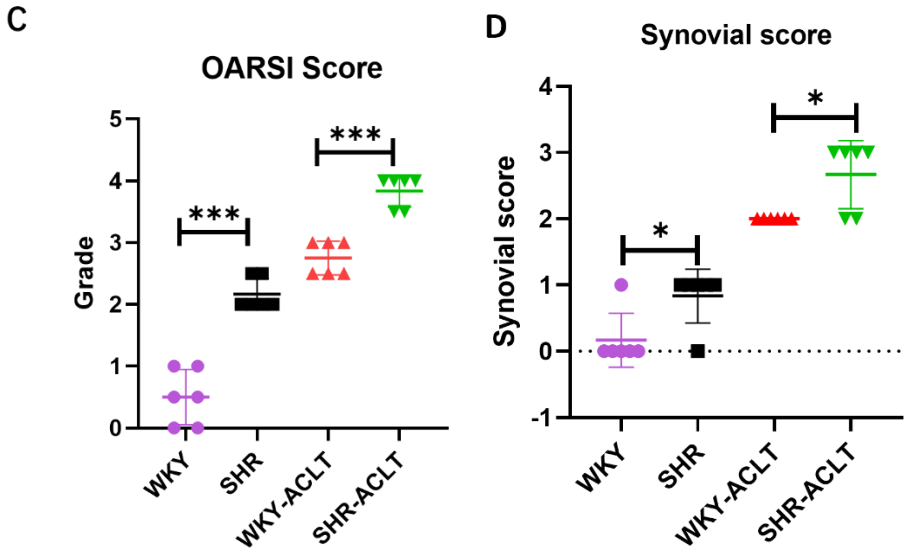
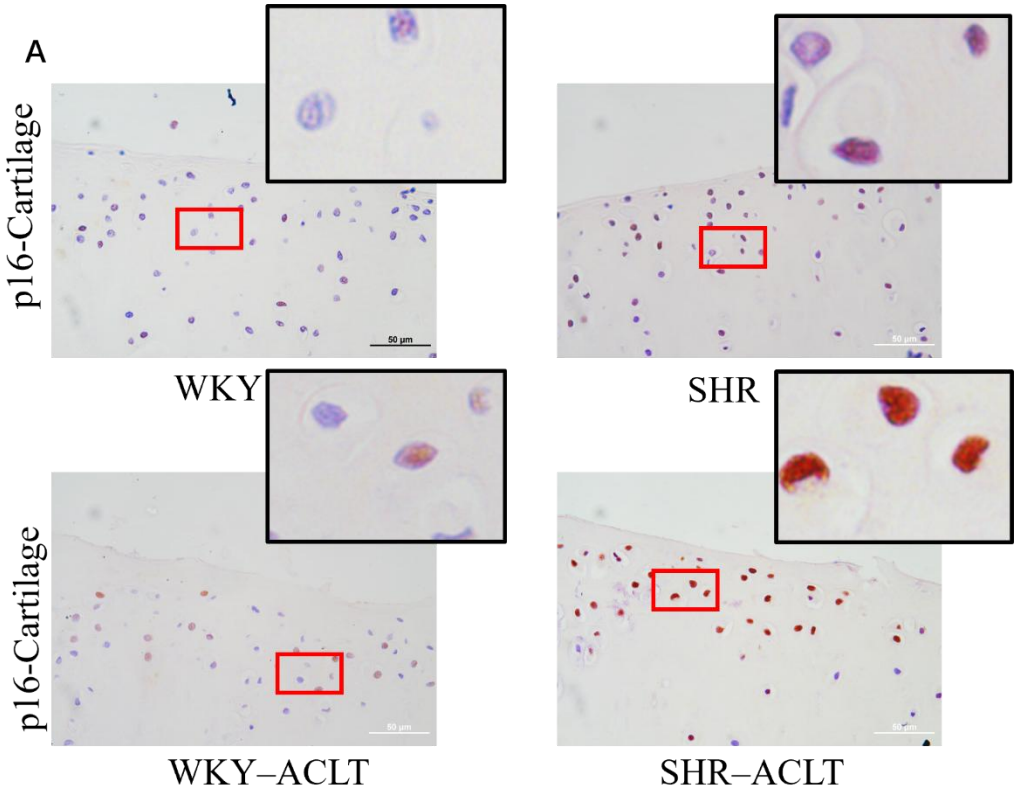
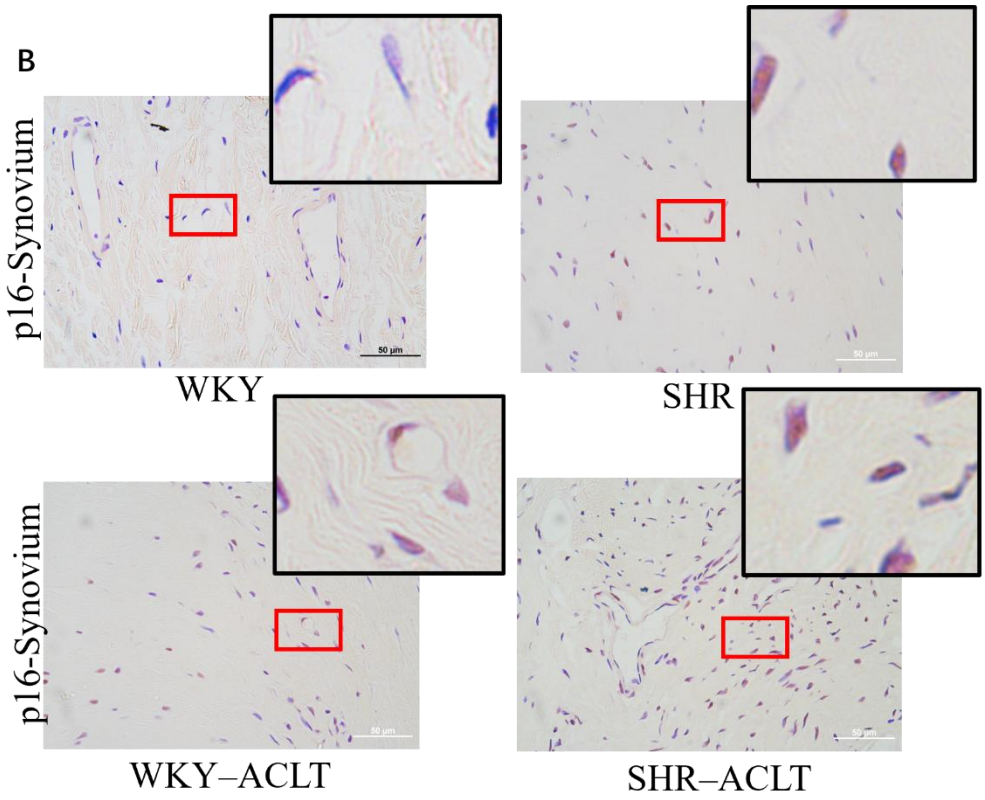
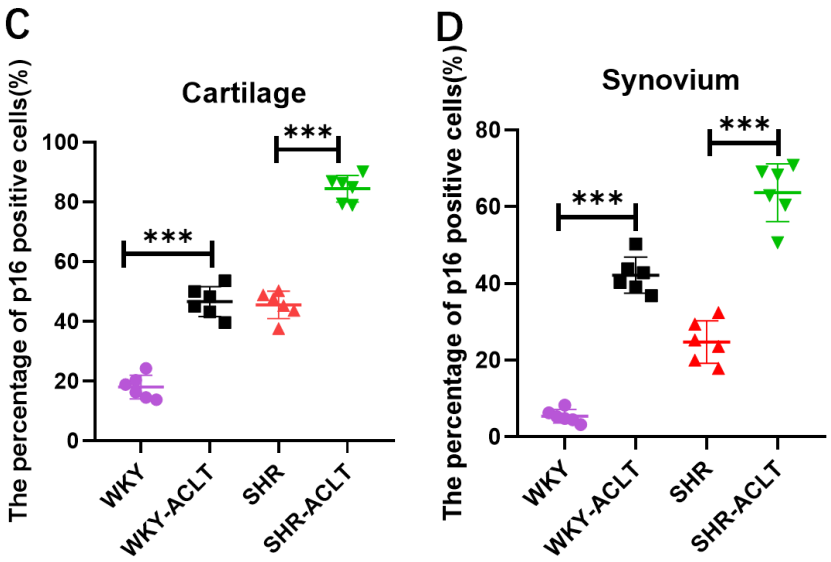


Figure 4. 1. Hypertensions aggravate osteoarthritis in cartilage and synovium. (A) Representative images of safranin-O/Fast Green staining for 9-month-old joints. (B) Representative images of hematoxylin and eosin staining for 9-month-old joints in synovium. (C) OARSI score grading system was adopted from {OARSI osteoarthritis cartilage histopathology assessment system: A biomechanical evaluation in the human

knee - Waldstein - 2016 - Journal of Orthopaedic Research - Wiley Online Library}. (D) Synovium score grading. [All data were presented as mean ± S.E.M. Two-tailed Student's t-test （unpaired） was performed for staining intensity and percentage of positively stained cells between different group. * p<0.05; ** p<0.005; *** p<0.001]




B
p16-Synovium
WKY
SHR
p16-Synovium
WKY–ACLT
SHR–ACLT


C
Cartilage
The percentage of p16 positive cells(%)
***
***
100
80
60
40
20
0
WKY
WKY-ACLT
SHR
SHR-ACLT
D
Synovium
The percentage of p16 positive cells(%)
***
***
80
60
40
20
0
WKY
WKY-ACLT
SHR
SHR-ACLT

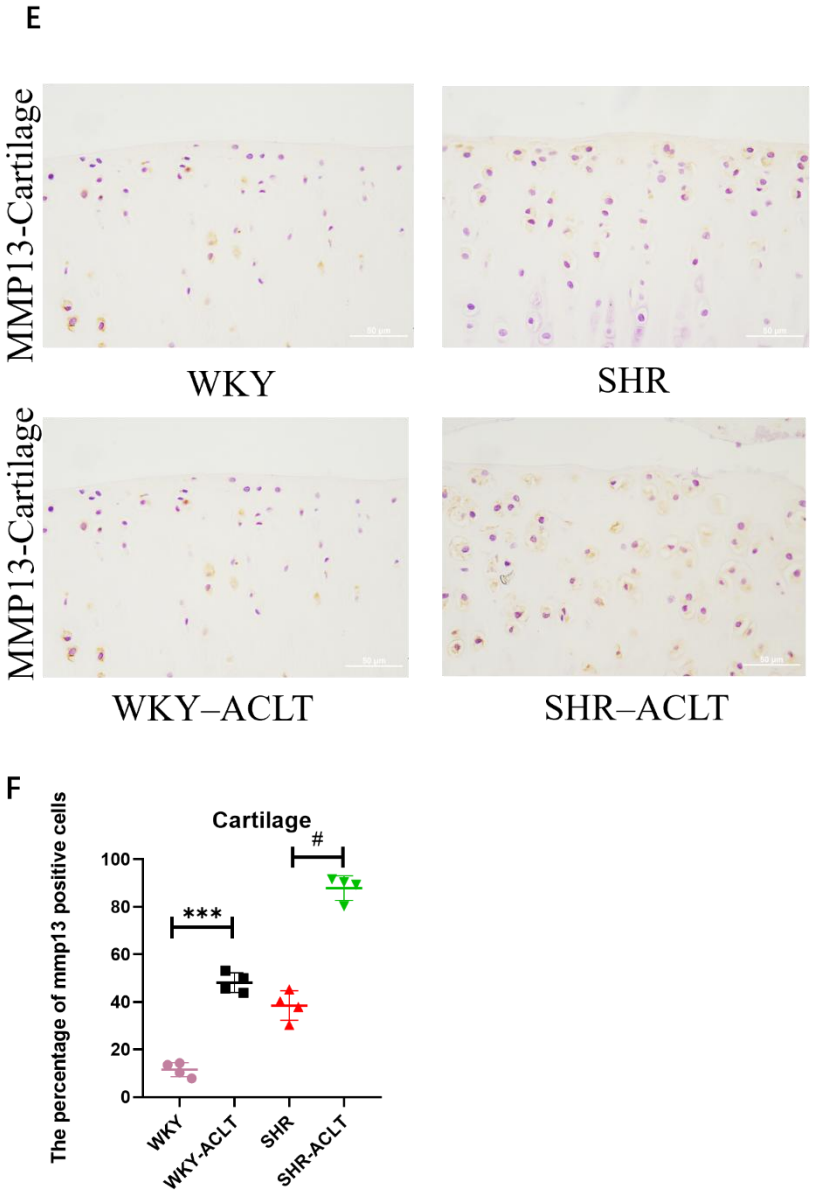


Figure 4. 2. Hypertension further aggravates the aging and degradation of cartilage and synovium in osteoarthritis. (A-D) Immunohistochemistry staining and quantification of p16ink4a in cartilage and synovium of 9-month-old rats. (E-F) Immunohistochemistry staining and quantification of MMP13 in cartilage of 9-month-old rats. [All data were presented as mean ± S.E.M. Two-tailed Student's t-test （unpaired） was performed for staining intensity and percentage of positively stained cells between different group. * $p<0.05$; ** $p<0.005$; *** $p<0.001$]

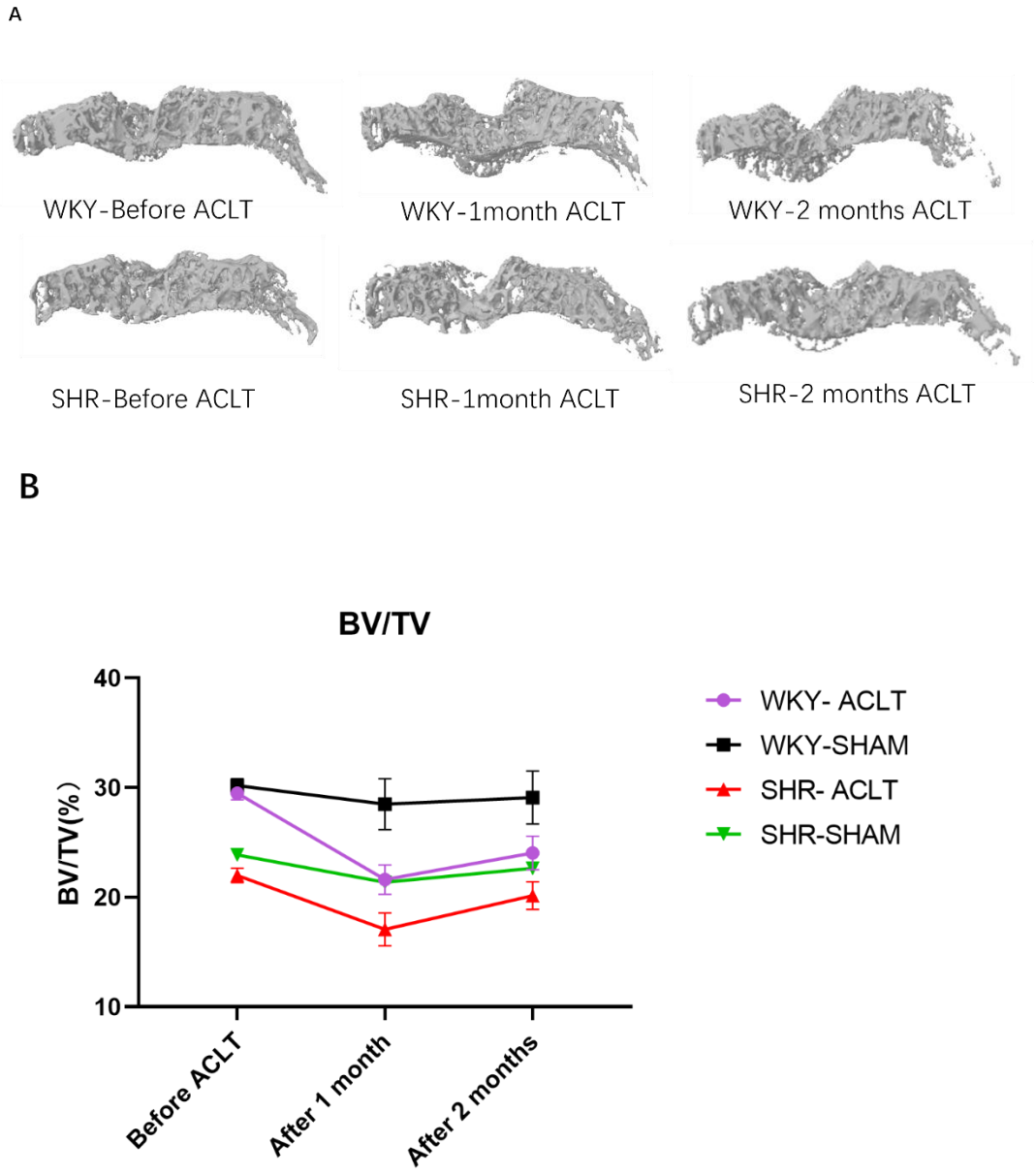


Figure 4. 3. Hypertension further exacerbates bone loss in osteoarthritis. (A-B) Representative images and quantification of Micro-CT result for subchondral bone of joint for baseline, one-month ACLT and two months ACLT. [All data were presented as mean ± S.E.M. Two-tailed Student's t-test （unpaired） was performed for staining intensity and percentage of positively stained cells between different group. * $p<0.05$; ** $p<0.005$; *** $p<0.001$]

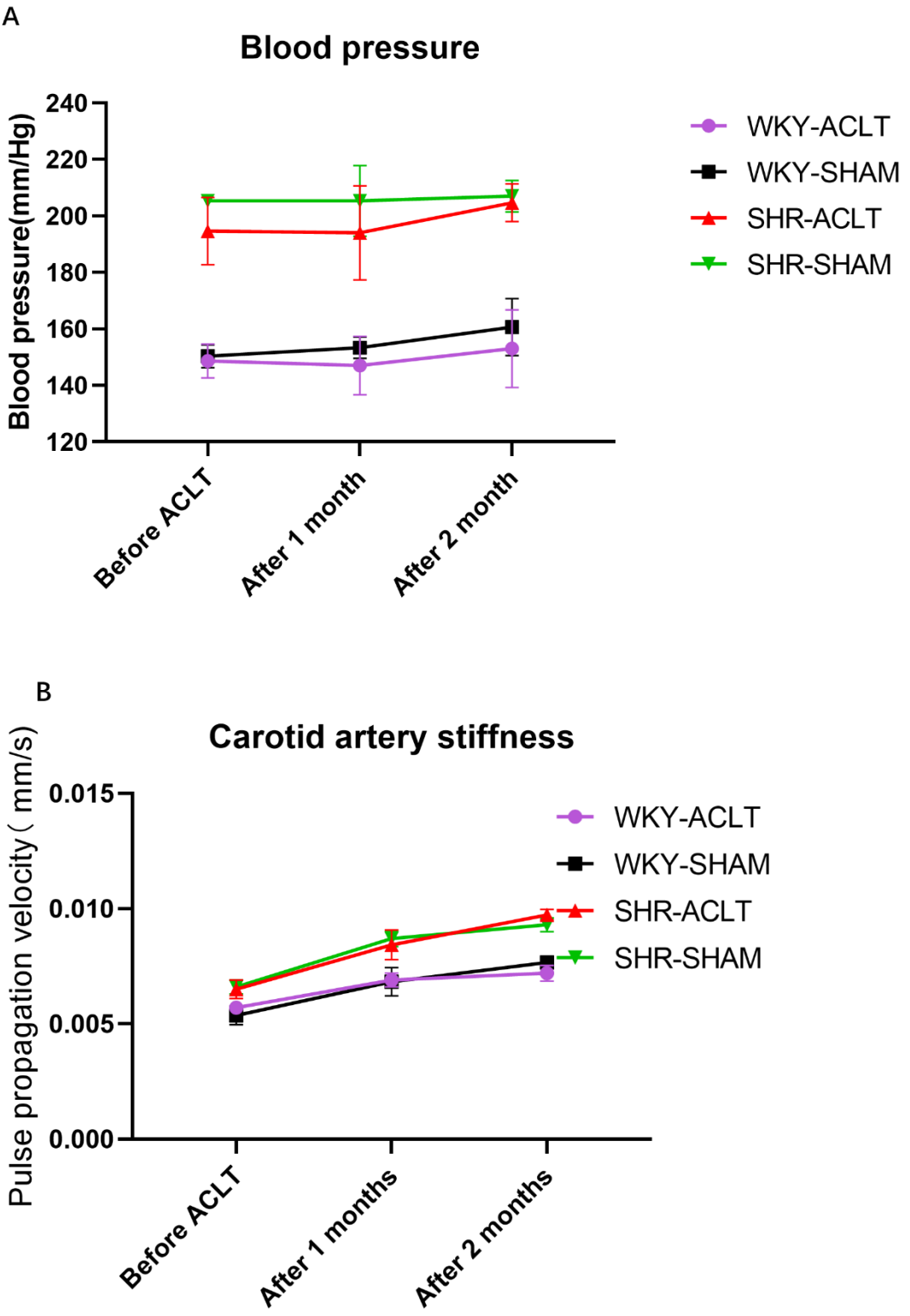


Figure 4. 4. Hypertensive rats suffering from OA will not further aggravate

hypertension. (A) Systolic blood pressure measured by tail-cuff method for baseline, one-month ACLT and two months ACLT. (B) Carotid artery stiffness for baseline, one-month ACLT and two months ACLT.

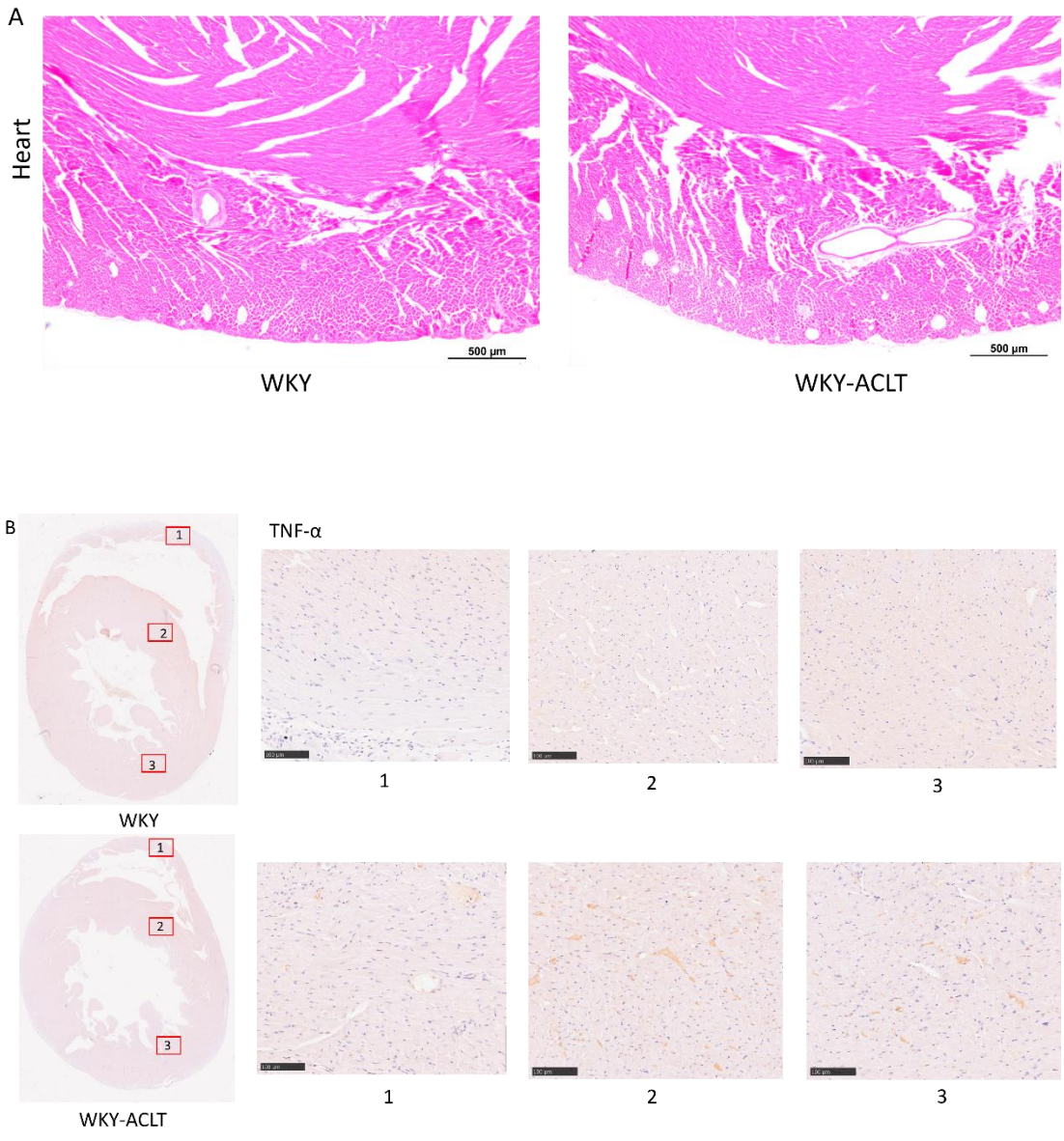


Figure 4. 5.OA aggravates inflammation in various parts of the heart. (A) Representative images of hematoxylin and eosin staining for 9-month-old heart of WKY and WKY-ACLT group. (B) Representative immunofluorescent staining of TNF-

α for 9-month-old heart of WKY and WKY-ACLT group.

Hypertensions aggravate already existing osteoarthritis

From the safranin-O/Fast Green staining and hematoxylin and eosin staining, it can be seen that for joints with existing OA, the state of both cartilage and synovium in hypertensive rats is worse than that in WKY rats. The cartilage layer is more severely damaged, and the loss of proteoglycan is more serious. At the same time, more layers of synovial cells appear in the synovium, and the cell density increases. This is also verified by the OARSI score and Synovium score. (Figure 4.1A-D). At the same time, for rats in the SHR-ACLT group, the levels of p16ink4a in cartilage and synovium were also higher than those in the WKY-ACLT group (Figure 4.2A-D). The cartilage-degrading enzyme MMP13 was also higher in the SHR-ACLT group than in the WKY-ACLT group (Figure 4.2E-F). Micro-CT results showed that the bone mass of the OA group decreased one month after surgery, and then increased in the second month, which is consistent with the decrease in early OA bone mass followed by an increase. However, during this process, the bone mass of SHR was always lower than that of WKY, whether in the surgical or non-surgical group (Figure 4.3A-B).

Hypertensive rats suffering from OA will not further aggravate hypertension,

The most intuitive manifestation of hypertension is the blood pressure value. From the blood pressure measurement results, whether it is WKY or SHR, the blood pressure difference between the surgical and non-surgical groups is not large (Figure 4.4A). At

the same time, we also measured carotid artery stiffness. Although the carotid artery stiffness of all rats increases with age, there is not much difference between the surgical and non-surgical groups (Figure 4.4B). This shows that OA has no effect on hypertension.

However, we stained the heart tissue sections and observed that the number of blood vessels in the heart was significantly increased in the WKY and WKY-ACLT groups (Figure 4.5A). We also observed that inflammatory factors also showed a significant upward trend in the heart (Figure 4.5B).

5.4 Conclusion and discussion

We found that for rats with existing OA, hypertension further aggravated OA, including more severe cartilage damage, increased senescent cells, and synovium. At the same time, the bone mass of SHR rats was always lower than that of WKY rats, both in the OA group and the non-OA group.

But in turn, OA did not further aggravate hypertension. The most important reference standard for hypertension is the level of blood pressure. There was almost no difference in blood pressure between the OA and non-OA groups, both in WKY and SHR rats. There was no significant difference in vascular stiffness before and after surgery.

However, there are many articles showing that people with OA are more likely to suffer

from cardiovascular disease[245]. An article found that among 350,000 people, the probability of cardiovascular disease increased by 24% in patients with osteoarthritis compared with those without osteoarthritis[246]. The statement that OA is associated with a 24% increased risk of any CVD event and a 42% increased risk of heart failure compared to non-OA populations is supported by a meta-analysis conducted by Hall et al. This study systematically reviewed and analyzed data from over 32 million individuals, concluding that OA patients have a significantly higher prevalence of CVD. Specifically, individuals with OA were found to be almost three times as likely to have heart failure (relative risk [RR]: 2.80; 95% confidence interval [CI]: 2.25 to 3.49) and had a 78% increased risk of ischemic heart disease (RR: 1.78; 95% CI: 1.18 to 2.69) compared to matched non-OA cohorts[247].

The connection between OA and CVD is complex and multifactorial, with several overlapping mechanisms implicated in their pathogenesis:

1. Systemic Inflammation

Chronic low-grade inflammation is a hallmark of both OA and CVD. In OA, inflammatory mediators, such as IL-6 and TNF-α, are elevated not only locally in joint tissues but also systemically. This systemic inflammation can contribute to endothelial dysfunction, atherosclerosis, and plaque instability in the cardiovascular system. Additionally, inflammation plays a critical role in arterial stiffening, a risk factor for hypertension and heart disease, suggesting a bidirectional relationship between joint and vascular health.

2. Oxidative Stress

Oxidative stress, characterized by the overproduction of reactive oxygen species (ROS), is involved in the progression of both OA and CVD. In joint tissues, ROS contributes to cartilage breakdown and synovial inflammation, accelerating OA progression. Similarly, oxidative stress is a key driver of atherosclerosis and other vascular pathologies, leading to endothelial dysfunction and promoting vascular aging. The shared oxidative stress pathways underscore the interconnected nature of OA and CVD pathogenesis.

3. Metabolic Dysregulation and Obesity

Metabolic syndrome, obesity, and insulin resistance are common comorbidities in OA and CVD patients. Adipose tissue in obese individuals produces pro-inflammatory cytokines, such as IL-6 and TNF-α, which contribute to systemic inflammation and are implicated in both OA and CVD. Furthermore, excess weight places additional mechanical stress on joints, worsening OA symptoms and limiting physical activity, which is essential for cardiovascular health. Insulin resistance, frequently observed in obese and diabetic patients, is also associated with impaired endothelial function and increased risk of CVD.

4. Vascular Dysfunction and Subchondral Bone Changes

Vascular health is crucial for maintaining joint integrity. In OA, reduced blood flow to

the subchondral bone may contribute to bone remodeling and cartilage degradation. Vascular dysfunction is also a key feature of CVD, particularly in atherosclerosis, where impaired blood flow leads to tissue ischemia. This suggests a possible link between vascular impairment in OA joints and systemic cardiovascular health, although further research is needed to clarify this connection.

5. Physical Inactivity and Reduced Mobility

Pain and stiffness in OA often lead to reduced physical activity, which is a known risk factor for CVD. Physical inactivity contributes to obesity, dyslipidemia, and hypertension, all of which are major cardiovascular risk factors. The lack of exercise not only exacerbates OA symptoms but also increases the risk of CVD, creating a cycle that further deteriorates health outcomes for OA patients.

We conducted histological staining of heart sections from both WKY and WKY-ACLT groups and observed a marked increase in inflammatory markers throughout various regions of the heart in the OA group. Although blood pressure in OA rats was not elevated compared to non-OA controls, cardiovascular inflammation was notably more pronounced. Given that our observations were made two months post-surgery, it is possible that extending the study duration might reveal even more significant cardiovascular functional impairment. This suggests that OA may contribute to cardiovascular inflammation, potentially exacerbating cardiac risk with prolonged disease progression.

Chapter 6 Conclusion，limitation and future plan

Using both a spontaneously hypertensive rat model and a drug-induced hypertensive model, we validated that hypertension leads to early-stage vascular dysfunction, characterized by increased oxidative stress, endothelial cell senescence, and elevated vascular stiffness. In later stages, joint aging, along with cartilage degradation and bone loss consistent with OA symptoms, was observed. Administration of the antihypertensive drug captopril showed improvements in both vascular and joint aging, as well as reduced oxidative stress. Subsequent human cell experiments confirmed that endothelial cell senescence further induces chondrocyte aging; however, captopril alleviates chondrocyte senescence indirectly by reducing endothelial cell aging, as it does not directly impact chondrocyte aging. Both transcriptomic and proteomic analyses indicated that the endothelial cell-chondrocyte crosstalk may be mediated by oxidative stress and IGFBP6.

1.Animal model

In our study, there are some limitations. For instance, we did not account for the influence of sex differences. In fact, hypertension incidence in younger and middle-aged individuals is typically higher in males than in females. However, postmenopausal women, particularly in older age, exhibit a marked increase in hypertension incidence, surpassing that of men. This shift is likely due to the reduction in estrogen levels following menopause. We chose male hypertensive rats for our study because, after measuring blood pressure in WKY and SHR rats from 3 to 9 months of age, we

observed a more pronounced elevation in blood pressure among male SHR rats compared to females (Supplementary Figure 1). We anticipated that higher blood pressure might lead to more pronounced osteoarthritic manifestations, which informed our decision to use male hypertensive rats. In future studies, we plan to include female SHR rats to evaluate whether sex differences influence the impact of hypertension on osteoarthritic changes in the joints.

2.Cell model

There are several limitations in our choice of cell models. We observed elevated oxidative stress in the aorta of early-stage spontaneously hypertensive rats, and later also noted increased oxidative stress in the vasculature of the synovial tissue. This finding led us to induce oxidative stress and senescence in endothelial cells using H_2O_2 in our cell model. However, hypertension is a complex, multifactorial condition that is challenging to fully replicate in a 2D cell culture model. The impact of hypertension on joints involves not only biochemical signaling pathways but also nutritional supply, biomechanical loading, and other factors that are difficult to reproduce in conventional cell cultures.

A potential solution could be the use of microfluidic chip technology to create a 3D joint model that includes vascular structures, enabling more accurate simulation of joint and vascular interactions under hypertensive conditions. However, current technology still cannot fully replicate the complex crosstalk between hypertension and joint health,

and many underlying factors remain to be explored. This emphasizes the need for further advancements in biomimetic modeling to better capture the multifaceted nature of hypertension-induced joint effects.

We have identified that IGFBP6 plays a role in the crosstalk between hypertension and joint health. However, a systematic understanding of how IGFBP6 exerts its regulatory effects in this context, including its molecular pathways, upstream and downstream signaling molecules, and detailed mechanisms of action, remains lacking. This gap represents a crucial area for future investigation. Our observations show that IGFBP6 levels increase throughout the process, raising the question of whether targeted knockout of IGFBP6 in animal models might interrupt the pathway through which endothelial cell senescence influences chondrocyte aging. This is a compelling area for further exploration to elucidate the potential of IGFBP6 as a therapeutic target in the management of hypertension-induced joint aging.

In our investigation of whether OA in rats might, in turn, exacerbate hypertension, we sacrificed the rats two months after the onset of OA. The results showed no increase in blood pressure or vascular stiffness, leading us to consider that the duration of OA might not have been sufficient for these changes to manifest. However, we did observe an increase in inflammatory markers in the heart, indicating that OA may indeed have a worsening effect on cardiovascular health. In future studies, we plan to extend the duration of OA to further examine its impact on cardiovascular disease and to explore

the underlying mechanisms driving this interaction.

3. Symptomatic OA

Until now, our discussion has primarily focused on the impact of hypertension on radiographic OA, including structural changes in joint cartilage. However, we have not yet addressed the relationship between hypertension and symptomatic OA. It is important to note that the effect of hypertension on radiographic OA remains controversial. For instance, individual-level data from 384,838 unrelated participants in the UK Biobank study indicates an inverse correlation between systolic blood pressure and the incidence of knee and hip OA[261]. This finding specifically pertains to symptomatic OA rather than radiographic OA. Additionally, a separate study involving 991 participants demonstrated that high diastolic blood pressure is associated with an increased incidence of symptomatic OA in both men and women[262]. These findings suggest that hypertension may have differential effects on radiographic versus symptomatic OA, warranting further research to clarify the mechanisms and clinical implications of these associations.

Along with another researcher, Yvette, we conducted a series of basic behavioral tests on rats divided into four groups: WKY, SHR, WKY-ACLT, and SHR-ACLT. These tests included the pain sensitivity assessment, specifically using the Von Frey test. Our results indicated differential pain responses following ACLT surgery between the WKY and SHR groups. Post-surgery, WKY rats exhibited heightened sensitivity to pain,

whereas SHR rats displayed a notably reduced sensitivity to pain (Supplementary Figure 2). Additionally, we observed delayed wound healing in the SHR group. In response, Yvette extracted the dorsal root ganglion (DRG) from both SHR and WKY rats for RNA sequencing analysis. Our findings revealed significantly lower levels of calcitonin gene-related peptide (CGRP) in the DRG of SHR rats compared to WKY rats (Supplementary Figure 3). This discrepancy in CGRP levels may contribute to the observed differences in pain perception and wound healing between the two strains following ACLT surgery.

CGRP is a neuropeptide with vasodilatory and pro-inflammatory properties, widely studied for its role in migraine and other pain conditions. However, recent research has also implicated CGRP in joint pain and inflammation associated with OA. CGRP is released from primary sensory neurons within joint tissues and has been shown to modulate inflammation, pain perception, and cartilage degeneration, positioning it as a promising therapeutic target in OA.

1. CGRP Structure, Function, and Distribution

1.1 Structure and Expression of CGRP

CGRP is a 37-amino acid neuropeptide derived from the calcitonin gene. It exists in two isoforms, α-CGRP and β-CGRP, with α-CGRP predominantly found in sensory neurons and β-CGRP primarily in enteric neurons. In the context of OA, α-CGRP is of particular interest due to its localization in dorsal root ganglia (DRG) and peripheral

sensory nerves that innervate joint tissues.

1.2 CGRP in the Nervous System and Joints

CGRP is widely expressed in the peripheral nervous system, particularly in the DRG and sensory nerve endings that innervate synovium, subchondral bone, and periarticular tissues[263]. In response to nociceptive stimuli, CGRP is released and acts on its receptor complex, comprising the calcitonin receptor-like receptor (CLR) and receptor activity-modifying protein 1 (RAMP1). Through this signaling pathway, CGRP influences pain transmission, blood flow, and inflammatory responses in joint tissues, linking it directly to OA-related pain and inflammation.

2. CGRP and Pain Perception in Osteoarthritis

2.1 Role of CGRP in Nociception and Hyperalgesia

One of CGRP's primary roles in OA is its involvement in pain signaling. CGRP is released from sensory neurons in response to mechanical stress and inflammation within the joint. It sensitizes nociceptors and enhances pain perception, contributing to hyperalgesia—a heightened sensitivity to pain. Studies have shown elevated levels of CGRP in synovial fluid and DRG neurons of OA patients, suggesting its role in OA-related pain[264].

2.2 Mechanisms of CGRP-Mediated Pain in OA

CGRP exerts its nociceptive effects by increasing the excitability of pain-sensing

neurons. In OA, cartilage degradation and inflammation release factors that activate sensory nerves, leading to increased CGRP release[265]. CGRP then binds to receptors on pain neurons, amplifying pain signals transmitted to the central nervous system. This process underlies the chronic and sometimes severe pain experienced by OA patients. Blocking CGRP or its receptor has shown efficacy in reducing pain in animal models of OA, supporting its potential as a therapeutic target for OA pain.

3. CGRP and Joint Inflammation in Osteoarthritis

3.1 Pro-inflammatory Effects of CGRP in Joint Tissues

CGRP not only modulates pain but also has significant effects on joint inflammation. In OA, CGRP is involved in inflammatory processes within the synovium and subchondral bone. CGRP can stimulate synovial cells to release pro-inflammatory cytokines such as IL-6 and TNF-α, exacerbating synovial inflammation and joint degeneration[266]. The elevated inflammatory environment contributes to a vicious cycle of pain and tissue damage, accelerating OA progression.

3.2 CGRP's Role in Immune Cell Modulation

CGRP has been shown to influence immune cell activity, particularly in regulating macrophages and T cells within joint tissues[267]. In OA, CGRP promotes a pro-inflammatory phenotype in macrophages, leading to the release of factors that damage cartilage and promote bone resorption. Additionally, CGRP modulates T cell responses, skewing the immune response towards inflammation and away from tissue repair.

These findings highlight CGRP's multifaceted role in amplifying inflammation within OA-affected joints.

4. CGRP and Cartilage Degradation

4.1 Impact of CGRP on Chondrocyte Activity

Chondrocytes, the primary cells within cartilage, play a crucial role in maintaining the extracellular matrix. CGRP has been shown to influence chondrocyte behavior, particularly under inflammatory conditions[263]. In OA, elevated CGRP levels contribute to increased MMP production by chondrocytes, leading to accelerated cartilage breakdown. Additionally, CGRP suppresses the anabolic activity of chondrocytes, hindering cartilage repair processes and perpetuating joint degeneration.

4.2 Effects on Subchondral Bone and Osteophyte Formation

CGRP also impacts subchondral bone remodeling, which is a key feature of OA progression. CGRP promotes osteoclast differentiation and activity, leading to increased bone resorption in the subchondral region. The increased bone turnover contributes to osteophyte formation and subchondral sclerosis, both of which are associated with advanced OA and increased joint pain. Thus, CGRP plays a direct role in the structural changes observed in OA joints.

5. Therapeutic Implications of Targeting CGRP in Osteoarthritis

5.1 CGRP Antagonists for Pain Relief

Given CGRP's role in pain signaling, CGRP antagonists have emerged as promising therapeutic agents for OA-related pain. Several CGRP receptor antagonists, originally developed for migraine treatment, are being explored for their potential in OA. These antagonists inhibit CGRP binding to its receptor, reducing nociceptive signaling and offering relief from chronic pain. Preclinical studies have demonstrated that CGRP antagonists can significantly reduce pain behavior in OA models, suggesting a potential application in OA management[268].

5.2 Anti-CGRP Therapy for Modulating Inflammation

Beyond pain relief, anti-CGRP therapies may also reduce inflammation within OA joints. By blocking CGRP's effects on synovial and immune cells, these therapies could attenuate the pro-inflammatory environment in OA joints, potentially slowing disease progression. Future research is needed to confirm whether CGRP antagonists can effectively modulate joint inflammation in clinical settings.

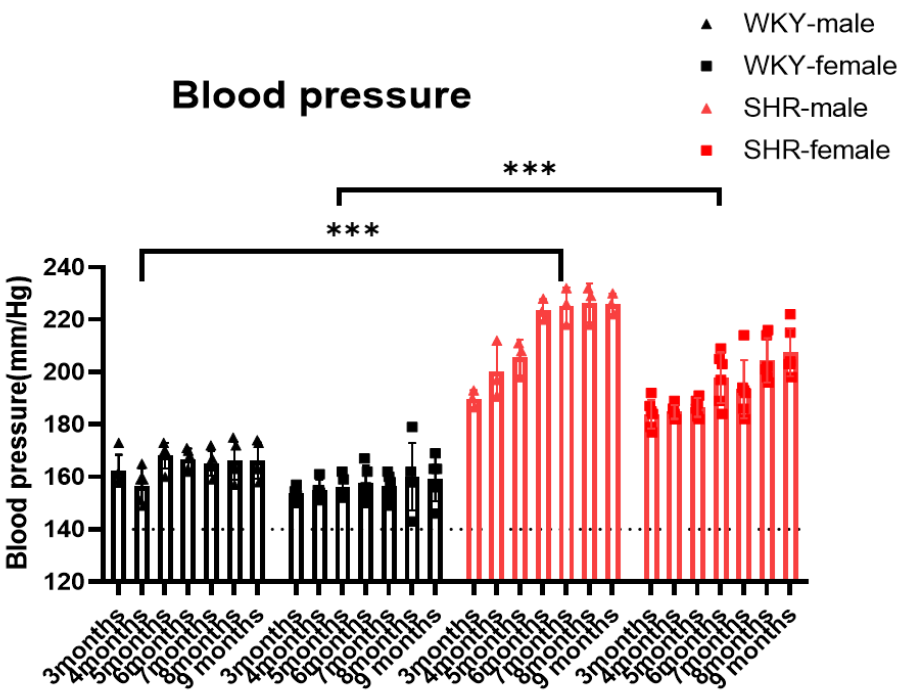
5.3 Combination Therapy with Current OA Treatments

The integration of CGRP-targeted therapies with current OA treatments, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and physical therapy, may provide a more comprehensive approach to managing OA. CGRP antagonists could be particularly beneficial for OA patients with severe pain and inflammation that are unresponsive to standard treatments. This multimodal approach may help alleviate pain, reduce inflammation, and protect joint structure, improving quality of life for OA

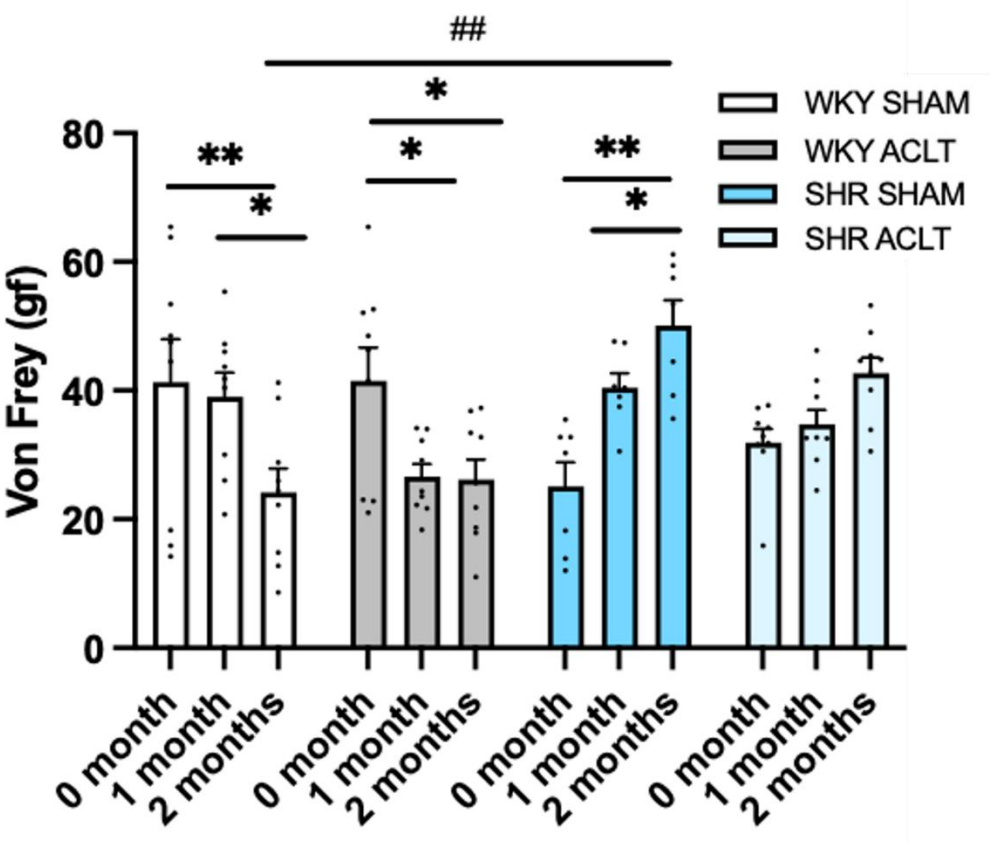
patients.

In summary, the deficiency of CGRP observed in SHR rats may be a critical factor in the impact of hypertension on symptomatic OA. In future experiments, we plan to locally administer CGRP within the joints of SHR rats to examine whether pain sensitivity in hypertensive rats can be restored to levels similar to those of normotensive WKY rats. Additionally, we aim to assess whether joint cartilage dysfunction can be alleviated through this intervention. Given CGRP's short half-life, we are concerned that direct intraperitoneal injection may not produce a sustained effect on joint tissues. Therefore, we plan to develop a nanomaterial-based delivery system to encapsulate CGRP, allowing for its controlled and gradual release within the joint. This approach is intended to enhance the local efficacy of CGRP, potentially providing a more reliable and sustained therapeutic effect for managing pain and preserving cartilage function in hypertensive OA models

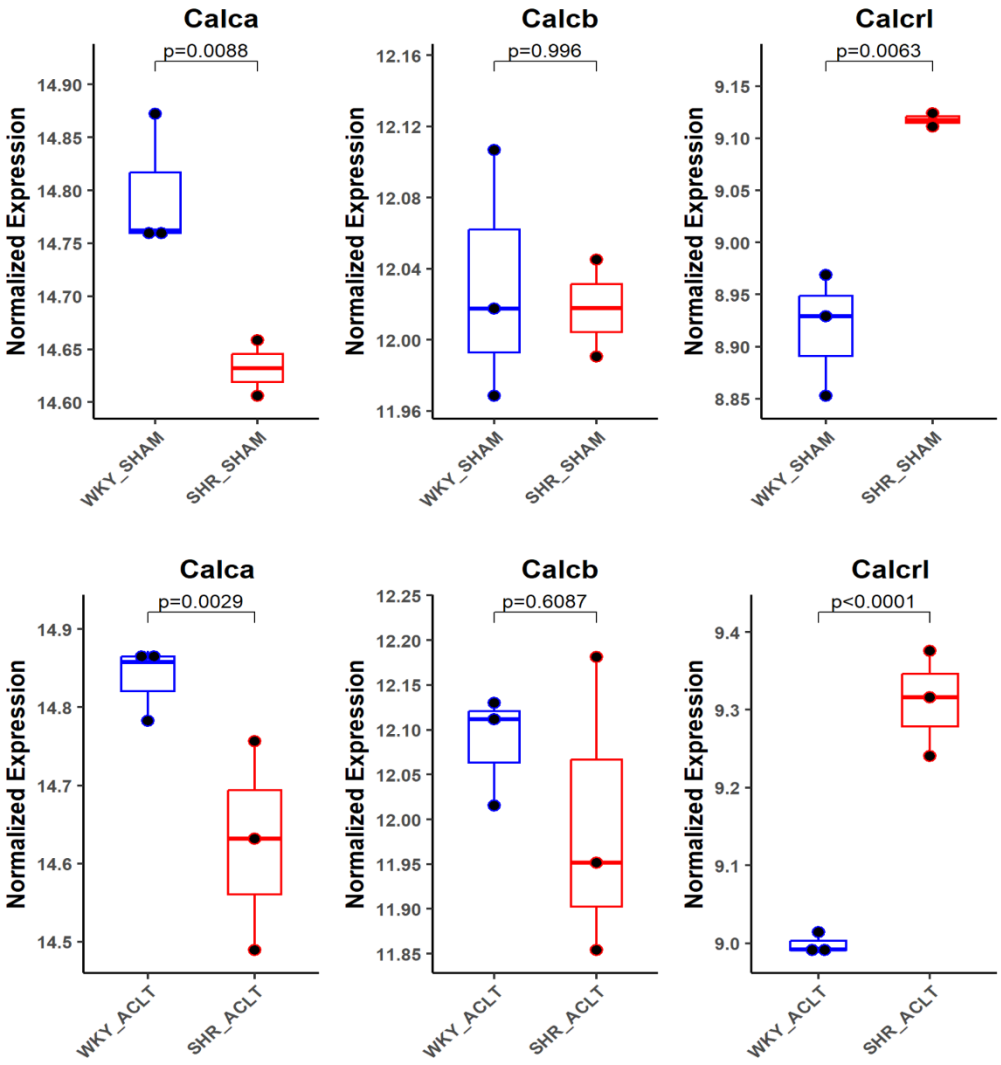
Supplementary Figure



Supplementary Figure 1. Blood pressure in male and female WKY and SHR rats aged 3-9 months.



Supplementary Figure 2. Von Frey test result for WKY, SHR, WKY-ACLT and SHR-ACLT group after ACLT surgery from baseline to 2 months.



Supplementary Figure 3. RNA-Seq result of CGRP for WKY, SHR, WKY-ACLT and SHR-ACLT group.

Chapter 7 Significance

Our findings provide experimental evidence that hypertension contributes to joint dysfunction, including symptoms such as chondrocyte senescence and degradation. Specifically, vascular dysfunction induces cartilage aging through biochemical crosstalk. In brief, this study opens new avenues for OA management strategies by demonstrating the essential role of vascular health in maintaining cartilage homeostasis. We also highlighted the anti-senescent effects of antihypertensive drugs on endothelial cells and their subsequent protective impact on chondrocytes. Our investigation into vascular-joint crosstalk supports the concept of addressing localized joint dysfunction through systemic therapies, laying a foundation for advancements in OA management.

Furthermore, we observed that hypertension exacerbates OA pathology in rats with pre-existing OA, while OA, in turn, intensifies cardiovascular inflammation. This bidirectional influence between hypertension and OA offers compelling evidence for the reciprocal impact of these conditions on each other's progression. These insights emphasize the need for an integrated approach to managing both cardiovascular and joint health in patients affected by these interconnected diseases.

Reference

1. Organization, W.H. *Osteoarthritis*. 2023; Available from: https://www.who.int/news-room/fact-sheets/detail/osteoarthritis.
2. Disease, G.B.o. *Explore result from the 2019 Global Burden of Disease*. 2019; Available from: https://vizhub.healthdata.org/gbd-results/.
3. China, N.H.C.o.t.P.s.R.o. *World Arthritis Day: Pay attention to joint health*. 2014; Available from: http://www.nhc.gov.cn/gjyr/mtbd/201410/3c35619fc52c4df1b13c4551c6fded75.shtml.
4. Li, D., et al., *The Prevalence of Symptomatic Knee Osteoarthritis in Relation to Age, Sex, Area, Region, and Body Mass Index in China: A Systematic Review and Meta-Analysis.* Front Med (Lausanne), 2020. **7**: p. 304.
5. Jevsevar, D.S., et al., *The American Academy of Orthopaedic Surgeons Evidence-Based Guideline on: Treatment of Osteoarthritis of the Knee, 2nd Edition.* JBJS, 2013. **95**(20).
6. Initiative, U.S.B.a.J. *The Burden of Musculoskeletal Diseases in the United States (BMUS).* 2018; Available from: https://www.boneandjointburden.org/fourth-edition.
7. Liu, S., et al., *Burden of Cardiovascular Diseases in China, 1990-2016: Findings From the 2016 Global Burden of Disease Study.* JAMA Cardiol, 2019. **4**(4): p. 342-352.
8. X.R., G. *How Many Artificial Knee and Hip Replacement Surgeries Did China Do in 2018? What Is the Amount?* accessed on 1 December 2020; Available from: https://www.haodf.com/zhuanjiaguandian/gaoxurendr_7591652124.htm
9. Valdes, A.M. and T.D. Spector, *Genetic epidemiology of hip and knee osteoarthritis.* Nature Reviews Rheumatology, 2011. **7**(1): p. 23-32.
10. Felson, D.T., et al., *Osteoarthritis: new insights. Part 1: the disease and its risk factors.* Ann Intern Med, 2000. **133**(8): p. 635-46.
11. Mei, Y., et al., *Roles of Hormone Replacement Therapy and Menopause on Osteoarthritis and Cardiovascular Disease Outcomes: A Narrative Review.* Front Rehabil Sci, 2022. **3**: p. 825147.
12. Cooper, C., et al., *Risk factors for the incidence and progression of radiographic knee osteoarthritis.* Arthritis Rheum, 2000. **43**(5): p. 995-1000.
13. Kapoor, M., et al., *Role of proinflammatory cytokines in the pathophysiology of osteoarthritis.* Nat Rev Rheumatol, 2011. **7**(1): p. 33-42.
14. Hunter, D.J. and S. Bierma-Zeinstra, *Osteoarthritis.* Lancet, 2019. **393**(10182): p. 1745-1759.
15. Alberti, K.G., et al., *Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity.* Circulation, 2009. **120**(16): p. 1640-5.
16. Berenbaum, F., *Diabetes-induced osteoarthritis: from a new paradigm to a new phenotype.* Ann Rheum Dis, 2011. **70**(8): p. 1354-6.
17. Wei, G., et al., *Risk of metabolic abnormalities in osteoarthritis: a new perspective to understand its pathological mechanisms.* Bone Research, 2023. **11**(1): p. 63.
18. Collins, K.H., et al., *Adipose tissue is a critical regulator of osteoarthritis.* Proc Natl Acad

Sci U S A, 2021. **118**(1).
19. Thijssen, E., A. van Caam, and P.M. van der Kraan, *Obesity and osteoarthritis, more than just wear and tear: pivotal roles for inflamed adipose tissue and dyslipidaemia in obesity-induced osteoarthritis.* Rheumatology (Oxford), 2015. **54**(4): p. 588-600.
20. Wang, T. and C. He, *Pro-inflammatory cytokines: The link between obesity and osteoarthritis.* Cytokine Growth Factor Rev, 2018. **44**: p. 38-50.
21. González, P., et al., *Hyperglycemia and Oxidative Stress: An Integral, Updated and Critical Overview of Their Metabolic Interconnections.* Int J Mol Sci, 2023. **24**(11).
22. Li, Y., et al., *The age-related changes in cartilage and osteoarthritis.* Biomed Res Int, 2013. **2013**: p. 916530.
23. Magnusson, K., et al., *Shared genetic factors between osteoarthritis and cardiovascular disease may underlie common etiology.* Nature Communications, 2024. **15**(1): p. 9569.
24. Wang, H., et al., *Osteoarthritis and the risk of cardiovascular disease: a meta-analysis of observational studies.* Scientific Reports, 2016. **6**(1): p. 39672.
25. Loeser, R.F., J.A. Collins, and B.O. Diekman, *Ageing and the pathogenesis of osteoarthritis.* Nat Rev Rheumatol, 2016. **12**(7): p. 412-20.
26. Sewell, J., et al., *Association between arthritis and cardiovascular risk factors in community-based adults: an opportunity to target cardiovascular risk.* BMC Cardiovascular Disorders, 2022. **22**(1): p. 232.
27. Fernandes, G.S. and A.M. Valdes, *Cardiovascular disease and osteoarthritis: common pathways and patient outcomes.* Eur J Clin Invest, 2015. **45**(4): p. 405-14.
28. Constantino de Campos, G., et al., *Osteoarthritis, mobility-related comorbidities and mortality: an overview of meta-analyses.* Ther Adv Musculoskelet Dis, 2020. **12**: p. 1759720x20981219.
29. Kelwick, R., et al., *The ADAMTS (A Disintegrin and Metalloproteinase with Thrombospondin motifs) family.* Genome Biology, 2015. **16**(1): p. 113.
30. Li, G., et al., *Subchondral bone in osteoarthritis: insight into risk factors and microstructural changes.* Arthritis Res Ther, 2013. **15**(6): p. 223.
31. Gao, L., M. Cucchiarini, and H. Madry, *Cyst formation in the subchondral bone following cartilage repair.* Clin Transl Med, 2020. **10**(8): p. e248.
32. Sanchez-Lopez, E., et al., *Synovial inflammation in osteoarthritis progression.* Nat Rev Rheumatol, 2022. **18**(5): p. 258-275.
33. Wu, C.L., et al., *The role of macrophages in osteoarthritis and cartilage repair.* Osteoarthritis and Cartilage, 2020. **28**(5): p. 544-554.
34. Zhen, G., et al., *Mechanical stress determines the configuration of TGFβ activation in articular cartilage.* Nat Commun, 2021. **12**(1): p. 1706.
35. Scanzello, C.R. and S.R. Goldring, *The role of synovitis in osteoarthritis pathogenesis.* Bone, 2012. **51**(2): p. 249-57.
36. Zhen, G. and X. Cao, *Targeting TGFβ signaling in subchondral bone and articular cartilage homeostasis.* Trends Pharmacol Sci, 2014. **35**(5): p. 227-36.
37. Fan, W.J., et al., *Exosomes in osteoarthritis: Updated insights on pathogenesis, diagnosis, and treatment.* Front Cell Dev Biol, 2022. **10**: p. 949690.
38. Lisowska, B., A. Lisowski, and K. Siewruk, *Substance P and Chronic Pain in Patients with Chronic Inflammation of Connective Tissue.* PLoS One, 2015. **10**(10): p. e0139206.

39. Charlier, E., et al., *Chondrocyte dedifferentiation and osteoarthritis (OA).* Biochem Pharmacol, 2019. **165**: p. 49-65.
40. Rim, Y.A., Y. Nam, and J.H. Ju, *The Role of Chondrocyte Hypertrophy and Senescence in Osteoarthritis Initiation and Progression.* Int J Mol Sci, 2020. **21**(7).
41. Cambier, S., M. Gouwy, and P. Proost, *The chemokines CXCL8 and CXCL12: molecular and functional properties, role in disease and efforts towards pharmacological intervention.* Cellular & Molecular Immunology, 2023. **20**(3): p. 217-251.
42. Li, Y.S., et al., *T Cells in Osteoarthritis: Alterations and Beyond.* Front Immunol, 2017. **8**: p. 356.
43. Maruotti, N., A. Corrado, and F.P. Cantatore, *Osteoblast role in osteoarthritis pathogenesis.* J Cell Physiol, 2017. **232**(11): p. 2957-2963.
44. Luo, P., et al., *Effects of Immune Cells and Cytokines on Different Cells in OA.* J Inflamm Res, 2023. **16**: p. 2329-2343.
45. Wen, C., et al., *Insulin-like growth factor-1 in articular cartilage repair for osteoarthritis treatment.* Arthritis Res Ther, 2021. **23**(1): p. 277.
46. Martel-Pelletier, J., et al., *IGF/IGFBP axis in cartilage and bone in osteoarthritis pathogenesis.* Inflamm Res, 1998. **47**(3): p. 90-100.
47. Dixit, M., S.B. Poudel, and S. Yakar, *Effects of GH/IGF axis on bone and cartilage.* Mol Cell Endocrinol, 2021. **519**: p. 111052.
48. Wen, C., et al., *Insulin-like growth factor-1 in articular cartilage repair for osteoarthritis treatment.* Arthritis Research & Therapy, 2021. **23**(1): p. 277.
49. Olney, R.C., et al., *Chondrocytes from osteoarthritic cartilage have increased expression of insulin-like growth factor I (IGF-I) and IGF-binding protein-3 (IGFBP-3) and -5, but not IGF-II or IGFBP-4.* The Journal of clinical endocrinology and metabolism, 1996. **81 3**: p. 1096-103.
50. Alunno, A., et al., *Insulin-Like Growth Factor Binding Protein 6 in Rheumatoid Arthritis: A Possible Novel Chemotactic Factor?* Front Immunol, 2017. **8**: p. 554.
51. Fortier, L.A., et al., *Insulin-like growth factor-I enhances cell-based repair of articular cartilage.* J Bone Joint Surg Br, 2002. **84**(2): p. 276-88.
52. Loeser, R.F., *Aging and osteoarthritis: the role of chondrocyte senescence and aging changes in the cartilage matrix.* Osteoarthritis Cartilage, 2009. **17**(8): p. 971-9.
53. Xu, X., et al., *Up-regulation of IGF2BP2 by multiple mechanisms in pancreatic cancer promotes cancer proliferation by activating the PI3K/Akt signaling pathway.* J Exp Clin Cancer Res, 2019. **38**(1): p. 497.
54. Grimberg, A., et al., *IGFBP-3 mediates p53-induced apoptosis during serum starvation.* Int J Oncol, 2002. **21**(2): p. 327-35.
55. Schedlich, L.J., et al., *Molecular basis of the interaction between IGFBP-3 and retinoid X receptor: Role in modulation of RAR-signaling.* Archives of Biochemistry and Biophysics, 2007. **465**(2): p. 359-369.
56. Kwon, A., et al., *Insulin-like growth factor binding protein-3 induces senescence by inhibiting telomerase activity in MCF-7 breast cancer cells.* Sci Rep, 2023. **13**(1): p. 8739.
57. Dya, G.A., et al., *Specific cleavage of IGFBP-4 by papp-a in nervous tissue.* Biochemical and Biophysical Research Communications, 2024. **733**: p. 150655.
58. Sanada, F., et al., *IGF Binding Protein-5 Induces Cell Senescence.* Front Endocrinol

(Lausanne), 2018. **9**: p. 53.
59. Sureshbabu, A., et al., *Relative Roles of TGF-β and IGFBP-5 in Idiopathic Pulmonary Fibrosis.* Pulm Med, 2011. **2011**: p. 517687.
60. Evdokimova, V., et al., *IGFBP7 binds to the IGF-1 receptor and blocks its activation by insulin-like growth factors.* Sci Signal, 2012. **5**(255): p. ra92.
61. Siraj, Y., et al., *IGFBP7 is a key component of the senescence-associated secretory phenotype (SASP) that induces senescence in healthy cells by modulating the insulin, IGF, and activin A pathways.* Cell Commun Signal, 2024. **22**(1): p. 540.
62. Zhou, Y., et al., *Wnt/β-catenin Signaling in Osteoarthritis and in Other Forms of Arthritis.* Curr Rheumatol Rep, 2017. **19**(9): p. 53.
63. Clevers, H. and R. Nusse, *Wnt/β-catenin signaling and disease.* Cell, 2012. **149**(6): p. 1192-205.
64. Lories, R.J. and F.P. Luyten, *The bone-cartilage unit in osteoarthritis.* Nat Rev Rheumatol, 2011. **7**(1): p. 43-9.
65. Escribano-Núñez, A., et al., *IGF1 drives Wnt-induced joint damage and is a potential therapeutic target for osteoarthritis.* Nature Communications, 2024. **15**(1): p. 9170.
66. Chen, S., et al., *Wnt/β-catenin signaling pathway promotes abnormal activation of fibroblast-like synoviocytes and angiogenesis in rheumatoid arthritis and the intervention of Er Miao San.* Phytomedicine, 2023. **120**: p. 155064.
67. Choi, M.C., et al., *NF-κB Signaling Pathways in Osteoarthritic Cartilage Destruction.* Cells, 2019. **8**(7).
68. Bondeson, J., et al., *The role of synovial macrophages and macrophage-produced cytokines in driving aggrecanases, matrix metalloproteinases, and other destructive and inflammatory responses in osteoarthritis.* Arthritis Research & Therapy, 2006. **8**(6): p. R187.
69. Boyce, B.F., Z. Yao, and L. Xing, *Functions of nuclear factor kappaB in bone.* Ann N Y Acad Sci, 2010. **1192**: p. 367-75.
70. Wang, W., D. Rigueur, and K.M. Lyons, *TGFβ signaling in cartilage development and maintenance.* Birth Defects Res C Embryo Today, 2014. **102**(1): p. 37-51.
71. Henrotin, Y., B. Kurz, and T. Aigner, *Oxygen and reactive oxygen species in cartilage degradation: friends or foes?* Osteoarthritis and Cartilage, 2005. **13**(8): p. 643-654.
72. Zahan, O.M., et al., *The evaluation of oxidative stress in osteoarthritis.* Med Pharm Rep, 2020. **93**(1): p. 12-22.
73. Renaudin, F., et al., *NADPH oxidase 4 deficiency attenuates experimental osteoarthritis in mice.* RMD Open, 2023. **9**(1).
74. Wang, X., W.M. Oo, and J.M. Linklater, *What is the role of imaging in the clinical diagnosis of osteoarthritis and disease management?* Rheumatology, 2018. **57**(suppl_4): p. iv51-iv60.
75. Chien, A., et al., *Magnetic resonance imaging of the knee.* Pol J Radiol, 2020. **85**: p. e509-e531.
76. Sarzi-Puttini, P., et al., *Osteoarthritis: An Overview of the Disease and Its Treatment Strategies.* Seminars in Arthritis and Rheumatism, 2005. **35**(1, Supplement 1): p. 1-10.
77. Organization, W.H. *Hypertension*. 2023; Available from: https://www.who.int/news-room/fact-sheets/detail/hypertension.
78. baike.baidu. *Hypertension*. 2024; Available from:

https://baike.baidu.com/item/%E9%AB%98%E8%A1%80%E5%8E%8B/195863.
79. Office of Policy, P., and Evaluation. *Health Topics-High Blood Pressure*. 2024; Available from: https://www.cdc.gov/policy/polaris/healthtopics/highbloodpressure/index.html.
80. Wang, Y. and J.G. Wang, *Genome-Wide Association Studies of Hypertension and Several Other Cardiovascular Diseases*. Pulse (Basel), 2019. **6**(3-4): p. 169-186.
81. Weir, M.R. and V.J. Dzau, *The renin-angiotensin-aldosterone system: a specific target for hypertension management.* American Journal of Hypertension, 1999. **12**(S9): p. 205S-213S.
82. Triposkiadis, F., et al. *The Sympathetic Nervous System in Hypertensive Heart Failure with Preserved LVEF*. Journal of Clinical Medicine, 2023. **12**, DOI: 10.3390/jcm12206486.
83. Drożdż, D., M. Drożdż, and M. Wójcik, *Endothelial dysfunction as a factor leading to arterial hypertension*. Pediatric Nephrology, 2023. **38**(9): p. 2973-2985.
84. Hall, J.E., *Renal Dysfunction, Rather Than Nonrenal Vascular Dysfunction, Mediates Salt-Induced Hypertension.* Circulation, 2016. **133**(9): p. 894-906.
85. Schulz, E., T. Gori, and T. Münzel, *Oxidative stress and endothelial dysfunction in hypertension.* Hypertension Research, 2011. **34**(6): p. 665-673.
86. Tran, N., et al., *Endothelial Nitric Oxide Synthase (eNOS) and the Cardiovascular System: in Physiology and in Disease States*. Am J Biomed Sci Res, 2022. **15**(2): p. 153-177.
87. Kostov, K., *The Causal Relationship between Endothelin-1 and Hypertension: Focusing on Endothelial Dysfunction, Arterial Stiffness, Vascular Remodeling, and Blood Pressure Regulation*. Life (Basel), 2021. **11**(9).
88. Sandoo, A., et al., *The endothelium and its role in regulating vascular tone.* Open Cardiovasc Med J, 2010. **4**: p. 302-12.
89. Dos Passos, R.R., et al., *Immunomodulatory Activity of Cytokines in Hypertension: A Vascular Perspective.* Hypertension, 2024. **81**(7): p. 1411-1423.
90. Gallo, G., M. Volpe, and C. Savoia, *Endothelial Dysfunction in Hypertension: Current Concepts and Clinical Implications*. Front Med (Lausanne), 2021. **8**: p. 798958.
91. Zhang, Z., et al., *Role of inflammation, immunity, and oxidative stress in hypertension: New insights and potential therapeutic targets*. Front Immunol, 2022. **13**: p. 1098725.
92. Williams, B., et al., *2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH).* European Heart Journal, 2018. **39**(33): p. 3021-3104.
93. Messerli Franz, H., et al., *Angiotensin-Converting Enzyme Inhibitors in Hypertension.* Journal of the American College of Cardiology, 2018. **71**(13): p. 1474-1482.
94. Ondetti, M.A., B. Rubin, and D.W. Cushman, *Design of specific inhibitors of angiotensin-converting enzyme: new class of orally active antihypertensive agents*. Science, 1977. **196**(4288): p. 441-4.
95. Xanthakis, V. and R.S. Vasan, *Aldosterone and the risk of hypertension.* Curr Hypertens Rep, 2013. **15**(2): p. 102-7.
96. Weinberger, M.H., *Role of sympathetic nervous system activity in the blood pressure response to long-term captopril therapy in severely hypertensive patients.* Am J Cardiol, 1982. **49**(6): p. 1542-3.
97. Charkoudian, N. and J.A. Rabbitts, *Sympathetic neural mechanisms in human*

cardiovascular health and disease. Mayo Clin Proc, 2009. **84**(9): p. 822-30.
98. Elliott, W.J. and C.V. Ram, *Calcium channel blockers.* J Clin Hypertens (Greenwich), 2011. **13**(9): p. 687-9.
99. Li, H., et al., *Metabolic syndrome and components exacerbate osteoarthritis symptoms of pain, depression and reduced knee function.* Ann Transl Med, 2016. **4**(7): p. 133.
100. Lo, G.H., et al., *Systolic and pulse pressure associate with incident knee osteoarthritis: data from the Osteoarthritis Initiative.* Clin Rheumatol, 2017. **36**(9): p. 2121-2128.
101. Lo, K., et al., *Association between hypertension and osteoarthritis: A systematic review and meta-analysis of observational studies.* J Orthop Translat, 2022. **32**: p. 12-20.
102. Ni, R., et al., *Hemodynamic stress shapes subchondral bone in osteoarthritis: An emerging hypothesis.* J Orthop Translat, 2022. **32**: p. 85-90.
103. Ni, R., et al., *Hemodynamic stress shapes subchondral bone in osteoarthritis: An emerging hypothesis.* Journal of Orthopaedic Translation, 2022. **32**: p. 85-90.
104. Hugle, T. and J. Geurts, *What drives osteoarthritis?-synovial versus subchondral bone pathology.* Rheumatology, 2017. **56**(9): p. 1461-1471.
105. Hu, Y., et al., *Subchondral bone microenvironment in osteoarthritis and pain.* Bone Research, 2021. **9**(1): p. 13.
106. Hu, W.H., et al., *Microenvironment in subchondral bone: predominant regulator for the treatment of osteoarthritis.* Annals of the Rheumatic Diseases, 2021. **80**(4): p. 413-422.
107. Goldring, M.B. and S.R. Goldring, *Articular cartilage and subchondral bone in the pathogenesis of osteoarthritis*. Skeletal Biology and Medicine, ed. M. Zaidi. Vol. 1192. 2010, Hoboken: Wiley-Blackwell. 230-237.
108. Zhou, F., et al., *Associations of osteoclastogenesis and nerve growth in subchondral bone marrow lesions with clinical symptoms in knee osteoarthritis.* Journal of Orthopaedic Translation, 2022. **32**: p. 69-76.
109. Chan, P.M.B., et al., *Is subchondral bone cyst formation in non-load-bearing region of osteoarthritic knee a vascular problem?* Med Hypotheses, 2017. **109**: p. 80-83.
110. Muratovic, D., et al., *Bone matrix microdamage and vascular changes characterize bone marrow lesions in the subchondral bone of knee osteoarthritis.* Bone, 2018. **108**: p. 193-201.
111. Chan, P., et al., *ROLE OF SYSTEMIC HYPERTENSION IN CELL SENESCENCE AND SUBCHONDRAL BONE DISTURBANCE OF KNEE JOINT.* Osteoarthritis and Cartilage, 2018. **26**: p. S118-S118.
112. Turner, J.E., V.A. Lira, and P.C. Brum, *New Insights into the Benefits of Physical Activity and Exercise for Aging and Chronic Disease.* Oxidative Medicine and Cellular Longevity, 2017. **2017**: p. 3.
113. Pautz, A., H.G. Li, and H. Kleinert, *Regulation of NOS expression in vascular diseases.* Frontiers in Bioscience-Landmark, 2021. **26**(5): p. 85-101.
114. Al-Afify, A.S.A., et al., *Avocado soybean unsaponifiables ameliorates cartilage and subchondral bone degeneration in mono-iodoacetate-induced knee osteoarthritis in rats.* Tissue & Cell, 2018. **52**: p. 108-115.
115. de Sa, G.A., et al., *Angiotensin II triggers knee joint lesions in experimental osteoarthritis.* Bone, 2021. **145**: p. 10.
116. Sin, A., et al., *The emerging role of endothelin-1 in the pathogenesis of subchondral bone*

disturbance and osteoarthritis. Osteoarthritis and Cartilage, 2015. **23**(4): p. 516-524.
117. Au, M., et al., *Endothelin-1 induces chondrocyte senescence and cartilage damage via endothelin receptor type B in a post-traumatic osteoarthritis mouse model.* Osteoarthritis and Cartilage, 2020. **28**(12): p. 1559-1571.
118. Mazidi, M., et al., *VEGF, the underlying factor for metabolic syndrome; fact or fiction?* Diabetes & Metabolic Syndrome-Clinical Research & Reviews, 2017. **11**: p. S61-S64.
119. Liu, S.C., et al., *Melatonin abolished proinflammatory factor expression and antagonized osteoarthritis progression in vivo.* Cell Death & Disease, 2022. **13**(3): p. 10.
120. Hamilton, J.L., et al., *Targeting VEGF and Its Receptors for the Treatment of Osteoarthritis and Associated Pain.* Journal of Bone and Mineral Research, 2016. **31**(5): p. 911-924.
121. Shabestari, M., et al., *Altered protein levels in bone marrow lesions of hip osteoarthritis: Analysis by proteomics and multiplex immunoassays.* International Journal of Rheumatic Diseases, 2020. **23**(6): p. 788-799.
122. Lu, J.S., et al., *Positive-Feedback Regulation of Subchondral H-Type Vessel Formation by Chondrocyte Promotes Osteoarthritis Development in Mice.* Journal of Bone and Mineral Research, 2018. **33**(5): p. 909-920.
123. Li, Y.J., et al., *Synovial macrophages in cartilage destruction and regeneration-lessons learnt from osteoarthritis and synovial chondromatosis.* Biomedical Materials, 2022. **17**(1): p. 9.
124. Pap, T., et al., *Synovial fibroblasts and articular tissue remodelling: Role and mechanisms.* Seminars in Cell & Developmental Biology, 2020. **101**: p. 140-145.
125. Kleine, S.A. and S.C. Budsberg, *Synovial Membrane Receptors as Therapeutic Targets: A Review of Receptor Localization, Structure, and Function.* Journal of Orthopaedic Research, 2017. **35**(8): p. 1589-1605.
126. Gao, S.M. and H. Song, *Integrated comparison of the mRNAome in cartilage, synovium, and macrophages in osteoarthritis.* Zeitschrift Fur Rheumatologie: p. 9.
127. Feldt, J., et al., *Production and Secretion of Gelsolin by Both Human Macrophage- and Fibroblast-like Synoviocytes and GSN Modulation in the Synovial Fluid of Patients with Various Forms of Arthritis.* Biomedicines, 2022. **10**(3): p. 13.
128. Farah, H., et al., *Differential Metabotypes in Synovial Fibroblasts and Synovial Fluid in Hip Osteoarthritis Patients Support Inflammatory Responses.* International journal of molecular sciences, 2022. **23**(6).
129. Woodell-May, J.E. and S.D. Sommerfeld, *Role of Inflammation and the Immune System in the Progression of Osteoarthritis.* Journal of Orthopaedic Research, 2020. **38**(2): p. 253-257.
130. Su, S.W., et al., *Resolvin D1 inhibits the proliferation of osteoarthritis fibroblast-like synoviocytes through the Hippo-YAP signaling pathway.* Bmc Musculoskeletal Disorders, 2022. **23**(1): p. 9.
131. Sanchez-Lopez, E., et al., *Synovial inflammation in osteoarthritis progression.* Nature Reviews Rheumatology, 2022. **18**(5): p. 258-275.
132. Matsuzaki, T., et al., *Transthyretin deposition promotes progression of osteoarthritis.* Aging Cell, 2017. **16**(6): p. 1313-1322.
133. Masi, S., M. Uliana, and A. Virdis, *Angiotensin II and vascular damage in hypertension: Role of oxidative stress and sympathetic activation.* Vascular Pharmacology, 2019. **115**: p.

13-17.
134. Zhang, P., et al., *Association of Inpatient Use of Angiotensin-Converting Enzyme Inhibitors and Angiotensin II Receptor Blockers With Mortality Among Patients With Hypertension Hospitalized With COVID-19.* Circulation Research, 2020. **126**(12): p. 1671-1681.
135. Terenzi, R., et al., *Angiotensin II type 2 receptor (AT2R) as a novel modulator of inflammation in rheumatoid arthritis synovium.* Scientific Reports, 2017. **7**: p. 11.
136. Li, M., et al., *Identification of potential target genes associated with the pathogenesis of osteoarthritis using microarray based analysis.* Molecular Medicine Reports, 2017. **16**(3): p. 2799-2806.
137. Wang, Y.H., et al., *Apelin Affects the Progression of Osteoarthritis by Regulating VEGF-Dependent Angiogenesis and miR-150-5p Expression in Human Synovial Fibroblasts.* Cells, 2020. **9**(3): p. 16.
138. Tsai, C.H., et al., *High glucose induces vascular endothelial growth factor production in human synovial fibroblasts through reactive oxygen species generation.* Biochimica Et Biophysica Acta-General Subjects, 2013. **1830**(3): p. 2649-2658.
139. Gao, J.L. and S.L. Xia, *Reduced miR-519d-3p levels in the synovium and synovial fluid facilitate the progression of post-traumatic osteoarthritis by targeting VEGF.* Experimental and Therapeutic Medicine, 2021. **22**(6): p. 9.
140. Takano, S., et al., *Vascular endothelial growth factor expression and their action in the synovial membranes of patients with painful knee osteoarthritis.* Bmc Musculoskeletal Disorders, 2018. **19**: p. 8.
141. Yang, L.L., et al., *Extensive cytokine analysis in synovial fluid of osteoarthritis patients.* Cytokine, 2021. **143**: p. 10.
142. Gurzu, S., et al., *Different synovial vasculogenic profiles of primary, rapidly destructive and osteonecrosis-induced hip osteoarthritis. An immunohistochemistry study.* International Orthopaedics, 2017. **41**(6): p. 1107-1112.
143. Zhang, L., et al., *Characteristics of sensory innervation in synovium of rats within different knee osteoarthritis models and the correlation between synovial fibrosis and hyperalgesia.* Journal of Advanced Research, 2022. **35**: p. 141-151.
144. Nagao, M., et al., *Vascular Endothelial Growth Factor in Cartilage Development and Osteoarthritis.* Scientific Reports, 2017. **7**: p. 16.
145. Buckwalter, J.A., H.J. Mankin, and A.J. Grodzinsky, *Articular cartilage and osteoarthritis.* Instr Course Lect, 2005. **54**: p. 465-80.
146. Buckwalter, J.A. and H.J. Mankin, *Articular cartilage: tissue design and chondrocyte-matrix interactions.* Instr Course Lect, 1998. **47**: p. 477-86.
147. Lefebvre, V. and P. Smits, *Transcriptional control of chondrocyte fate and differentiation.* Birth Defects Res C Embryo Today, 2005. **75**(3): p. 200-12.
148. Yang, G., et al., *Osteogenic fate of hypertrophic chondrocytes.* Cell Research, 2014. **24**(10): p. 1266-1269.
149. Sophia Fox, A.J., A. Bedi, and S.A. Rodeo, *The basic science of articular cartilage: structure, composition, and function.* Sports Health, 2009. **1**(6): p. 461-8.
150. Buckwalter, J.A. and H.J. Mankin, *Articular cartilage: degeneration and osteoarthritis, repair, regeneration, and transplantation.* Instr Course Lect, 1998. **47**: p. 487-504.
151. Jones, D.G., *Articular cartilage degeneration: etiologic association with obesity.* Ochsner J,

2009. **9**(3): p. 137-9.
152. Wang, Y., et al., *Nutrition and degeneration of articular cartilage.* Knee Surg Sports Traumatol Arthrosc, 2013. **21**(8): p. 1751-62.
153. Imhof, H., et al., *Subchondral bone and cartilage disease: a rediscovered functional unit.* Invest Radiol, 2000. **35**(10): p. 581-8.
154. Findlay, D.M., *Vascular pathology and osteoarthritis.* Rheumatology, 2007. **46**(12): p. 1763-1768.
155. Pufe, T., et al., *Vascular endothelial growth factor (VEGF) induces matrix metalloproteinase expression in immortalized chondrocytes.* J Pathol, 2004. **202**(3): p. 367-74.
156. !!! INVALID CITATION !!! .
157. Sheng, J., et al., *Egr-1 increases angiogenesis in cartilage via binding Netrin-1 receptor DCC promoter.* Journal of Orthopaedic Surgery and Research, 2018. **13**(1): p. 125.
158. Hasegawa, A., et al., *Anterior cruciate ligament changes in the human knee joint in aging and osteoarthritis.* Arthritis Rheum, 2012. **64**(3): p. 696-704.
159. Nagelli, C.V., et al., *Does Anterior Cruciate Ligament Innervation Matter for Joint Function and Development of Osteoarthritis?* J Knee Surg, 2017. **30**(4): p. 364-371.
160. Alonso, B., et al., *Osteoarthritis-related biomarkers profile in chronic anterior cruciate ligament injured knee.* Knee, 2020. **27**(1): p. 51-60.
161. Skyhar, M.J., et al., *Nutrition of the anterior cruciate ligament: Effects of continuous passive motion.* The American Journal of Sports Medicine, 1985. **13**(6): p. 415-418.
162. Schulze-Tanzil, G., *Intraarticular Ligament Degeneration Is Interrelated with Cartilage and Bone Destruction in Osteoarthritis.* Cells, 2019. **8**(9).
163. Krishnasamy, P., M. Hall, and S.R. Robbins, *The role of skeletal muscle in the pathophysiology and management of knee osteoarthritis.* Rheumatology (Oxford), 2018. **57**(suppl_4): p. iv22-iv33.
164. Cairns, D.M., et al., *The role of muscle cells in regulating cartilage matrix production.* J Orthop Res, 2010. **28**(4): p. 529-36.
165. Cairns, D.M., et al., *Muscle cells enhance resistance to pro-inflammatory cytokine-induced cartilage destruction.* Biochem Biophys Res Commun, 2010. **392**(1): p. 22-8.
166. Simão, A.P., et al., *Involvement of BDNF in knee osteoarthritis: the relationship with inflammation and clinical parameters.* Rheumatol Int, 2014. **34**(8): p. 1153-7.
167. Peake, J.M., et al., *Cytokine expression and secretion by skeletal muscle cells: regulatory mechanisms and exercise effects.* Exerc Immunol Rev, 2015. **21**: p. 8-25.
168. Banday, A.A. and M.F. Lokhandwala, *Oxidative stress causes renal angiotensin II type 1 receptor upregulation, Na+/H+ exchanger 3 overstimulation, and hypertension.* Hypertension, 2011. **57**(3): p. 452-9.
169. Cichoż-Lach, H. and A. Michalak, *Oxidative stress as a crucial factor in liver diseases.* World J Gastroenterol, 2014. **20**(25): p. 8082-91.
170. Luo, H., et al., *Oxidative stress causes imbalance of renal renin angiotensin system (RAS) components and hypertension in obese Zucker rats.* J Am Heart Assoc, 2015. **4**(2).
171. Sukhanov, S., et al., *Angiotensin II, oxidative stress and skeletal muscle wasting.* Am J Med Sci, 2011. **342**(2): p. 143-7.
172. Cassis, L.A., et al., *Local adipose tissue renin-angiotensin system.* Curr Hypertens Rep, 2008. **10**(2): p. 93-8.

173. Leung, P.S., *The physiology of a local renin-angiotensin system in the pancreas.* J Physiol, 2007. **580**(Pt 1): p. 31-7.
174. Hsieh, T.J., et al., *High glucose stimulates angiotensinogen gene expression via reactive oxygen species generation in rat kidney proximal tubular cells.* Endocrinology, 2002. **143**(8): p. 2975-85.
175. Wu, Y., et al., *Renin-angiotensin system in osteoarthritis: A new potential therapy.* International Immunopharmacology, 2019. **75**: p. 105796.
176. Birk, M., et al., *Angiotensin II Induces Oxidative Stress and Endothelial Dysfunction in Mouse Ophthalmic Arteries via Involvement of AT1 Receptors and NOX2.* Antioxidants (Basel), 2021. **10**(8).
177. Wu, Y., et al., *Renin-angiotensin system in osteoarthritis: A new potential therapy.* Int Immunopharmacol, 2019. **75**: p. 105796.
178. Akagi, T., et al., *Effect of Angiotensin II on Bone Erosion and Systemic Bone Loss in Mice with Tumor Necrosis Factor-Mediated Arthritis.* Int J Mol Sci, 2020. **21**(11).
179. Lepetsos, P. and A.G. Papavassiliou, *ROS/oxidative stress signaling in osteoarthritis.* Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 2016. **1862**(4): p. 576-591.
180. Scioli, M.G., et al., *Oxidative Stress and New Pathogenetic Mechanisms in Endothelial Dysfunction: Potential Diagnostic Biomarkers and Therapeutic Targets.* J Clin Med, 2020. **9**(6).
181. Qi, Z., et al., *The role and intervention of mitochondrial metabolism in osteoarthritis.* Mol Cell Biochem, 2024. **479**(6): p. 1513-1524.
182. Liu, L., et al., *The role of oxidative stress in the development of knee osteoarthritis: A comprehensive research review.* Front Mol Biosci, 2022. **9**: p. 1001212.
183. Kim, Y.W. and T.V. Byzova, *Oxidative stress in angiogenesis and vascular disease.* Blood, 2014. **123**(5): p. 625-31.
184. Macvanin, M., et al., *New insights on the cardiovascular effects of IGF-1.* Front Endocrinol (Lausanne), 2023. **14**: p. 1142644.
185. Tavera, C., et al., *IGF and IGF-binding protein system in the synovial fluid of osteoarthritic and rheumatoid arthritic patients.* Osteoarthritis Cartilage, 1996. **4**(4): p. 263-74.
186. Gao, H.L., et al., *Luteolin Attenuates Hypertension via Inhibiting NF-κB-Mediated Inflammation and PI3K/Akt Signaling Pathway in the Hypothalamic Paraventricular Nucleus.* Nutrients, 2023. **15**(3).
187. Ansari, M.Y., N. Ahmad, and T.M. Haqqi, *Oxidative stress and inflammation in osteoarthritis pathogenesis: Role of polyphenols.* Biomed Pharmacother, 2020. **129**: p. 110452.
188. Zhang, H. and N.S. Dhalla, *The Role of Pro-Inflammatory Cytokines in the Pathogenesis of Cardiovascular Disease.* Int J Mol Sci, 2024. **25**(2).
189. Berenbaum, F., *Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!).* Osteoarthritis and Cartilage, 2013. **21**(1): p. 16-21.
190. Mobasheri, A., et al., *Chondrosenescence: Definition, hallmarks and potential role in the pathogenesis of osteoarthritis.* Maturitas, 2015. **80**(3): p. 237-244.
191. Ashraf, S., et al., *Regulation of senescence associated signaling mechanisms in chondrocytes for cartilage tissue regeneration.* Osteoarthritis Cartilage, 2016. **24**(2): p.

196-205.
192. Yang, H., et al., *Navitoclax (ABT263) reduces inflammation and promotes chondrogenic phenotype by clearing senescent osteoarthritic chondrocytes in osteoarthritis.* Aging (Albany NY), 2020. **12**(13): p. 12750-12770.
193. Maurer, S., et al., *Senolytic therapy combining Dasatinib and Quercetin restores the chondrogenic phenotype of human osteoarthritic chondrocytes by promoting the release of pro-anabolic mediators*. 2024.
194. Um, H.D., *Bcl-2 family proteins as regulators of cancer cell invasion and metastasis: a review focusing on mitochondrial respiration and reactive oxygen species.* Oncotarget, 2016. **7**(5): p. 5193-203.
195. Miura, Y., et al., *Clearance of senescent cells with ABT-263 improves biological functions of synovial mesenchymal stem cells from osteoarthritis patients.* Stem Cell Research & Therapy, 2022. **13**(1): p. 222.
196. Liu, S., et al., *The PI3K-Akt pathway inhibits senescence and promotes self-renewal of human skin-derived precursors in vitro.* Aging Cell, 2011. **10**(4): p. 661-74.
197. Zhang, Y., et al., *Expression of Clusterin suppresses Cr(VI)-induced premature senescence through activation of PI3K/AKT pathway.* Ecotoxicology and Environmental Safety, 2019. **183**: p. 109465.
198. Bruning, A., *Inhibition of mTOR signaling by quercetin in cancer treatment and prevention.* Anticancer Agents Med Chem, 2013. **13**(7): p. 1025-31.
199. Wang, H., et al., *Quercetin prevents osteoarthritis progression possibly via regulation of local and systemic inflammatory cascades.* J Cell Mol Med, 2023. **27**(4): p. 515-528.
200. Bourgeois, B. and T. Madl, *Regulation of cellular senescence via the FOXO4-p53 axis*. FEBS Lett, 2018. **592**(12): p. 2083-2097.
201. Baar, M.P., et al., *Targeted Apoptosis of Senescent Cells Restores Tissue Homeostasis in Response to Chemotoxicity and Aging.* Cell, 2017. **169**(1): p. 132-147.e16.
202. Miwa, S., et al., *Mitochondrial dysfunction in cell senescence and aging.* J Clin Invest, 2022. **132**(13).
203. Iske, J., et al., *Senolytics prevent mt-DNA-induced inflammation and promote the survival of aged organs following transplantation.* Nature Communications, 2020. **11**(1): p. 4289.
204. Hu, X., et al., *The JAK/STAT signaling pathway: from bench to clinic.* Signal Transduction and Targeted Therapy, 2021. **6**(1): p. 402.
205. Xu, M., et al., *JAK inhibition alleviates the cellular senescence-associated secretory phenotype and frailty in old age.* Proc Natl Acad Sci U S A, 2015. **112**(46): p. E6301-10.
206. Han, Y. and S.Y. Kim, *Endothelial senescence in vascular diseases: current understanding and future opportunities in senotherapeutics*. Experimental & Molecular Medicine, 2023. **55**(1): p. 1-12.
207. Coppé, J.P., et al., *The senescence-associated secretory phenotype: the dark side of tumor suppression.* Annu Rev Pathol, 2010. **5**: p. 99-118.
208. Zhan, H., et al., *Ataxia telangiectasia mutated (ATM)-mediated DNA damage response in oxidative stress-induced vascular endothelial cell senescence.* J Biol Chem, 2010. **285**(38): p. 29662-70.
209. Donato, A.J., et al., *Cellular and molecular biology of aging endothelial cells.* J Mol Cell Cardiol, 2015. **89**(Pt B): p. 122-35.

210. Valdes, A.M., et al., *Association of Beta-Blocker Use With Less Prevalent Joint Pain and Lower Opioid Requirement in People With Osteoarthritis.* Arthritis Care Res (Hoboken), 2017. **69**(7): p. 1076-1081.
211. Nakafero, G., et al., *β-blocker prescription is associated with lower cumulative risk of knee osteoarthritis and knee pain consultations in primary care: a propensity score–matched cohort study.* Rheumatology, 2021. **60**(12): p. 5686-5696.
212. Zhou, L., et al., *Lack of evidence that beta blocker use reduces knee pain, areas of joint pain, or analgesic use among individuals with symptomatic knee osteoarthritis.* Osteoarthritis and Cartilage, 2020. **28**(1): p. 53-61.
213. Zhang, Y., et al., *Effects of blood pressure and antihypertensive drugs on osteoarthritis: a mendelian randomized study.* Aging Clin Exp Res, 2023. **35**(11): p. 2437-2444.
214. Bendtzen, K., P.R. Hansen, and K. Rieneck, *Spironolactone inhibits production of proinflammatory cytokines, including tumour necrosis factor-alpha and interferon-gamma, and has potential in the treatment of arthritis.* Clin Exp Immunol, 2003. **134**(1): p. 151-8.
215. Li, M., et al., *The effects of different antihypertensive drugs on pain and joint space width of knee osteoarthritis - A comparative study with data from Osteoarthritis Initiative.* J Clin Hypertens (Greenwich), 2021. **23**(11): p. 2009-2015.
216. Tang, Y., X. Hu, and X. Lu, *Captopril, an angiotensin-converting enzyme inhibitor, possesses chondroprotective efficacy in a rat model of osteoarthritis through suppression local renin-angiotensin system.* Int J Clin Exp Med, 2015. **8**(8): p. 12584-92.
217. Poulter, N.R., D. Prabhakaran, and M. Caulfield, *Hypertension.* The Lancet, 2015. **386**(9995): p. 801-812.
218. Amador, C.A., et al., *Spironolactone decreases DOCA-salt-induced organ damage by blocking the activation of T helper 17 and the downregulation of regulatory T lymphocytes.* Hypertension, 2014. **63**(4): p. 797-803.
219. Barrett, K.V., A.T. McCurley, and I.Z. Jaffe, *Direct contribution of vascular mineralocorticoid receptors to blood pressure regulation.* Clin Exp Pharmacol Physiol, 2013. **40**(12): p. 902-9.
220. Cole, T.J., P.J. Fuller, and M.J. Young, *Chapter 20 - Mutations of the Human Mineralocorticoid Receptor and Targeted Deletion in Model Organisms*, in *Stress: Genetics, Epigenetics and Genomics*, G. Fink, Editor. 2021, Academic Press. p. 229-239.
221. Ray, E.C., et al., *Sodium retention and volume expansion in nephrotic syndrome: implications for hypertension.* Adv Chronic Kidney Dis, 2015. **22**(3): p. 179-84.
222. Bae, E.H., et al., *Altered regulation of renal sodium transporters and natriuretic peptide system in DOCA–salt hypertensive rats.* Regulatory Peptides, 2009. **157**(1): p. 76-83.
223. Belden, Z., et al., *The Role of the Mineralocorticoid Receptor in Inflammation: Focus on Kidney and Vasculature.* Am J Nephrol, 2017. **46**(4): p. 298-314.
224. DeLalio, L.J., A.F. Sved, and S.D. Stocker, *Sympathetic Nervous System Contributions to Hypertension: Updates and Therapeutic Relevance.* Can J Cardiol, 2020. **36**(5): p. 712-720.
225. Kita, S., et al., *Effects of endothelin-1 on norepinephrine-induced vasoconstriction in deoxycorticosterone acetate–salt hypertensive rats.* European Journal of Pharmacology, 1998. **344**(1): p. 53-57.
226. Igbekele, A.E., et al., *Mineralocorticoid Receptor Activation in Vascular Insulin Resistance*

and Dysfunction. Int J Mol Sci, 2022. **23**(16).
227. Zhang, A., et al., *Relative contributions of mitochondria and NADPH oxidase to deoxycorticosterone acetate-salt hypertension in mice*. Kidney Int, 2011. **80**(1): p. 51-60.
228. Lingappan, K., *NF-κB in Oxidative Stress.* Curr Opin Toxicol, 2018. **7**: p. 81-86.
229. Huang, L.-I., et al., *Protective effects of grape seed proanthocyanidins on cardiovascular remodeling in DOCA-salt hypertension rats.* The Journal of Nutritional Biochemistry, 2015. **26**(8): p. 841-849.
230. Westhoff, J.H., et al., *Hypertension induces somatic cellular senescence in rats and humans by induction of cell cycle inhibitor p16INK4a.* Hypertension, 2008. **52**(1): p. 123-9.
231. Bae, E.H., et al., *Rosiglitazone prevents the progression of renal injury in DOCA-salt hypertensive rats.* Hypertens Res, 2010. **33**(3): p. 255-62.
232. Dhand, N. and M. Khatkar, *Statulator: An online statistical calculator.* Sample size calculator for estimating a single proportion, 2014.
233. Price, J.S., et al., *The role of chondrocyte senescence in osteoarthritis.* Aging Cell, 2002. **1**(1): p. 57-65.
234. Dhand, N.K.K., M.S. *Statulator: An online statistical calculator. Sample Size Calc. Comp. Two Indep. Means*. 2014.
235. Liu, Z., et al., *Photoacoustic imaging of synovial tissue hypoxia in experimental post-traumatic osteoarthritis.* Prog Biophys Mol Biol, 2019. **148**: p. 12-20.
236. Waldstein, W., et al., *OARSI osteoarthritis cartilage histopathology assessment system: A biomechanical evaluation in the human knee.* J Orthop Res, 2016. **34**(1): p. 135-40.
237. Ye, Z., H. Lu, and P. Liu, *Association between essential hypertension and bone mineral density: a systematic review and meta-analysis*. Oncotarget, 2017. **8**(40): p. 68916-68927.
238. Cappuccio, F.P., et al., *High blood pressure and bone-mineral loss in elderly white women: a prospective study. Study of Osteoporotic Fractures Research Group.* Lancet, 1999. **354**(9183): p. 971-5.
239. Chai, H., et al., *Hypertension is associated with osteoporosis: a case-control study in Chinese postmenopausal women.* BMC Musculoskeletal Disorders, 2021. **22**(1): p. 253.
240. Long, J.T., et al., *Hypertrophic chondrocytes serve as a reservoir for marrow-associated skeletal stem and progenitor cells, osteoblasts, and adipocytes during skeletal development*. Elife, 2022. **11**.
241. Yang, L., et al., *Hypertrophic chondrocytes can become osteoblasts and osteocytes in endochondral bone formation.* Proc Natl Acad Sci U S A, 2014. **111**(33): p. 12097-102.
242. Taddei, S., et al., *Hypertension causes premature aging of endothelial function in humans.* Hypertension, 1997. **29**(3): p. 736-43.
243. Schreckenberger, Z.J., et al., *Mitophagy in Hypertension-Associated Premature Vascular Aging.* American Journal of Hypertension, 2020. **33**(9): p. 804-812.
244. Flynn, J.T., *Hypertension, growth, and skeletal maturation in the young: a new look at an old idea.* Hypertension, 2009. **54**(6): p. 1204-5.
245. Rahman, M.M., et al., *The relationship between osteoarthritis and cardiovascular disease in a population health survey: a cross-sectional study.* BMJ Open, 2013. **3**(5): p. e002624.
246. Wang, H., et al., *Osteoarthritis and the risk of cardiovascular disease: a meta-analysis of observational studies.* Sci Rep, 2016. **6**: p. 39672.
247. Hall, A.J., et al., *Association between osteoarthritis and cardiovascular disease: Systematic*

review and meta-analysis. European Journal of Preventive Cardiology, 2016. **23**(9): p. 938-946.

248. Li, R., et al., *SOAP: short oligonucleotide alignment program.* Bioinformatics, 2008. **24**(5): p. 713-714.
249. Langmead, B. and S.L. Salzberg, *Fast gapped-read alignment with Bowtie 2.* Nat Methods, 2012. **9**(4): p. 357-9.
250. Li, B. and C.N. Dewey, *RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome.* BMC Bioinformatics, 2011. **12**(1): p. 323.
251. Love, M.I., W. Huber, and S. Anders, *Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2.* Genome Biol, 2014. **15**(12): p. 550.
252. Falcon, S. and R. Gentleman, *Using GOstats to test gene lists for GO term association.* Bioinformatics, 2007. **23**(2): p. 257-258.
253. Au, M., et al., *Endothelin-1 induces chondrocyte senescence and cartilage damage via endothelin receptor type B in a post-traumatic osteoarthritis mouse model.* Osteoarthritis Cartilage, 2020. **28**(12): p. 1559-1571.
254. Konno, T., et al., *Intracellular Sources of ROS/H(2)O(2) in Health and Neurodegeneration: Spotlight on Endoplasmic Reticulum.* Cells, 2021. **10**(2).
255. Shi, T., et al., *DNA damage and oxidant stress activate p53 through differential upstream signaling pathways.* Free Radic Biol Med, 2021. **172**: p. 298-311.
256. Liso, A., et al., *IGFBP-6: At the Crossroads of Immunity, Tissue Repair and Fibrosis.* Int J Mol Sci, 2022. **23**(8).
257. Paz-González, R., et al., *Proteomic profiling of human menisci from mild joint degeneration and end-stage osteoarthritis versus healthy controls.* Osteoarthritis and Cartilage Open, 2023. **5**(4): p. 100417.
258. Oliva, C.R., et al., *IGFBP6 controls the expansion of chemoresistant glioblastoma through paracrine IGF2/IGF-1R signaling.* Cell Communication and Signaling, 2018. **16**(1): p. 61.
259. Longhitano, L., et al., *The Crosstalk between GPR81/IGFBP6 Promotes Breast Cancer Progression by Modulating Lactate Metabolism and Oxidative Stress.* Antioxidants (Basel), 2022. **11**(2).
260. Jeon, H.J., et al., *Insulin-like growth factor binding protein-6 released from human mesenchymal stem cells confers neuronal protection through IGF-1R-mediated signaling.* Int J Mol Med, 2017. **40**(6): p. 1860-1868.
261. Funck-Brentano, T., et al., *Causal Factors for Knee, Hip, and Hand Osteoarthritis: A Mendelian Randomization Study in the UK Biobank.* Arthritis Rheumatol, 2019. **71**(10): p. 1634-1641.
262. Niu, J., et al., *Metabolic Syndrome, Its Components, and Knee Osteoarthritis: The Framingham Osteoarthritis Study.* Arthritis Rheumatol, 2017. **69**(6): p. 1194-1203.
263. Russell, F.A., et al., *Calcitonin gene-related peptide: physiology and pathophysiology.* Physiol Rev, 2014. **94**(4): p. 1099-142.
264. Benschop, R.J., et al., *Development of a novel antibody to calcitonin gene-related peptide for the treatment of osteoarthritis-related pain.* Osteoarthritis and Cartilage, 2014. **22**(4): p. 578-585.
265. Bullock, C.M., et al., *Peripheral calcitonin gene-related peptide receptor activation and mechanical sensitization of the joint in rat models of osteoarthritis pain.* Arthritis

Rheumatol, 2014. **66**(8): p. 2188-200.
266. Ebbinghaus, M., et al., *Interleukin-6-dependent influence of nociceptive sensory neurons on antigen-induced arthritis.* Arthritis Research & Therapy, 2015. **17**(1): p. 334.
267. Lu, Y.Z., et al., *CGRP sensory neurons promote tissue healing via neutrophils and macrophages.* Nature, 2024. **628**(8008): p. 604-611.
268. Bullock, C.M. and S. Kelly, *Calcitonin gene-related peptide receptor antagonists: beyond migraine pain--a possible analgesic strategy for osteoarthritis?* Curr Pain Headache Rep, 2013. **17**(11): p. 375.