CHAPTER II

LITERATURE REVIEW

2.1 Osteoarthritis (OA)

OA is one of the significant contributors to years lived with disability among the musculoskeletal conditions. As OA is more prevalent in older people, global prevalence is expected to increase with the ageing of populations. OA is a degenerative joint condition. It causes pain, swelling of the damage tissue that lost it's stiffness, affecting a person's ability to move freely. Symptoms of OA include pain, swelling, stiffness and trouble moving the affected joint. As a consequence of reduced movement, muscles often lose strength and people become less able to perform physical activities. OA can affect any joint but is most common in the knees, hips, spine and small joints in the hands. Muscles and tissue around the joint are often affected. Osteophyte development and induced bone ends eventually rub against one another because chondrocytes are unable to repair tissue (Hunter & Felson, 2006; Frech & Clegg, 2007). Mainly characterized by articular cartilage degradation, OA is a heterogeneous disease that impacts all component tissues of the articular joint organ (He et al., 2020).

2.1.1 Articular cartilage

Normal adult articular cartilage is made up of extracellular matrix: ECM (water, collagen, proteoglycans and a very small component of calcium salt) and chondrocytes (Golding & Marcu, 2009). While, proteoglycan turnover is rapid collagen turnover is relatively slow. (Mow, Yong GU, & Hui Chen, 2005). The normal turnover of matrix components is regulated by chondrocytes, which produce both the components and the proteolytic enzymes responsible for their degradation. In turn, chondrocytes are influenced by several factors, such as polypeptide growth factors, cytokines, structural and physical stimuli, and even the matrix components themselves (Wise, 2010). OA occurs due to the failure of chondrocytes to maintain the balance between synthesis and degradation of ECM components (Heijink, Gomall, & Madry, 2012). In osteoarthritic cartilage, both anabolic and catabolic

activities increase. Initially, compensatory mechanisms, such as elevated synthesis of matrix molecules like collagen, proteoglycans, and hyaluronate (Goldring & Goldring, 2007), along with the proliferation of chondrocytes in the deeper cartilage layers, help preserve the integrity of the articular cartilage. However, over time, the loss of chondrocytes and alterations in the ECM become dominant, leading to the progression of osteoarthritic changes (Man & Mologhianu, 2014).

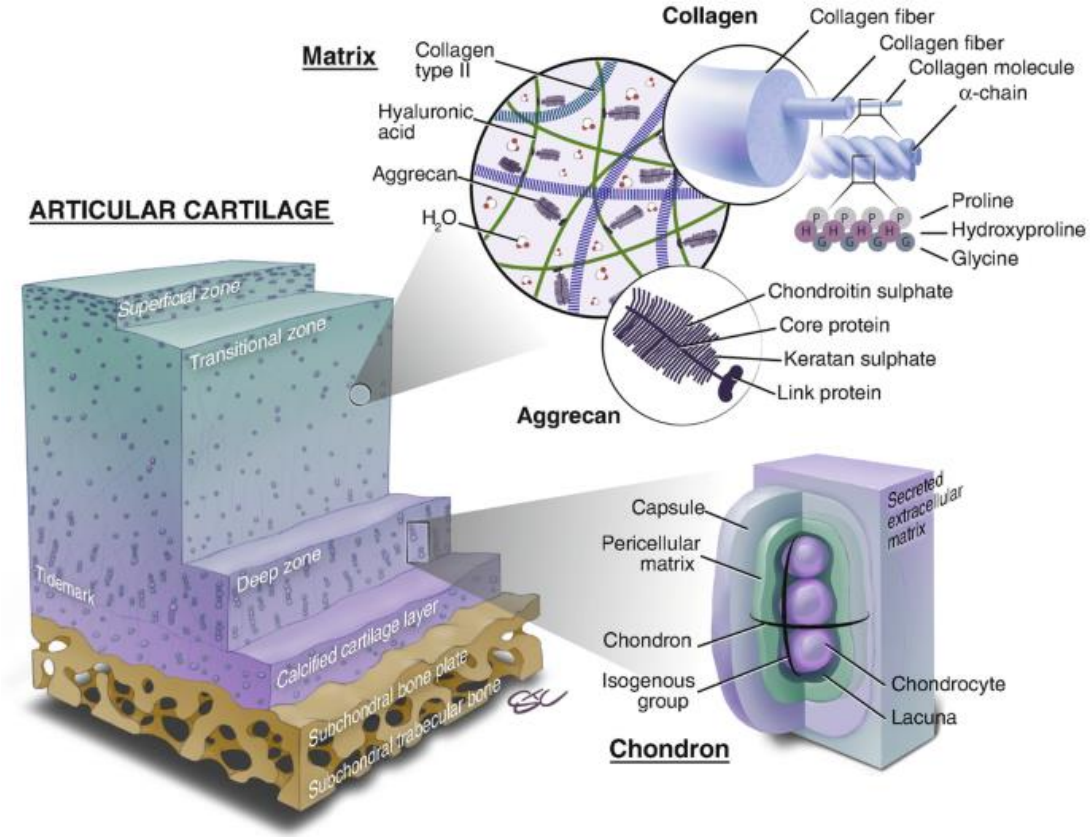

Figure 2.1 Structure of articular cartilage. (Baumann et al., 2019).

2.1.1.1 ECM: The dense ECM primarily consists of collagen type II alpha 1 chain (Col2a1) and the sulfated proteoglycan: aggrecan (ACAN) (Naba et al., 2015; Ariosa-Morejon et al., 2021). This matrix is critical for the biomechanical properties of cartilage, providing essential elastic support to distribute pressure and shear stress during joint movement. The ECM is produced by chondrocytes, specialized cells responsible for maintaining cartilage homeostasis by balancing

anabolic and catabolic activities (Wieland et al., 2005). Type II collagen, a fibrillar collagen, is secreted as triple-helical homotrimers of Col2a1, which associate with collagen type XI alpha 1 chain (Col11a1) and collagen type IX alpha 1 chain (Col9a1) IX collagens to form heterotypic fibrils within the cartilage tissue (Zhang, Hu, & Athanasiou, 2009; Bacenkova et al., 2023). ACAN, another major ECM component, has a long core protein with approximately 200 glycosaminoglycan (GAG) chains, primarily composed of dermatan and chondroitin sulfate (Cortes-Medina et al., 2023; Shen et al., 2023). These GAG chains carry a high negative charge, attracting cations (mainly sodium) and water, which are essential for maintaining the tissue's hydration and mechanical properties.

2.1.1.2 Chondrocytes: Chondrocytes, derived primarily from the mesoderm, are the sole cellular components of normal cartilage. Their terminal differentiation defines the type of cartilage they produce-whether hyaline, fibrous, or elastic (Heinegard & Saxne, 2011; McNulty & Guilak, 2015; Nimeskern et al. 2015; Nahian & Sapra, 2023). In articular cartilage, chondrocytes typically persist without further division once skeletal maturity is reached. However, in the epiphyseal growth plate, these cells differentiate to support endochondral ossification, after which they either undergo apoptosis or transform into osteoblasts (Shum & Nuckolls, 2001; Goldring, 2007). Chondrocytes are essential for both the synthesis and degradation of ECM components, including collagen and proteoglycans, as well as matrix-degrading enzymes like collagenase, neutral proteinases, and cathepsins (Green & Lund, 2005; Lu et al., 2011; Troeberg & Nagase, 2012). This dual role positions chondrocytes as key regulators of cartilage homeostasis (Goldring, 2007; Teng et al., 2017; Nahian & Sapra, 2023), balancing synthesis with breakdown. The pericellular matrix surrounding each chondrocyte contains type VI collagen, along with proteoglycans such as decorin and ACAN (Fujii et al., 2022). During chondrogenesis, chondrocytes actively synthesize the ECM, and they continue to regulate matrix turnover in mature cartilage to ensure the tissue's proper function. The organization and renewal of ECM components are critical to maintaining normal cartilage properties (Gentili & Cancedda, 2009; Sophia Fox, Bedi, & Rodeo, 2009; Michelacci, Baccarin, & Rodrigues, 2023). Proliferating chondrocytes exhibit strong expression of essential ECM genes, including Acan, Col2a1, Col9a1, and Col11a1. In contrast, hypertrophic chondrocytes

predominantly express collagen type X alpha 1 chain (Col10a1), osteopontin, and matrix metalloproteinases-9 (MMP-9) and metalloproteinases-13 (MMP-13). These gene expression patterns are regulated by master transcription factors, which orchestrate the differentiation of MSCs into chondrocytes and subsequently guide the transition from proliferating chondrocytes to hypertrophic chondrocytes (de Crombrugghe et al., 2000; Leung et al., 2011; Liu et al., 2017; Michelacci, Baccarin, & Rodrigues, 2023).

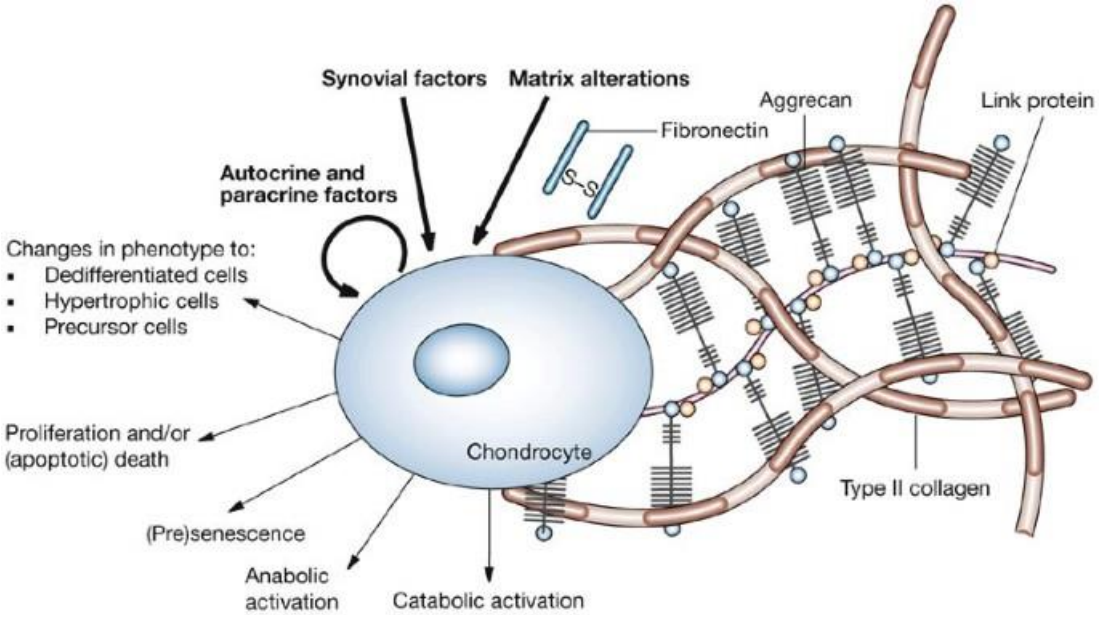

Figure 2.2 Chondrocytes in the pathogenesis of OA. (Aigner et al., 2007).

2.2 Regenerative therapy for OA

Treatments for OA are typically determined by the severity of the disease and guided by a physician's recommendations, which may include medication, physical therapy, or surgery. However, many patients experience recurrence or complications following treatment. Therefore, novel and more effective procedures, such as cartilage cell application, are essential. Unfortunately, obtaining these cells involves invasive surgery, making the process both complicated and expensive (Ebihara et al., 2012; Wong et al., 2020; Chen et al., 2022; Cong et al., 2023; Wang et al., 2024). Stem cell technology is currently regarded as a promising tool for cell-based therapy. Mesenchymal stromal cells (MSCs) have gained significant attention due to their potential for transdifferentiation

from one phenotype to another, offering exciting possibilities for cellular therapies (Talèns-Visconti et al., 2006; Musial-Wysocka, Kot, & Majka, 2019; Zhuang et al., 2021). Ongoing research is investigating the potential of MSCs for treating OA. These cells exhibit several desirable properties, including tri-mesodermal differentiation into osteoblasts, chondrocytes, and adipocytes, plastic adherence, self-renewal capabilities, and the expression of unrestricted MSC markers (Baddoo et al., 2003; Dominici et al., 2006; Ambrosi et al., 2017; Wolock et al., 2019; Azadniv et al., 2020). For numerous of these reasons, MSCs are currently an excellent candidate for therapeutic purpose in clinical applications. MSCs possess the unique ability to stimulate the growth of cartilage cells and other types of cells.

2.3 Mesenchymal stem cells (MSCs)

MSCs, are multipotent adult stem cells with the ability to differentiate into multiple specialized cell types and to self-renew, at least in mesodermal lineages of cells. (Morrison et al., 1997; Urbani et al., 2006; Guadix, Zugaza, & Gálvez-Martin, 2017). MSCs were first isolated from mouse bone marrow in 1976 by Friedenstein and colleagues. (Friedenstein, Gorskaja, & Kulagina, 1976). MSCs have the ability to adhere to plastic culture dishes and resemble fibroblasts in that they are spindle-shaped cells. Additionally, the adherent cells were defined as colony-forming unit fibroblasts due to their clonogenic feature or clonal density form (Friedenstein, Chailakhjan, & Lalykina. 1970). Moreover, the cells in this group have been defined as multipotent stromal precursor cells. Subsequent research over the years has revealed that these cells have the ability to develop *in vitro* into mesodermal lineages cells like oseteoblasts, adipocytes, and chondrocytes (Ashton et al., 1980; Castro-Malaspina et al., 1980; Bab et al., 1986; Pittenger et al., 1999). MSCs are extensively interesting in their biology, phenotypes, and features ever since Friedenstein and colleagues conducted pilot research on them in 1976. According to additional research, MSCs can be isolated from the bone marrow of rodents as well as from monkey, goat, sheep, dog, pig, and human (Rozemuller et al., 2010).

2.3.1 Sources and characteristics of MSCs

Although bone marrow was the original source of MSCs, other adult connective tissues have also been identified as potential sources of MSCs, including adipose tissue (Zuk et al., 2001; Romanov et al., 2005; Gruber et al., 2010), muscle (Asakura, Komaki, & Rudnicki, 2001), dental pulp (Perry et al., 2008; Ponnaiyan et al., 2012), and peripheral blood (Tondreau et al., 2005; Chong et al., 2012). Furthermore, MSCs can be obtained from tissues of fetal origin, including placenta (In't Anker et al., 2004), amniotic fluid (Tsai et al., 2004), umbilical cord blood (Lee et al., 2004), and Wharton's jelly (Troyer & Weiss, 2008; Ishige et al., 2009; Witkowska-Zimny & Wrobel, 2011; Mishra et al., 2020; Mebarki et al., 2021; Yea et al., 2023). Because MSCs have been investigated in laboratories using varying sources and culture techniques, several studies have reported various immunophenotype of MSCs. According to these findings, MSCs have absence of unique specific antigens but slight different expression of cell surface antigens depending on their origins (Hass et al., 2011). The International Society for Cellular Therapy (ISCT) established minimal criteria for characterizing MSCs in 2006 in order to clarify any confusion (Dominici et al., 2006). The following is a minimum definition of MSC characteristics. Firstly, after being cultured under standard culture conditions, MSCs must adhere to plastic culture flasks. Secondly, they must be negative stained for human haematopoietic stem cells surface antigens such as CD34 and CD45 and positive stained for some surface antigens such as CD44, CD73, CD90, and CD105. They also need to be negative stained for human leukocytes surface antigens such as CD11b, CD14, CD19, and CD79α and for human major histocompatibility complex (MHC) class II antigen or HLA-DR. Thirdly, *in vitro* differentiation into mesodermal-lineage cells such as osteoblasts, adipocytes, and chondroblasts must be possible.

In general, MSCs that have been isolated from various tissues have similar characteristics. There have been some differences observed in the proliferation and differentiation capability of adult and fetal MSCs *in vitro*. The proliferation rate of human first-trimester fetal MSCs was higher than that of adult MSCs. This finding can be explained that fetal MSCs have longer telomeres than adult MSCs, which relates to their more primitive origins (Campagnoli et al., 2001; Guillot et al., 2007). Furthermore, MSCs isolated from bone marrow and adipose tissue shown a higher

capacity for adipogenic differentiation than MSCs obtained from umbilical cord blood (Kern et al., 2006; Rebelatto et al., 2008). Based on the fundamental standards for MSC characterization, MSCs from various origins nevertheless have certain characteristics in common even though they have certain distinct properties.

2.3.1.1 Wharton's jelly mesenchymal stem cells (WJ-MSCs)

WJ-MSCs represent a primitive stromal population with several key characteristics defined by the International Society for Cellular Therapy. They exhibit mesenchymal morphology, self-renewal capabilities, and the ability to differentiate into various cell types, including bone, cartilage, and muscle. WJ-MSCs also support hematopoietic stem cell expansion and are well-tolerated by the immune system, with the ability to home to tumors. Compared to bone marrow-derived MSCs, WJ-MSCs have superior expansion potential, faster *in vitro* growth, and a unique cytokine synthesis profile. Preclinical studies show their therapeutic efficacy in models of neurodegenerative diseases (Staff, Jones, & Singer, 2019; Mattei, & Delle Monache, 2024), cancer (Lin et al., 2019; Lim, & Khoo, 2021; Vicinanza et al., 2022), heart disease (Bagno et al., 2018; Correia et al., 2023), and OA (Wang et al., 2022; Lv et al., 2023; Copp, Robb, & Viswanathan, 2023), primarily through trophic rescue and immune modulation. WJ-MSCs fulfill the criteria for MSCs and demonstrate promising therapeutic potential due to their enhanced expansion capabilities. Further research is necessary to ascertain their long-term engraftment and multipotency *in vivo*, confirming whether they constitute a true stem cell population (Taghizadeh, Cetrulo, & Cetrulo, 2011; Marino et al., 2019; Kamal & Kassem, 2020; Drobiova et al., 2023).

WJ-MSCs were isolated from Wharton's jelly, a gelatinous substance found within the umbilical cord. Umbilical cord-derived MSCs are valuable tools in stem cell research due to their easy isolation, high proliferation rates, multi-lineage differentiation potential, and immunomodulatory properties, along with the absence of ethical concerns. Among the various compartments of the umbilical cord, Wharton's jelly has recently been identified as the best source of MSCs (Davies, Walker, & Keating, 2017; Bharti et al., 2018; Drobiova et al., 2023).

2.3.2 Differentiation abilities

MSCs are multipotent progenitor cells that can differentiate into several lineages, including bone, cartilage, fat, and muscle (Fig 2.3). MSCs are known for their

ability to differentiate into various cell types, particularly those within the mesodermal lineage. Key differentiation pathways including osteogenic differentiation, chondrogenic differentiation, and adipogenic differentiation (Benayahu, 2022). Osteogenic differentiation: MSCs can become osteoblasts, which are responsible for bone formation (Pittenger et al., 2019). Chondrogenic differentiation: MSCs can differentiate into chondrocytes, which form cartilage, making them ideal for cartilage repair therapies (Augello & Bari, 2010). Adipogenic differentiation: MSCs are also capable of transforming into adipocytes, which store energy as fat (Tivig et al., 2024). These cells retain high plasticity and exhibit regenerative potential by responding to specific environmental cues *in vitro* or *in vivo*. This versatility positions them as promising candidates for various therapeutic applications (Yang et al., 2018).

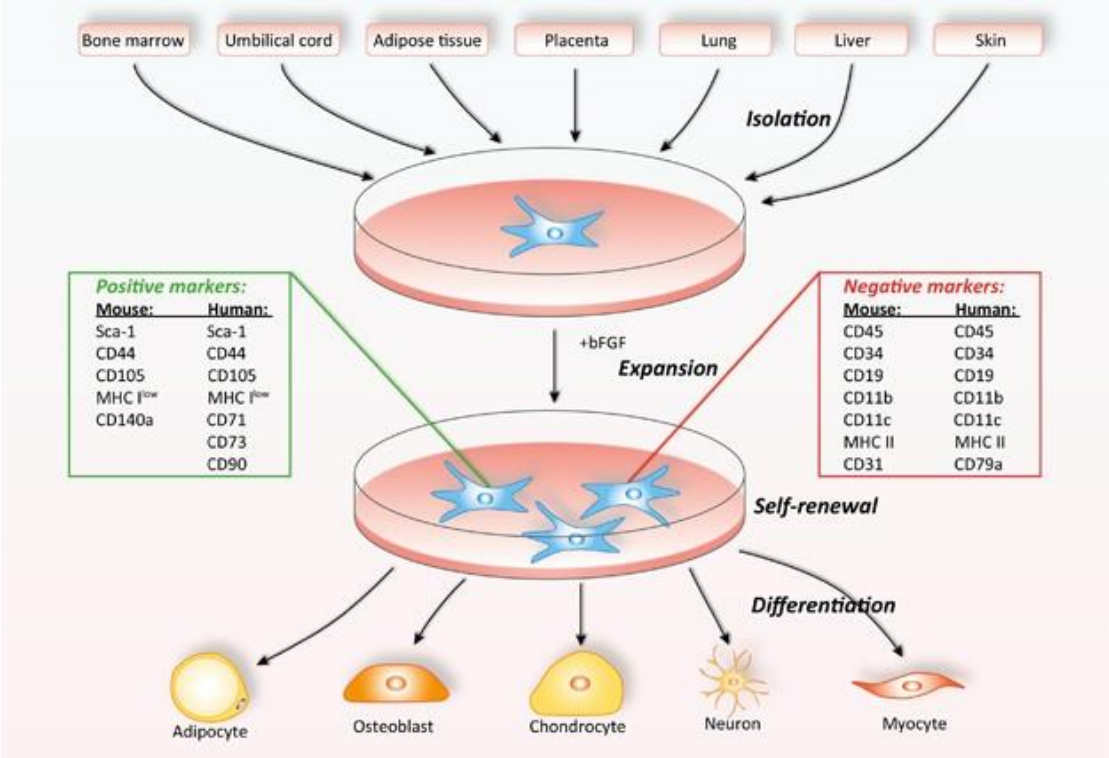

Figure 2.3 Isolation, expansion, and differentiation of MSCs (Chen et al., 2016).

MSCs are a major focus in regenerative therapies due to their robust differentiation abilities. MSCs can differentiate into mesodermal lineages, including osteoblasts (bone cells), chondrocytes (cartilage cells), and adipocytes (fat cells). This makes them essential for bone and cartilage repair (Pittenger et al., 2019; Saeedi, Halabian, & Imani Fooladi, 2019). MSCs can release bioactive molecules that promote the growth and differentiation of other stem cells, enhancing tissue regeneration (Vasanthan et al., 2020; Margiana et al., 2022). Under controlled conditions, MSCs display regenerative potential both *in vitro* and after implantation *in vivo*. This dual capability supports their therapeutic use in treating degenerative diseases, such as OA (Pittenger et al., 2019; Maldonado et al., 2023). MSCs exhibit self-renewal properties and can modulate immune responses, making them ideal for reducing inflammation and promoting tissue repair (Maldonado et al., 2023).

WJ-MSCs show high potential for chondrogenic differentiation, which is critical for cartilage repair. WJ-MSCs grown on silk scaffolds and supplemented with L-ascorbic acid (LAA) or platelet-rich plasma (PRP) demonstrated enhanced chondrogenic differentiation. These scaffolds provide a suitable 3D environment that mimics the natural ECM for cartilage formation (Barlian et al., 2020). WJ-MSCs have superior chondrogenic potential compared to bone marrow-derived MSCs. This makes WJ-MSCs preferable for therapeutic applications aimed at cartilage regeneration (Liau et al., 2019; Pelusi et al., 2021). WJ-MSCs retain their hypoimmunogenic nature during differentiation, making them suitable for allogeneic transplants without significant immune rejection risks (Voisin et al., 2020). These characteristics make WJ-MSCs promising candidates for future applications in regenerative medicine, especially for treating joint disorders and cartilage injuries.

2.4 Chondrogenesis

Chondrogenesis (Fig 2.4) refers to the process by which chondrocytes and cartilage tissues are formed. It begins with the recruitment and migration of MSCs, followed by the condensation of progenitor cells. This process then proceeds through chondrocyte differentiation and concludes with the maturation of these cells (Barna, & Niswander, 2007; Zuscik et al., 2008; Chen et al., 2009; Goldring, 2012; Yang et al., 2022). The regulation of chondrogenic differentiation in MSCs involves

intracellular proteins, receptor ligands, and transcription factors. Any disruption in these signaling pathways can lead to defective chondrocyte production (Green et al., 2015; Yang et al., 2022). The most important biological regulators of chondrogenesis and their interactions involve multiple signaling pathways and transcriptional regulation. Key signaling pathways include fibroblast growth factors (FGFs), transforming growth factor-ß (TGF-ß)/bone morphogenic proteins (BMPs), and the Wnt/ß-catenin pathway (Mariani, Pulsatelli, & Facchini, 2014; Green et al., 2015). The transcriptional regulation of chondrogenesis is crucial, involving key factors such as the SRY-related high-mobility group-box gene 9 (Sox9) and Runt-related transcription factors (Runx) (Zhou, Zheng, & Engin, 2006; Ohba et al., 2015; Nagata et al., 2022).

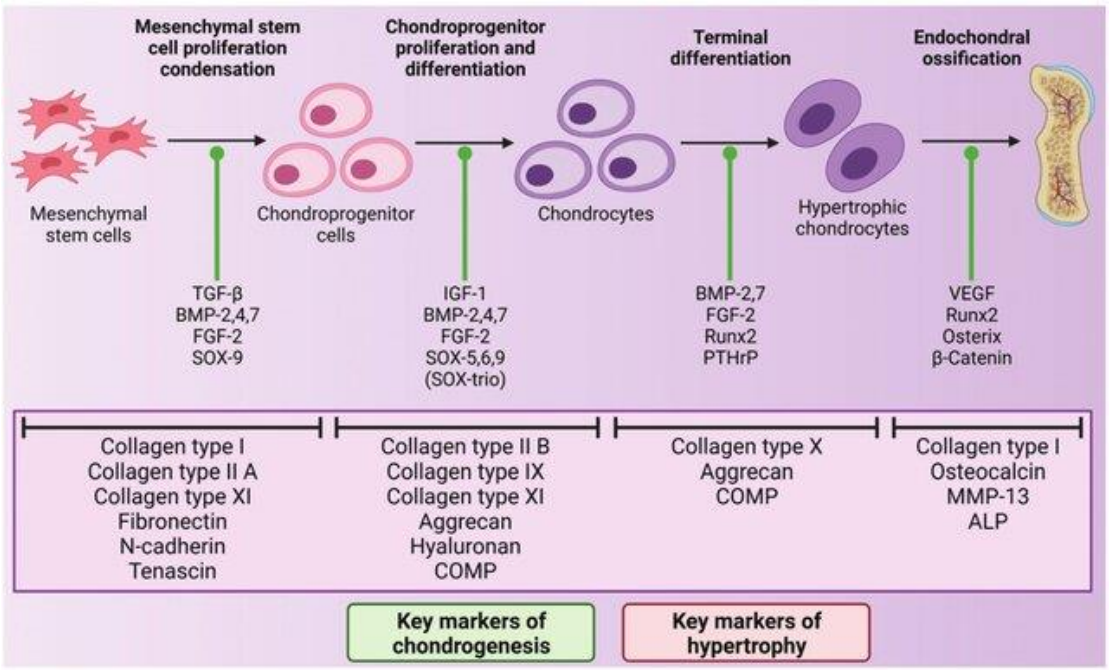

Figure 2.4 A schematic of the process of chondrogenesis (O'Shea, Curtin, & O'Brien, 2022).

2.4.1 Signaling pathways

2.4.1.1 WNT signaling

Wnt signaling can both promote and suppress the chondrogenic differentiation of progenitor cells in both embryonic and adult stages (Hartmann, & Tabin, 2000; Fischer, Boland, & Tuan, 2002; Yano, Kugimiya, & Ohba, 2005; Leijten et al.,

2012; Cheng et al., 2022; Liu et al., 2022). The antagonistic effects may result from various factors, such as the target cells' differentiation phase, the level of Wnt activity, the specific type of Wnt signal, and crosstalk with other signaling pathways like the TGF-ß/BMP pathway (Fischer, Boland, & Tuan, 2002; Zhou, Eid, & Glowacki, 2004; Hartmann, 2006; Wang et al., 2014; Vlashi et al., 2023; Wu et al., 2024). Although the role of Wnt signaling in the early phase of chondrogenesis remains poorly understood, studies on specific Wnt proteins indicate that Wnt plays a crucial role in promoting the migration and condensation of chondrogenic cells (Timothy et al., 2005; Bradley, & Driss, 2011; Kamel, Hoyos, & Rochard, 2013; Green et al., 2015; Hu et al., 2024). Wnt5b has been shown to regulate chondroprogenitor cell migration by activating the planar cell polarity pathway. It also reduces cell adhesion by suppressing the expression of a destabilizing cadherin receptor and modulates the activity of various other cadherins (Lee, Kim, & Cho, 2008; Bradley & Drissi, 2011; Green et al., 2015; Suthon et al., 2021). Wnt proteins such as Wnt1, Wnt3a, Wnt4, Wnt5b, and Wnt8 have been demonstrated to activate the canonical pathway *in vitro* (Hartmann, & Tabin, 2000; Maiese et al., 2008; Zhang et al., 2021; Qin et al., 2023). Wnt3a and Wnt5b promote chondrocyte differentiation and suppress chondrocyte hypertrophy, a critical step in the later stages of chondrogenesis (Veeman, Axelrod, & Moon, 2003; Hartmann, 2007; Green et al., 2015). In contrast, Wnt1, Wnt4, and Wnt8 have been reported to inhibit chondrocyte differentiation while promoting hypertrophy.

2.4.1.2 FGF signaling

FGF are a family of heparin-binding growth factors which play a role in the proliferation and differentiation of a variety of tissues. In general, FGFs signaling are known to be positive regulators of chondrogenic differentiation and hypertrophy (Ornitz & Marie, 2015; Green et al., 2015). The development of chondrocyte differentiation and maturation has been demonstrated to be affected by the sequential production of three FGFs: FGF2, FGF9, and FGF18 (Hellingman, Koevoet, & Kops, 2010; Oseni et al., 2011; Correa, Somoza, & Lin, 2015). FGF2 has been shown to effectively enhance chondrocyte proliferation and ready chondroprogenitor cells for terminal differentiation. However, until recently, the mechanisms through which FGF2 produces these effects have remained largely unknown (Oseni et al., 2011). FGF2 can trigger a sequence of intracellular signals, such as MAPK/ERK, that promote cell cycle

progression, thus accelerating chondrocyte proliferation. Additionally, FGF2 appears to modulate the activity of other growth factors like TGF- ß, enhance TGF-ß1 expression (Stevens, Marini, & Martin, 2004; Solchaga et al., 2009; Handorf, & Li, 2011; Green et al., 2015; Chen et al., 2021) which typically counterbalances proliferation to favor differentiation. FGF2 has been shown to downregulate TGF-ß2 expression, which contributes to FGF2-induced chondrocyte differentiation (Ito et al., 2008). Recent findings indicate that FGF2 can promote both chondrocyte differentiation and proliferation when TGF-ß3 is present, though it concurrently inhibits chondrocyte hypertrophy (Richter et al., 2009). Furthermore, FGF2 regulates chondrogenic differentiation via MAPK and Wnt signaling pathways (Solchaga et al., 2005).

2.4.1.3 TGF-ß /BMP signaling

The TGF-ß superfamily, in particular TGF-ß and BMP, are essential for multiple stages of embryonic chondrogenesis (Goldring, Tsuchimochi, & Ijiri, 2006; Cleary, van Osch, & Brama, 2015). This pathway, particularly the TGF-ß/BMP signaling pathway, is frequently used to promote chondrocyte differentiation in both MSCs and expanded chondrocyte populations (Goldring, Tsuchimochi, & Ijiri, 2006; Karamboulas, Dranse, & Underhill, 2010; Green et al., 2015). Chondrogenesis and osteogenesis are known to be majorly regulated by TGF-ß1 and TGF-ß2 (Sandberg, Autio-Harmainen, & Vuorio, 1998; Carrington, Roberts, & Flanders, 1988). TGF-ß also signals the transition from proliferation to the onset of chondrocyte differentiation (Cleary, van Osch, & Brama, 2015). This progression has been widely studied both *in vivo*, during embryonic cartilage development, and *in vitro*, using MSCs (Augustyniak, Trzeciak, & Richter, 2015). TGF-ß has been demonstrated to mediate the differentiation of chondroprogenitor cells into chondrocytes in addition to inhibiting proliferation (Rosen, Stempien, & Thompson, 1986). TGF- ß has long served as the primary growth factor model for inducing chondrogenesis in MSCs *in vitro* (Johnstone, Hering, & Caplan, 1998). TGF-ß, especially TGF-ß1 and TGF-ß3, activates Smad-dependent pathways (e.g., Smad2/3 and Smad1/5/8) to initiate and regulate chondrogenic differentiation (Cleary, van Osch, & Brama, 2015; Green et al., 2015; Chen et al., 2021). In MSCs, TGF-ß activates MAPK proteins, including p38, ERK, and JNK, which subsequently downregulate N-cadherin expression by inhibiting Wnt-mediated ß-catenin nuclear translocation (Tuli, Tuli, & Nandi, 2003; Rodríguez-García et al., 2017; Luo, 2017; Loh et

al., 2019). This reduction in N-cadherin expression is a critical step required for the transition from cell condensation to differentiation (Oh, Chang, & Yoon, 2000; Tufan, &Tuan, 2001; Tufan et al., 2002; Loh et al., 2019).

BMPs have been identified as key positive regulators in ectopic chondrogenesis and endochondral ossification (Guo et al., 2014). Inhibiting BMP signaling has been shown to reduce cartilage formation. BMP family members are essential for chondro-progenitor cell condensation and chondrocyte differentiation *in vivo* and *in vitro* (Yoon, Ovchinnikov, & Yoshii, 2005; Hidaka, & Goldring, 2008; Long, & Ornitz, 2013). Joint development, which also involves chondrogenesis, depends on BMPs such as BMP-2, BMP-4, and GDF5 (Coleman & Tuan, 2003; Hidaka, & Goldring, 2008). Application of BMP-2, -4, -6, -7, -9, -13, and -15 enhance type II collagen synthesis in articular chondrocytes *in vitro* (Chubinskaya, & Kuettner, 2003; Gründer, Gaissmaier, & Fritz, 2004; Hidaka, & Goldring, 2008; Cleary, van Osch, & Brama, 2015). The implications of BMP signaling are probably concentration-dependent and time-dependent, although BMPs participate in the condensation and differentiation stages, which are displayed to be mutually exclusive (Hidaka, & Goldring, 2008).

2.4.2 Transcriptional regulation

2.4.2.1 Sox9

Sox9 is a prochondrogenic transcription factor that is arguably the single most important regulator of chondrogenesis. During chondrogenesis, multiple signaling pathways regulate the expression and activity of Sox9 (Fig 2.5), (Augello, & De Bari, 2010; Kozhemyakina, Lassar, & Zelzer, 2015). Full expression of the chondrocyte phenotype requires high levels of Sox9 (Lefebvre, & Dvir-Ginzberg, 2016). Sox9 expression is induced by compressive force and is influenced by a variety of prochondrogenic factors, including RelA, Pref-1, and Arib5b, as well as two other Sox transcription factors, Sox5 and Sox6 (Ushita, Saito, & Ikeda, 2009; Wang, & Sul, 2009; Yamashita, Miyaki, & Kato, 2012; Hata, Takashima, & Amano, 2013; Juhász, Matta, & Somogyi, 2014). Sox9, as a transcription factor, directly regulates the genes it interacts with through its mechanism of action. It activates elements in the promoters of Col2a1, Col9a1, Col11a2, Bbf2h7-sec23a, and ACAN by interacting with two binding sites for HMG-domain proteins (Akiyama, Chaboissier, & Martin, 2002; Akiyama, 2008; Leung et al., 2011; Hino, Saito, & Kido, 2014). Sox9 primarily regulates chondrocyte proliferation

and differentiation by directly controlling the expression of various chondrocyte-specific genes. Furthermore, many studies have highlighted Sox9's role in the earlier phases of chondrogenesis, particularly during condensation. Research indicates that Sox9 expression levels are significantly elevated in condensing mesenchymal progenitors, both *in vitro* and *in vivo*, whereas Sox9 knockout mice are unable to undergo mesenchymal cell condensation (Mori-Akiyama et al., 2003; Akiyama, Lyons, & Mori-Akiyama, 2004; Quintana, Zur Nieden, & Semino, 2009). Sox9 induces MSCs differentiate into chondrocytes and promotes proliferation (Chen et al., 2021) FGF2 induces chondrocyte proliferation by upregulating the expression levels of Sox9 (Pan, Yu, & Chen, 2008; Shi, Wang, & Acton, 2015). Sox9 enhances the prochondrogenic properties of BMP2 while also inhibiting BMP2-induced osteogenic differentiation and endochondral ossification (Liao, Hu, & Zhou, 2014). Sox9 blocks the activation of the transcription factor Runx2, which is the key factor in reducing chondrocyte maturation, thereby repressing chondrocyte hypertrophy (Zhou et al., 2006). Sox9 inhibits Wnt signaling by interacting with β-catenin, which is known to promote chondrocyte hypertrophy (Topol et al., 2009). Sox9 may inhibit the expression of genes associated with hypertrophic chondrocytes, including Col10a1 and VEGFA (Hattori et al., 2010; Leung et al., 2011).

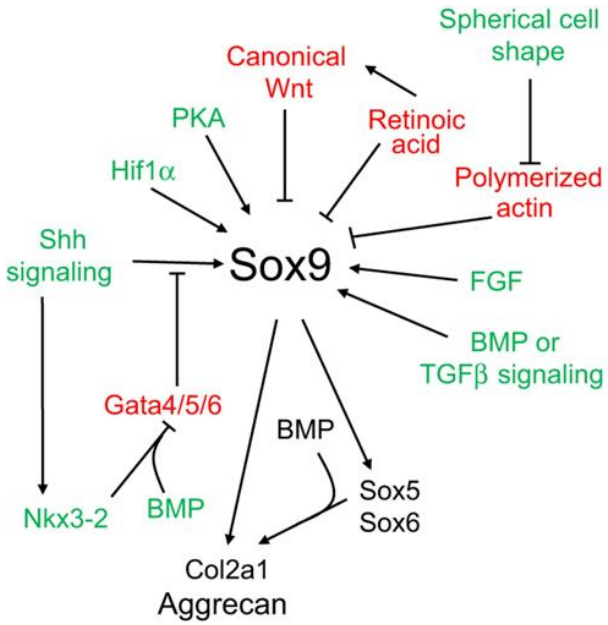

Figure 2.5 Multiple signaling pathways regulate the expression and activity of Sox9 during chondrogenesis (Kozhemyakina, Lassar, & Zelzer, 2015).

2.4.2.2 Runt-related transcription factors (Runx)

Runx proteins, which include Runx2 and Runx3, are a family of transcription factors that play crucial roles in chondrogenesis and are essential for the maturation of chondrocytes (Yoshida et al., 2004; Komori, 2018; Rashid, Chen, & Javed, 2021; Nakata et al., 2022, Komori, 2024). Runx2 regulates osteogenesis and chondrocyte proliferation through its interaction with PI3K-Akt signaling (Fujita, Azuma, & Fukuyama, 2004). Sox9 plays a dominant role in controlling Runx2, particularly in MSCs committed to the chondrogenic lineage (Akiyama et al., 2005; Zhou, Zheng, & Engin, 2006; Chan et al., 2021). This regulatory relationship becomes more complex with the involvement of CypA, which promotes the expression of both Runx2 and Sox9 in chondro-progenitor cells. As expected, knockdown of CypA results in reduced chondrogenesis and impaired endochondral ossification (Guo, Shen, & Kwak, 2015). Another regulatory mechanism for controlling Runx2 involves Nkx3.2. This factor represses Runx2 activity by directly interacting with its promoter, and such repression is essential for the progression of BMP-induced chondrogenesis (Lengner, Hassan, & Serra, 2005; Kawato et al., 2011; Rainbow, Won, & Zeng; 2014). The regulation of Runx2 expression plays a crucial role in chondrogenesis, as it influences multiple processes in MSCs. Runx2 is expressed at low levels in proliferating chondrocytes but increases as these cells exit the cell cycle. Notably, Runx2 expression in proliferating chondrocytes has been found to accelerate their transition to hypertrophy (Stricker et al., 2002; Hinoi et al., 2006; Chen et al., 2021; Rashid, Chen, & Javed, 2021; Komoti, 2022; Nagata et al., 2022; Rashid et al., 2024), the removal of RUNX2 inhibits normal mineralization of hypertrophic cartilage. Conversely, several studies show that ectopic expression of Runx2 in immature chondrocytes induces the expression of hypertrophic markers, including Col10a1, MMP13, and VEGF (Takeda et al., 2001; Stricker et al., 2002; Zheng, Zhou, & Morello, 2003; Li et al., 2011; Wang et al., 2014; Kozhemyakina, Lassar, & Zelzer, 2015; Rashid, Chen, & Javed, 2021; Chen et al., 2023).

2.5 Animal model for chondrocytes transplantation

cartilage regeneration. Mice, guinea pig, rabbits, dogs, goats, pigs, and horses models are frequently employed in cartilage repair studies due to their relevance to human joint mechanics and cartilage structure (Chu, Szczodry, & Bruno, 2010; Cook

et al., 2014; Kuyinu et al., 2016). Animal models provide valuable insights into developing and testing tissue-engineering techniques, helping researchers refine methods to repair damaged articular cartilage (Reinholz et al., 2004). A systematic review covering literature from 2000 to 2022 highlights advances and trends in using animal models for articular cartilage research, emphasizing their importance for improving transplantation techniques (Vinod et al., 2023).

Animal studies have provided valuable insights into the progression and treatment of OA in different models. Unilateral focal degeneration was noted in the medial tibial plateau of 2 out of 5 three-month-old guinea pigs, with age-related severity increasing to moderate-to-severe degeneration in all animals by 12-18 months, while the lateral knee joint remained unaffected (Bendele & Hulman, 1988). In animal study involving adult merino sheep, intraarticular hyaluronic acid (HA) injections post-unilateral medial meniscectomy were shown to mitigate changes in articular cartilage and subchondral bone associated with early OA (Armstrong, Read, & Ghosh, 1994). In 2003, Tessier et al. used MRI to assess longitudinal changes in knee joint integrity in guinea pigs with spontaneous OA, revealing cartilage swelling and fragmentation over time, culminating in a 36% loss of tibial cartilage volume between 9 and 12 months. A reliable histological scoring system was developed for OA assessment in guinea pigs (Kraus et al., 2010). Research by Ebihara et al., 2012 demonstrated that layered chondrocyte sheets can promote cartilage repair in a minipig model, though some defects remained. Sato et al., 2012 investigated the transplantation of MSCs in HA into guinea pig knees, finding partial cartilage repair in the HA-MSC group. Yan et al., 2014 documented age-related degeneration of cartilage and subchondral bone in female Dunkin Hartley guinea pigs, starting from 3 months, but found no significant correlations with estradiol levels or bone mineral density.

The use of Dunkin Hartley guinea pigs as an animal model for studying spontaneous cartilage degeneration in the knee joint, which is similar to OA in humans, has been well established (Tessier et al., 2003; Yan et al., 2014; Wang et al., 2021). Researchers reported that the knee joint of guinea pigs closely resembles that of humans affected by OA (Fernandez et al., 1997; Kraus et al., 2010). Moreover, spontaneous cartilage degeneration in the Dunkin-Hartley guinea pigs are used for study (Bendele & Hulman, 1988). Previous studies demonstrated that injecting MSCs

with hyaluronic acid (HA) into the articular cartilage of guinea pigs with OA led to recovery (Sato et al., 2012). HA-based formulations are currently delivered into the joint to relieve pain and improve joint mobility of OA patients by partial restoration of the rheological properties of the synovial fluid (La Gatta et al., 2021). These findings highlight how animal models, especially guinea pigs and sheep, are instrumental in studying OA mechanisms and developing new therapeutic strategies.

2.6 References

Aigner, T., Söder, S., Gebhard, P. M., McAlinden, A., & Hagg, J. (2007). Mechanisms of Disease: role of chondrocytes in the pathogenesis of osteoarthritis-structure, chaos and senescence. **Nat. Rev. Rheumatol.** 3: 391–399.

Akiyama, H. (2008). Control of chondrogenesis by the transcription factor Sox9. **Mod. Rheumatol.** 18: 213-219.

Akiyama, H., Chaboissier, M. C., & Martin, J. F. (2002). The transcription factor Sox9 has essential roles in successive steps of the chondrocyte differentiation pathway and is required for expression of Sox5 and Sox6. **Genes. Dev.** 16: 2813-2828.

Akiyama, H., Kim, J. E., Nakashima, K., Balmes, G., Iwai, N., Deng, J. M., Zhang, Z., Martin, J. F., Behringer, R. R., & Nakamura, T. (2005). Osteo-chondroprogenitor cells are derived from Sox9 expressing precursors. **Proc. Natl. Acad. Sci. USA.** 102: 14665-14670.

Akiyama, H., Lyons, J. P., & Mori-Akiyama, Y. (2004). Interactions between Sox9 and beta-catenin control chondrocyte differentiation. **Genes. Dev.** 18: 1072-1087.

Ambrosi, T. H., Scialdone, A., Graja, A., Gohlke, S., Jank, A.-M., Bocian, C., Woelk, L., Fan, H., Logan, D. W., Schürmann, A., Saraiva, L. S., & Schulz, T. J. (2017). Adipocyte Accumulation in the Bone Marrow during Obesity and Aging Impairs Stem Cell-Based Hematopoietic and Bone Regeneration. **Cell Stem Cell.** 20: 771-784.

Ariosa-Morejon, Y., Santos, A., Fischer, R., Davis, S., Charles, P., Thakker, R., Wann, A. K., & Vincent T. (2021). Age-dependent changes in protein incorporation into collagen-rich tissues of mice by *in vivo* pulsed SILAC labelling. **Elife.** 10: e66635.

Armstrong, S., Read, R., & Ghosh, P. (1994). The effects of intraarticular hyaluronan on cartilage and subchondral bone changes in an ovine model of early osteoarthritis. **J. Rheumatol.** 21: 680-688.

Asakura, A., Komaki, M. & Rudnicki, M. (2001). Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation. **Differentiation.** 68(4-5): 245-253.

Ashton, B. A., Allen, T. D., Howlett, C. R., Eaglesom, C. C., Hattori, A., & Owen, M. (1980). Formation of bone and cartilage by marrow stromal cells in diffusion chambers *in vivo*. **Clin. Orthop. Relat. Res.** 151: 294-307.

Augello, A., & De Bari, C. (2010). The regulation of differentiation in mesenchymal stem cells. **Hum. Gene. Ther.** 21(10): 1226-1238.

Augustyniak, E., Trzeciak, T., & Richter, M. (2015). The role of growth factors in stem cell-directed chondrogenesis: a real hope for damaged cartilage regeneration. **Int. Orthop.** 39: 995-1003.

Azadniv, M., Myers, J. R., McMurray, H. R., Guo, N., Rock, P., Coppage, M. L., Ashton, J., Becker, M. W., Calvi, L. M., & Liesveld, J. L. (2020). Bone Marrow Mesenchymal Stromal Cells from Acute Myelogenous Leukemia Patients Demonstrate Adipogenic Differentiation Propensity with Implications for Leukemia Cell Support. **Leukemia.** 34: 391-403.

Bab, I., Ashton, B. A., Gazit, D., Marx, G., Williamson, M. C. & Owen, M. E. (1986). Kinetics and differentiation of marrow stromal cells in diffusion chambers *in vivo*. **J. Cell Sci.** 84: 139-151.

Bacenkova, D., Trebunova, M., Demeterova, J., & Zivcak, J. (2023). Human Chondrocytes, Metabolism of Articular Cartilage, and Strategies for Application to Tissue Engineering. I**nt. J. Mol. Sci.** 24(23): 17096.

Baddoo, M., Hill, K., Wilkinson, R., Gaupp, D., Hughes, C., Kopen, G. C., & Phinney, D. G. (2003). Characterization of Mesenchymal Stem Cells Isolated from Murine Bone Marrow by Negative Selection. **J. Cell. Biochem.** 89: 1235-1249.

Bagno, L., Hatzistergos, K. E., Balkan, W., & Hare, J. M. (2018). Mesenchymal Stem Cell-Based Therapy for Cardiovascular Disease: Progress and Challenges. **Mol. Ther.** 26(7): 1610-1623.

Barlian, A., Judawisastra, H., Ridwan, A., Wahyuni, A. R., & Lingga, M. E. (2020). Chondrogenic differentiation of Wharton's Jelly mesenchymal stem cells on silk spidroin-fibroin mix scaffold supplemented with L-ascorbic acid and platelet rich plasma. **Sci. Rep.** 10(1): 19449.

Barna, M., & Niswander L. (2007). Visualization of cartilage formation: insight into cellular properties of skeletal progenitors and chondrodysplasia syndromes. **Dev. Cell.** 12: 931-941.

Baumann, C. A., Hinckel, B. B., Bozynski, C. C., & Farr, J. (2019). Articular Cartilage: Structure and Restoration. In Yanke, A., & Cole, B. (eds). *Joint Preservation of the Knee* (pp. 3-24). Cham: Springer.

Bendele, A. M., & Hulman, J. F. (1988). Spontaneous cartilage degeneration in guinea pigs. **Arthritis Rheum.** 31(4): 561-565.

Benayahu, D. (2022). Mesenchymal stem cell differentiation and usage for biotechnology applications: tissue engineering and food manufacturing. **Biomater. Transl.** 3(1): 17-23.

Bharti, D., Shivakumar, S. B., Park, J. K., Ullah, I., Subbarao, R. B., Park, J. S., Lee, S. L., Park, B. W., & Rho, G. J. (2018). Comparative analysis of human Wharton's jelly mesenchymal stem cells derived from different parts of the same umbilical cord. **Cell Tissue Res.** 372(1): 51-65.

Bradley, E. W., & Drissi, M. H. (2011). Wnt5b regulates mesenchymal cell aggregation and chondrocyte differentiation through the planar cell polarity pathway. **J. Cell. Physiol.** 226: 1683-1693.

Campagnoli, C., Roberts, I. A., Kumar, S., Bennett, P. R., Bellantuono, I. & Fisk, N. M. (2001). Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. **Blood.** 98(8): 2396-2402.

Carrington, J. L., Roberts, A. B., & Flanders, K. C. (1988). Accumulation, localization, and compartmentation of transforming growth factor beta during endochondral bone development. **J. Cell Biol.** 107: 1969-1975.

Castro-Malaspina, H., Gay, R. E., Resnick, G., Kapoor, N., Meyers, P., Chiarieri, D., McKenzie, S., Broxmeyer, H. E., & Moore, M. A. (1980). Characterization of human bone marrow fibroblast colony-forming cells (CFU-F) and their progeny. **Blood.** 56(2): 289-301.

Chan, W. C. W., Tan, Z., To, M. K. T., & Chan, D. (2021). Regulation and Role of Transcription Factors in Osteogenesis. **Int. J. Mol. Sci.** 22(11): 5445.

Chen, H., Tan, X-N., Hu, S., Liu, R-Q., Peng, L-H., Li, Y-M., & Wu, P. (2021). Molecular Mechanisms of Chondrocyte Proliferation and Differentiation. **Cell Dev. Biol.** 9: 664168.

Chen, N., Wu, R. W. H., Lam, Y., Chan, W. C. W., & Chan, D. (2023). Hypertrophic chondrocytes at the junction of musculoskeletal structures. **Bone Rep.** 19: 101698.

Chen, Q., Shou, P., Zheng, C., Jiang, M., Cao, G., Yang, Q., Cao, J., Xie, N., Velletri, T., Zhang, X., Xu, C., Zhang, L., Yang, H., Hou, J., Wang, Y., & Shi, Y. (2016). Fate decision of mesenchymal stem cells: Adipocytes or osteoblasts. **Cell Death Differ.** 23(7): 1-12.

Chen, W-H., Lai, M-T., Wu, A., Wu, C-C., Gelovani, J. G., Lin, C-T., Hung, S-C., Chiu, W-T., & Deng, W-P. (2009). *In vitro* stage-specific chondrogenesis of mesenchymal stem cells committed to chondrocytes. **Arthritis Rheumatol.** 60(2): 450-459.

Chen, Y., Zhang, C., Zhang, S., Qi, H., Zhang, D., Li, Y., & Fang, J. (2022). Novel advances in strategies and applications of artificial articular cartilage. **Front. Bioeng. Biotechnol.** 10: 987999.

Cheng, B., Feng, F., Shi, F., Huang, J., Zhang, S., Quan, Y., Tu, T., Liu, Y., Wang, J., Zhao, Y., & Zhang, M. (2022). Distinctive Roles of Wnt Signaling in Chondrogenic Differentiation of BMSCs under Coupling of Pressure and Platelet-Rich Fibrin. **Tissue Eng. Regen. Med.** 19(4): 823-837.

Chong, P. P., Selvaratnam, L., Abbas, A. A. & Kamarul, T. (2012). Human peripheral blood derived mesenchymal stem cells demonstrate similar characteristics and chondrogenic differentiation potential to bone marrow derived mesenchymal stem cells. **J. Orthop. Res.** 30(4): 634-642.

Chu, C. R., Szczodry, M., & Bruno, S. (2010). Animal models for cartilage regeneration and repair. **Tissue Eng. Part B Rev.** 16(1): 105-115.

Cleary, M. A., van Osch, G. J., & Brama, P. A. (2015). FGF, TGF-β and Wnt crosstalk: embryonic to *in vitro* cartilage development from mesenchymal stem cells. **J. Tissue Eng. Regen.** 9: 332-342.

Coleman, C. M. & Tuan, R. S. (2003). Growth/differentiation factor 5 enhances chondrocyte maturation. **Dev. Dyn.** 228: 208-216.

Cong, B., Sun, T., Zhao, Y., & Chen, M. (2023). Current and Novel Therapeutics for Articular Cartilage Repair and Regeneration. **Ther. Clin. Risk Manag.** 19: 485-502.

Cook, J. L., Hung, C. T., Kuroki, K., Stoker, A. M., Cook, C. R., Pfeiffer, F. M., Sherman, S. L., & Stannard, J. P. (2014). Animal models of cartilage repair. **Bone Joint Res.** 3(4): 89-94.

Copp, G., Robb, K. P. & Viswanathan, S. (2023). Culture-expanded mesenchymal stromal cell therapy: does it work in knee osteoarthritis? A pathway to clinical success. **Cell Mol. Immunol.** 20: 626-650.

Correa, D., Somoza, R. A., & Lin, P. (2015). Sequential exposure to fibroblast growth factors (FGF) 2, 9 and 18 enhances hMSC chondrogenic differentiation. **Osteoarthritis Cartilage.** 23: 443-453.

Correia, C. D., Ferreira, A., Fernandes, M. T., Silva, B. M., Esteves, F., Leitão, H. S., Bragança, J., & Calado, S. M. (2023). Human Stem Cells for Cardiac Disease Modeling and Preclinical and Clinical Applications-Are We on the Road to Success? **Cells.** 12(13): 1727.

Cortes-Medina, M., Bushman, A. R., Beshay, P. E., Adorno, J. J., Menyhert, M. M., Hildebrand, R. M., Agarwal, S. S., Avendano, A., & Song, J. W. (2023). Chondroitin sulfate, dermatan sulfate, and hyaluronic acid differentially modify the biophysical properties of collagen-based hydrogels. **bioRxiv.** 541626.

Chubinskaya, S., &Kuettner, K. E. (2003). Regulation of osteogenic proteins by chondrocytes. **Int. J. Biochem. Cell Biol.** 35: 1323-1340.

Davies, J. E., Walker, J. T., & Keating, A. (2017). Concise Review: Wharton's Jelly: The Rich, but Enigmatic, Source of Mesenchymal Stromal Cells. **Stem Cells Transl. Med.** 6(7): 1620-1630.

de Crombrugghe, B., Lefebvre, V., Behringer, R., Bi, W., Murakami, S., & Huang, W. (2000). Transcriptional mechanisms of chondrocyte differentiation. **Matrix Biol.** 19: 389-394.

Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D., Deans, R., Keating, A., Prockop, D., & Horwitz, E. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement. **Cytotherapy.** 8: 315-317.

Drobiova, H., Sindhu, S., Ahmad, R., Haddad, D., Al-Mulla, F., & Al Madhoun, A. (2023). Wharton's jelly mesenchymal stem cells: a concise review of their secretome and prospective clinical applications. **Cell Dev. Biol.** 11: 1211217.

Ebihara, G., Sato, M., Yamato, M., Mitani, G., Kutsuna, T., Nagai, T., Ito, S., Ukai, T., Kobayashi, M., & Kokubo, M. (2012). Cartilage repair in transplanted scaffold-free chondrocyte sheets using a minipig model. **Biomaterials.** 33: 3846–3851.

Fernandez, M. L., Vergara-Jimenez, M., Conde, K., Behr, T., & Abdel-Fattah, G. (1997). Regulation of apolipoprotein B-containing lipoproteins by dietary soluble fiber in guinea pigs. **Am. J. Clin. Nutr.** 65(3): 814-822.

Frech, T. M., & Clegg, D. O. (2007). The utility of nutraceuticals in the treatment of osteoarthritis. **Curr. Rheumatol. Rep.** 9(1): 25-30.

Friedenstein, A. J., Chailakhjan, R. K., & Lalykina, K. S. (1970). The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. **Cell Tissue Kinet.** 3: 393–403.

Friedenstein, A. J., Gorskaja, J. F., & Kulagina, N. N. (1976). Fibroblast precursors in normal and irradiated mouse hematopoietic organs. **Exp. Hematol.** 4(5): 267-274.

Fujii, Y., Liu, L., Yagasaki, L., Inotsume, M., Chiba, T., & Asahara, H. (2022). Cartilage Homeostasis and Osteoarthritis. **Int. J. Mol. Sci.** 23(11): 6316.

Fujita, T., Azuma, Y., & Fukuyama, R. (2004). Runx2 induces osteoblast and chondrocyte differentiation and enhances their migration by coupling with PI3K-Akt signaling. **J. Cell Biol.** 166: 85-95.

Gentili, C., & Cancedda, R. (2009). Cartilage and bone extracellular matrix. **Curr. Pharm. Des.** 15 (12): 1334-1348.

Goldring, M., Tsuchimochi, K., & Ijiri, K. (2006). The control of chondrogenesis. **J. Cell Biochem.** 97: 33-44.

Goldring, M. B. (2007). Update on the Chondrocyte Lineage and Implications for Cell Therapy in Osteoarthritis. In Sharma, L., & Berenbaum, F. (eds). *Osteoarthritis:*

A companion to rheumatology. (1st Ed, pp. 53-76) Philadelphia: Mosby, Inc., an affiliate of Elsevier, Inc.

Goldring, M. B., & Goldring, S. R. (2007). Osteoarthritis. **J. Cell Physiol.** 213: 626-634.

Goldring, M. B., & Marcu, K. B. (2009). Cartilage homeostasis in health and rheumatic diseases. **Arthritis Res. Ther.** 11:224.

Goldring, M. B. (2012). Chondrogenesis, chondrocyte differentiation, and articular cartilage metabolism in health and osteoarthritis. **Ther. Adv. Musculoskelet. Dis.** 4(4): 269-285.

Green, J. D., Tollemar, V., Dougherty, M., Yan, Z., Yin, L., Ye, J., Collier, Z., Mohammed, M. K., Haydon, R. C., Luu, H. H., Kang, R., Lee, M. J., Ho, S. H., He, T. C., Shi, L. L., & Athiviraham, A. (2015). Multifaceted signaling regulators of chondrogenesis: Implications in cartilage regeneration and tissue engineering. **Genes Dis.** 2(4): 307-327.

Green, K. A., & Lund, L. R. (2005). ECM degrading proteases and tissue remodelling in the mammary gland. **Bioessays.** 27: 894–903.

Gruber, H. E., Deepe, R., Hoelscher, G. L., Ingram, J. A., Norton, H. J., Scannell, B., Loeffler, B. J., Zinchenko, N., Hanley, E. N., & Tapp, H. (2010). Human adipose-derived mesenchymal stem cells: direction to a phenotype sharing similarities with the disc, gene expression profiling, and coculture with human annulus cells. **Tissue Eng. Part A.** 16: 2843-2860.

Gründer, T., Gaissmaier, C., & Fritz, J. (2004). Bone morphogenetic protein (BMP)-2 enhances the expression of type II collagen and aggrecan in chondrocytes embedded in alginate beads. **Osteoarthritis Cartilage.** 12: 559-567.

Guadix, J. A., Zugaza, J. L., & Gálvez-Martin, P. (2017). Characteristics, applications and prospects of mesenchymal stem cells in cell therapy. **Med. Clin. (English).** 148(9): 408-414.

Guillot, P.V., Gotherstrom, C., Chan, J., Kurata, H. & Fisk, N. M. (2007). Human first-trimester fetal MSC express pluripotency markers and grow faster and have longer telomeres than adult MSC. **Stem Cells.** 25(3): 646-654.

Guo, P., Shi, Z-L., Liu, A., Lin, T., Bi, F., Shi, M., & Yan, S. G. (2014). Effects of cartilage oligomeric matrix protein on bone morphogenetic protein-2-induced differentiation of mesenchymal stem cells. **Orthop. Surg.** 6(4): 280-287.

Guo, M., Shen, J., & Kwak, J. H. (2015). A novel role of Cyclophilin A in regulation of chondrogenic commitment and endochondral ossification. **Mol. Cell Biol.**

Handorf, A., & Li, W-J. (2011). Fibroblast Growth Factor-2 Primes Human Mesenchymal Stem Cells for Enhanced Chondrogenesis. **PloS one.** 6(7): e22887.

Hartmann, C., & Tabin, C. J. (2000). Dual roles of Wnt signaling during chondrogenesis in the chicken limb. **Development.** 127: 3141-3159.

Hartmann, C. A. (2006). Wnt canon orchestrating osteoblastogenesis. **Trends Cell Biol**. 16: 151-158.

Hartmann, C. (2007). Skeletal Development - Wnts Are in Control. **Mol. Cells.** 24(2): 177-184.

Hass, R., Kasper, C., Bohm, S. & Jacobs, R. (2011). Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. **Cell Commun. Signal.** 9: 8-12.

Hata, K., Takashima, R., & Amano, K. (2013). Arid5b facilitates chondrogenesis by recruiting the histone demethylase Phf2 to Sox9-regulated genes. **Nat. Commun.** 4: 2850.

Hattori, T., Müller, C., Gebhard, S., Bauer, E., Pausch, F., Schlund, B., Bösl, M. R., Hess, A., Surmann-Schmitt, C., von der Mark, H., de Crombrugghe, B., & von der Mark, K. (2010). SOX9 is a major negative regulator of cartilage vascularization, bone marrow formation and endochondral ossification. **Development.** 137(6): 901-911.

He, Y., Li, Z., Alexander, P. G., Ocasio-Nieves, B. D., Yocum, L., Lin, H., & Tuan, R. S. (2020). Pathogenesis of Osteoarthritis: Risk Factors, Regulatory Pathways in Chondrocytes, and Experimental Models. **Biology.** 9: 194.

Heijink, A., Gomoll, A. H., & Madry, H. (2012). Biomechanical considerations in the pathogenesis of osteoarthritis of the knee, Knee Surgery, Sports Traumatology. **Arthroscopy.** 20: 423-435.

Heinegard, D., & Saxne, T. (2011). The Role of the Cartilage Matrix in Osteoarthritis. **Nat. Rev. Rheumatol.** 7 (1): 50–56.

Hellingman, C. A., Koevoet, W., & Kops, N. (2010). Fibroblast growth factor receptors in *in vitro* and *in vivo* chondrogenesis: relating tissue engineering using adult

mesenchymal stem cells to embryonic development. **Tissue Eng. Part A.** 16: 545–556.

Hidaka, C., & Goldring, M. B. (2008). Regulatory mechanisms of chondrogenesis and implications for understanding articular cartilage homeostasis. **Curr. Rheumatol. Rev.** 4: 136-147.

Hino, K., Saito, A., & Kido, M. (2014). Master regulator for chondrogenesis, Sox9, regulates transcriptional activation of the endoplasmic reticulum stress transducer BBF2H7/CREB3L2 in chondrocytes. **J. Biol. Chem.** 289: 13810-13820.

Hinoi, E., Bialek, P., Chen, Y. T., Rached, M. T., Groner, Y., Behringer, R. R., Ornitz, D. M., & Karsenty, G. (2006). Runx2 inhibits chondrocyte proliferation and hypertrophy through its expression in the perichondrium. **Genes Dev.** 20(21): 2937-42.

Hu, L., Chen, W., Qian, A., & Li, Y-P. (2024). Wnt/ß-catenin signaling components and mechanisms in bone formation, homeostasis, and disease. **Bone Res.** 12: 39.

Hunter, D. J., & Felson, D. T. (2006). Osteoarthritis. **BMJ.** 332(7542): 639-642.

In't Anker, P. S., Scherjon, S. A., Kleijburg-van der Keur, C., de Groot-Swings, G. M., Claas, F. H., Fibbe, W. E. & Kanhai, H. H. (2004). Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. **Stem Cells.** 22(7): 1338-1345.

Ishige, I., Nagamura-Inoue, T., Honda, M. J., Harnprasopwat, R., Kido, M., Sugimoto, M., Nakauchi, H., & Tojo, A. (2009). Comparison of mesenchymal stem cells derived from arterial, venous, and Wharton's jelly explants of human umbilical cord. **Int. J. Hematol.** 90: 261-269.

Ito, T., Sawada, R., Fujiwara, Y., & Tsuchiya, T. (2008). FGF-2 increases osteogenic and chondrogenic differentiation potentials of human mesenchymal stem cells by inactivation of TGF-beta signaling. **Cytotechnology.** 56: 1-7.

Johnstone, B., Hering, T. M., & Caplan, A. I. (1998). *In vitro* chondrogenesis of bone marrow-derived mesenchymal progenitor cells. **Exp. Cell Res.** 238: 265-272.

Juhász, T., Matta, C., & Somogyi, C. (2014). Mechanical loading stimulates chondrogenesis via the PKA/CREB-Sox9 and PP2A pathways in chicken micromass cultures. **Cell. Signal**. 26: 468-482**.**

Kamal, M. M., & Kassem, D. H. (2020). Therapeutic Potential of Wharton's Jelly Mesenchymal Stem Cells for Diabetes: Achievements and Challenges. **Front. Cell Dev. Biol.** 8: 16.

Kamel, G., Hoyos, T., & Rochard, L. (2013). Requirements for frzb and fzd7a in cranial neural crest convergence and extension mechanisms during zebrafish palate and jaw morphogenesis. **Dev. Biol.** 381: 423-433.

Karamboulas, K., Dranse, H. J., & Underhill, T. M. (2010). Regulation of BMP-dependent chondrogenesis in early limb mesenchyme by TGF-β signals. **J. Cell Sci.** 123: 2068-2076.

Kawato, Y., Hirao, M., Ebina, K., Tamai, N., Shi, K., Hashimoto, J., Yoshikawa, H., & Myoui, A. (2011). Nkx3.2-induced suppression of Runx2 is a crucial mediator of hypoxia-dependent maintenance of chondrocyte phenotypes. **Res. Commun.** 416(1-2): 205-210.

Kern, S., Eichler, H., Stoeve, J., Kluter, H. & Bieback, K. (2006). Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. **Stem Cells.** 24(5): 1294-1301.

Kolasinski, S. L., Neogi, T., Hochberg, M. C., Oatis, C., Guyatt, G., Block, J., Callahan, L., Copenhaver, C., Dodge, C., & Felson, D. (2020). American college of rheumatology/arthritis foundation guideline for the management of osteoarthritis of the hand, hip, and knee. **Arthritis Rheumatol.** 72: 220–233.

Komori, T. (2018). Runx2, an inducer of osteoblast and chondrocyte differentiation. **Histochem. Cell Biol.** 49: 313-323.

Komori, T. (2022). Whole Aspect of Runx2 Functions in Skeletal Development. **Int. J. Mol. Sci.** 23(10): 5776.

Komori, T. (2024). Regulation of Skeletal Development and Maintenance by Runx2 and Sp7. **Int. J. Mol. Sci.** 25(18): 10102.

Kozhemyakina, E., Lassar, A. B., & Zelzer, E. (2015). A pathway to bone: signaling molecules and transcription factors involved in chondrocyte development and maturation, **Development**. 142(5): 817-831.

Kraus, V. B., Huebner, J. L., DeGroot, J., & Bendele, A. (2010). The OARSI histopathology initiative - recommendations for histological assessments of

osteoarthritis in the guinea pig. **Osteoarthritis Cartilage.** 18 Suppl 3(Suppl 3): S35-52.

Kuyinu, E. L., Narayanan, G., Nair, L. S., & Laurencin, C. T. (2016). Animal models of osteoarthritis: classification, update, and measurement of outcomes. **J. Orthop. Surg. Res.** 11: 19.

La Gatta, A., Stellavato, A., Vassallo, V., Di Meo, C., Toro, G., Iolascon, G., & Schiraldi, C. (2021). Hyaluronan and derivatives: an *in vitro* multilevel assessment of their potential in viscosupplementation. **Polymers.** 13: 3208.

Lee, O. K., Kuo, T. K., Chen, W. M., Lee, K. D., Hsieh, S. L., & Chen, T. H. (2004). Isolation of multipotent mesenchymal stem cells from umbilical cord blood. **Blood.** 103: 1669-1675.

Lee, J. M., Kim, J. Y., & Cho, K. W. (2008). Wnt11/Fgfr1b cross-talk modulates the fate of cells in palate development. **Dev. Biol.** 314: 341-350.

Lefebvre, V., & Dvir-Ginzberg, M. (2016). SOX9 and the many facets of its regulation in the chondrocyte lineage. **Connect. Tissue Res.**

Leijten, J. C., Emons, J., & Sticht, C. (2012), Gremlin 1, frizzled-related protein, and Dkk-1 are key regulators of human articular cartilage homeostasis. **Arthritis Rheum.** 64: 3302-3312.

Lengner, C. J., Hassan, M. Q., & Serra, R. W. (2005). Nkx3.2-mediated repression of Runx2 promotes chondrogenic differentiation. **J. Biol. Chem.** 280: 15872-15879.

Leung, V., Gao, B., Leung, K., Melhado, I., Wynn, S., Au, T., Dung, N., Lau, J., Mak, A., Chan, D., & Cheah, K. S. E. (2011). SOX9 governs differentiation stage-specific gene expression in growth plate chondrocytes via direct concomitant transactivation and repression. **PLoS Genet.** 7: e1002356.

Li, F., Lu, Y., Ding, M., Napierala, D., Abbassi, S., Chen, Y., Duan, X., Wang, S., Lee, B., & Zheng, Q. (2011). Runx2 contributes to murine Col10a1 gene regulation through direct interaction with its cis-enhancer. **J. Bone Miner. Res.** 26: 2899-2910.

Liao, J., Hu, N., & Zhou, N. (2014). Sox9 potentiates BMP2-induced chondrogenic differentiation and inhibits BMP2-induced osteogenic differentiation. **PLoS One.** 9: e89025.

Liau, L. L., Ruszymah, B. H. I., Ng, M. H., & Law, J. X. (2019). Characteristics and clinical applications of Wharton's jelly-derived mesenchymal stromal cells. **Curr. Res. Transl. Med.** 68(1): 5-16.

Lim, S. K., & Khoo, B. Y. (2021). An overview of mesenchymal stem cells and their potential therapeutic benefits in cancer therapy. **Oncol. Lett.** 22(5): 785.

Lin, W., Huang, L., Li, Y., Fang, B., Li, G., Chen, L., & Xu, L. (2019). Mesenchymal Stem Cells and Cancer: Clinical Challenges and Opportunities. **Biomed Res. Int.** 2019: 2820853.

Liu, C. F., Samsa, W. E., Zhou, G., & Lefebvre, V. (2017). Transcriptional control of chondrocyte specification and differentiation. **Semin. Cell Dev. Biol.** 62: 34-49.

Liu, J., Xiao, Q., Xiao, J. Niu, C., Li, Y., Zhang, X., Zhou, Z., Shu, G., & Yin, G. (2022). Wnt/ β-catenin signalling: function, biological mechanisms, and therapeutic opportunities. **Signal Transduct. Target Ther.** 7: 3.

Loh, C-Y., Chai, J. Y., Tang, T. F., Wong, W. F., Sethi, G., Shanmugam, M. K., Chong, P. P., & Looi, C. Y. (2019). The E-Cadherin and N-Cadherin Switch in Epithelial-to-Mesenchymal Transition: Signaling, Therapeutic Implications, and Challenges. **Cells**. 8(10): 1118.

Long, F., & Ornitz, D. M. (2013). Development of the endochondral skeleton. **Cold Spring Harb. Perspect Biol.** 5: a008334.

Lu, P., Takai, K., Weaver, V. M., & Werb, Z. (2011). Extracellular matrix degradation and remodeling in development and disease. **Cold Spring Harb. Perspect Biol.** 3(12): a005058.

Luo, K. (2017). Signaling Cross Talk between TGF-ß/Smad and Other Signaling Pathways. **Cold Spring Harb. Perspect Biol.** 9(1): a022137.

Lv, Z., Cai, X., Bian, Y., Wei, Z., Zhu, W., Zhao, X., & Weng, X. (2023). Advances in Mesenchymal Stem Cell Therapy for Osteoarthritis: From Preclinical and Clinical Perspectives. **Bioengineering (Basel).** 10(2): 195.

Maiese, K., Li, F., Chong, Z. Z., & Shang, Y. C. (2008). The Wnt signaling pathway: Aging gracefully as a protectionist? **Pharmacol. Ther.** 118(1): 58-81.

Maldonado, V. V., Patel, N. H., Smith, E. E., Barners, C. L., Gustafson, M. P., Rao, R. R., & Samsonraj, R. M. (2023). Clinical utility of mesenchymal stem/stromal cells in regenerative medicine and cellular therapy. **J. Biol. Eng.** 17: 44.

Man, G. S., & Mologhianu, G. (2014). Osteoarthritis pathogenesis – a complex process that involves the entire joint. **J. Med. Life.** 7(1): 37-41.

Margiana, R., Markov, A., Zekiy, A.O., Hamza, M-U., Al-Dabbagh, K. A., Al-Zubaidi, S. H., Hameed, N. M., Ahmad, I., Sivaraman, R., Kzar, H. H., Al-Gazally, M. E., Mustafa, Y-F., & Siahmansouri, H. (2022). Clinical application of mesenchymal stem cell in regenerative medicine: a narrative review. **Stem Cell Res. Ther.** 13: 366.

Mariani, E., Pulsatelli, L., & Facchini, A. (2014). Signaling Pathways in Cartilage Repair. **Int. J. Mol. Sci.** 15(5): 8667-8698.

Marino, L., Castaldi, M. A., Rosamilio, R., Ragni, E., Vitolo, R., Fulgione, C., Castaldi, S. G., Serio, B., Bianco, R., Guida, M., & Selleri. C. (2019). Mesenchymal Stem Cells from the Wharton's Jelly of the Human Umbilical Cord: Biological Properties and Therapeutic Potential. **Int. J. Stem Cells.** 12(2): 218-226.

Mattei, V., & Delle Monache, S. (2024). Mesenchymal Stem Cells and Their Role in Neurodegenerative Diseases. **Cells.** 13(9): 779.

McNulty, A. L., & Guilak, F. (2015). Mechanobiology of the Meniscus. **J. Biomech.** 48 (8).

Mebarki, M., Abadie, C., Larghero, J., & Cras, A. (2021). Human umbilical cord-derived mesenchymal stem/stromal cells: a promising candidate for the development of advanced therapy medicinal products. **Stem Cell Res. Ther.** 12: 152.

Michelacci, Y. M., Baccarin, R. Y. A., & Rodrigues, N. N. P. (2023). Chondrocyte Homeostasis and Differentiation: Transcriptional Control and Signaling in Healthy and Osteoarthritic Conditions. **Life (Basel).** 13(7): 1460.

Mishra, s., Sevak, J. K., Das, A., Arimbasseri, G. A., Bhatnagar, S., & Gopinath, S. D. (2020). Umbilical cord tissue is a robust source for mesenchymal stem cells with enhanced myogenic differentiation potential compared to cord blood. **Sci. Rep.** 10: 18978.

Mori-Akiyama, Y., Akiyama, H., Rowitch, D. H., de & Crombrugghe, B. (2003). Sox9 is required for determination of the chondrogenic cell lineage in the cranial neural crest. **Proc. Natl. Acad. Sci. U. S. A.** 100: 9360-9365.

Morrison, S. J., Shah, N. M., & Anderson, D. J. (1997). Regulatory mechanisms in stem cell biology. **Cell.** 88: 287-298.

Mow, V. C., Yong GU, W., & Hui Chen, F. (2005). Structure and function of articular carilage and meniscus. In Mow, V.C., & Huiskers, R. (eds). *Basic Orthopaedic Biomechanics and Mechano-Biology*. (3rd Ed, pp. 181-258). Philadelphia: Lippincot Williams & Wilkins.

Musial-Wysocka, A., Kot, M., & Majka, M. (2019). The Pros and Cons of Mesenchymal Stem Cell-Based Therapies. **Cell Transplant.** 28(7): 801-812.

Naba, A., Clauser, K. R., Ding, H., Whittaker, C. A., Carr, S. A., & Hynes, R. O. (2016). The extracellular matrix: Tools and insights for the “omics” era. **Matrix Biol.** 49:10-24.

Nagata, K., Hojo, H., Chang, S. H. Okada, H., Yano, F., Chijimatsu, R., Omata, Y., Mori, D., Makii, Y., Kawata, M., Kaneko, T., Iwanaga, Y., Nakamoto, H., Maenohara, Y., Tachibana, N., Ishikura, H., Higuchi, J., Taniguchi, Y., Ohba, S., Chung, U-I., Tanaka, S., & Saito, T. (2022). Runx2 and Runx3 differentially regulate articular chondrocytes during surgically induced osteoarthritis development. **Nat. Commun.** 13: 6187.

Nahian, A., & Sapra, A. (2023). Histology, Chondrocytes. (NBK557576) Island: StatPearls Publishing LLC.

Nimeskern, L., Pleumeekers, M. M., Pawson, D. J., Koevoet, W. L. M., Lehtoviita, I., Soyka, M. B., Roosli, C., Holzmann, D., van Osch, G. J. V. M., Muller, R., & Stok, K. S. (2015). Mechanical and Biochemical Mapping of Human Auricular Cartilage for Reliable Assessment of Tissue-Engineered Constructs. **J. Biomech.** 48 (10), 1721–1729.

Oh, C. D., Chang, S. H., & Yoon, Y. M. (2000). Opposing role of mitogen-activated protein kinase subtypes, erk-1/2 and p38, in the regulation of chondrogenesis of mesenchymes. **J. Biol. Chem.** 275: 5613-5619.

Ohba, S., He, X., Hojo, H., & McMahon, A. P. (2015). Distinct Transcriptional Programs Underlie Sox9 Regulation of the Mammalian Chondrocyte. **Cell Rep.** 12(2): 229-243.

Ornitz, D. M., & Marie, P. J. (2015). Fibroblast growth factor signaling in skeletal development and disease. **Genes Dev.** 29(14): 1463-86.

Oseni, A. O., Crowley, C., Boland, M. Z., Bulter, P. E., & Seifalian, A. M. (2011). Cartilage tissue engineering: the application of nanomaterials and stem cell technology. **Tissue Eng. Regen. Med.** 233-266.

O'Shea, D. G., Curtin, C. M., & O'Brien, F. J. (2022). Articulation inspired by nature: A review of biomimetic and biologically active 3D printed scaffolds for cartilage tissue engineering. **Biomater. Sci.** 10: 2462-2483.

Pan, Q., Yu, Y., & Chen, Q. (2008). Sox9, a key transcription factor of bone morphogenetic protein-2-induced chondrogenesis, is activated through BMP pathway and a CCAAT box in the proximal promoter. **J. Cell Physiol.** 217: 228-241.

Pelusi, L., Schiavone, V., Bologna, G., Cerritelli, P., Lezza, D., Pantalone, A., & Mandatori, D. (2021) Chondrogenic Differentiation of Human Wharton's Jelly and Bone Marrow-Derived Mesenchymal Stem Cells: Focus on the Role of an Acrylamide-Free Cross-Linked Hyaluronic Acid Hydrogel. **J. Orthop. Res. Ther.** 6: 1209.

Perry, B. C., Zhou, D., Wu, X., Yang, F. C., Byers, M. A., Chu, T. M., Hockema, J. J., Woods, E. J. & Goebel, W. S. (2008). Collection, cryopreservation, and characterization of human dental pulp-derived mesenchymal stem cells for banking and clinical use. **Tissue Eng. Part C: Methods.** 14(2): 149-156.

Pittenger, M. F., Mackay, A. M., Beck, S. C., Jaiswal, R. K., Douglas, R., Mosca, J. D., Moorman, M. A., Simonetti, D. W., Craig, S., & Marshak, D. R. (1999). Multilineage potential of adult human mesenchymal stem cells. **Science.** 284: 143-147.

Pittenger, M. F., Discher, D. E., Péault, B. M., Phinney, D. G., Hare, J. M., & Caplan, A. I. (2019). Mesenchymal stem cell perspective: cell biology to clinical progress. **Regen. Med.** 4: 22.

Ponnaiyan, D., Bhat, K. M., & Bhat, G. S. (2012). Comparison of immuno-phenotypes of stem cells from human dental pulp and periodontal ligament. **Int. J. Immunopathol. Pharmacol.** 25: 127-134.

Qin, K., Yu, M., Fan, J., Wang, H., Zhao, P., Zhao, G., Zeng, W., Chen, C., Wang, Y., Wang, A., Schwartz, Z., Hong, J., Song, L., Wagstaff, W., Haydon, R. C., Luu, H. H., Ho, S. H., Strelzow, J., Reid, R. R., He, T. C., & Shi, L. L. (2023). Canonical

and noncanonical Wnt signaling: Multilayered mediators, signaling mechanisms and major signaling crosstalk. **Genes Dis.** 11(1): 103-134.

Quintana, L., Zur Nieden, N. I., & Semino, C. E. (2009). Morphogenetic and regulatory mechanisms during developmental chondrogenesis: new paradigms for cartilage tissue engineering. **Tissue Eng. B.** 15 :29-41.

Rainbow, R. S., Won, H. K., & Zeng, L. (2014). The role of Nkx3.2 in chondrogenesis. **Front. Biol. (Beijing).** 9(5): 376-381.

Rashid, H., Chen, H., & Javed, A. (2021). Runx2 is required for hypertrophic chondrocyte mediated degradation of cartilage matrix during endochondral ossification. **Matrix Biol. Plus.** 12: 100088.

Rashid, H., Smith, C. M., Convers, V., Clark, K., & Javed, A. (2024). Runx2 deletion in hypertrophic chondrocytes impairs osteoclast mediated bone resorption. **Bone**. 181: 117014.

Rebelatto, C. K., Aguiar, A. M., Moretao, M. P., Senegaglia, A. C., Hansen, P., Barchiki, F., Oliveira, J., Martins, J., Kuligouski, C., Mansur, F., Christofis, A., Amaral, V. F., Brofman, P. S., Goldenberg, S., Nakao, L. S. & Correa, A. (2008). Dissimilar differentiation of mesenchymal stem cells from bone marrow, umbilical cord blood, and adipose tissue. **Exp. Biol. Med.** 233(7): 901-913.

Reinholz, G. G., Lu, L., Saris, D. B. F., Yaszemski, M. J., & O'Driscoll, S. W. (2004). Animal models for cartilage reconstruction. **Biomaterials.** 25(9): 1511-1521.

Richter, W., Bock, R., Hennig, T., & Weiss, S. (2009). Influence of FGF-2 and PTHrP on chondrogenic differentiation of human mesenchymal stem cells. **J. Bone Joint Surg. Br.** 91-B (suppl III): 444.

Rodríguez-García, A., Samsó, P., Fontova, P., Simon-Molas, H., Manzano, A., Castaño, E., Rosa, J. L., Martinez-Outshoorn, U., Ventura, F., Navarro-Sabaté, À., & Bartrons, R. (2017). TGF- β1 targets Smad, p38 MAPK, and PI3K/Akt signaling pathways to induce PFKFB3 gene expression and glycolysis in glioblastoma cells. **FEBS J.** 284(20): 3437-3454.

Romanov, Y. A., Darevskaya, A. N., Merzlikina, N. V., & Buravkova, B. (2005). Mesenchymal stem cells from human bone marrow and adipose tissue: isolation, characterization and differentiation potentialities. Bulletin **Exp. Biol. Med.** 140: 138-143.

Rosen, D. M., Stempien, S. A., & Thompson, A. Y. (1986). Differentiation of rat mesenchymal cells by cartilage-inducing factor. Enhanced phenotypic expression by dihydrocytochalasin B. **Exp. Cell Res.** 165: 127-138.

Rozemuller, H., Prins, H. J., Naaijkens, B., Staal, J., Buhring, H. J. & Martens, A. C. (2010). Prospective isolation of mesenchymal stem cells from multiple mammalian species using cross-reacting anti-human monoclonal antibodies. **Stem Cells Dev.** 19(12): 1911-1921.

Saeedi, P., Halabian, R., Imani Fooladi. A. A. (2019). A revealing review of mesenchymal stem cells therapy, clinical perspectives and Modification strategies. **Stem Cell Investig.** 6: 34.

Sandberg, M., Autio-Harmainen, H., & Vuorio, E. (1998). Localization of the expression of types I, III, and IV collagen, TGF-beta 1 and c-fos genes in developing human calvarial bones. **Dev. Biol.** 130: 324-334.

Sato, M., Uchida, K., Nakajima, H., Miyazaki, T., Guerrero, A. R., Watanabe, S., Roberts, S., & Baba, H. (2012). Direct transplantation of mesenchymal stem cells into the knee joints of Hartley strain guinea pigs with spontaneous osteoarthritis. **Arthritis Res. Ther.** 14(1): R31.

Shen, Q., Guo, Y., Wang, K., Zhang, C., & Ma, Y. (2023). A Review of Chondroitin Sulfate's Preparation, Properties, Functions, and Applications. **Molecules.** 28(20): 7093.

Shi, S., Wang, C., & Acton, A. J. (2015). Role of Sox9 in growth factor regulation of articular chondrocytes. **J. Cell. Biochem.** 116: 1391-1400.

Shum, L., & Nuckolls, G. (2002). The life cycle of chondrocytes in the developing skeleton. **Arthritis Res.** 4(2): 94-106.

Solchaga, L. A., Penick, K., Porter, J. D., Goldberg, V. M., Caplan, A. I., & Welter, J. F. (2005). FGF-2 enhances the mitotic and chondrogenic potentials of human adult bone marrow-derived mesenchymal stem cells. **J. Cell Physiol.** 203(2): 398-409.

Sophia Fox, A. J., Bedi, A., & Rodeo, S. A. (2009). The basic science of articular cartilage: structure, composition, and function. **Sports Health.** 1 (6): 461-468.

Staff, N. P., Jones, D. T., & Singer, W. (2019). Mesenchymal Stromal Cell Therapies for Neurodegenerative Diseases. **Mayo Clin. Proc.** 94(5): 892-905.

Stevens, M. M., Marini, R. P., & Martin, I. (2004). FGF-2 enhances TGF- β1-induced periosteal chondrogenesis. **J. Orthop. Res.** 25: 1114-1119.

Stricker, S., Fundele, R., Vortkamp, A., & Mundlos, S. (2002). Role of Runx genes in chondrocyte differentiation. **Dev. Biol.** 245(1): 95-108.

Suthon, S., Perkins, R. S., Bryja, V., Miranda-Carboni, G. A., & Krum, S. A. (2021). WNT5B in Physiology and Disease. **Front. Cell Dev. Biol.** 9: 667581.

Taghizadeh, R. R., Cetrulo, K. J., & Cetrulo, C. L. (2011). Wharton's Jelly stem cells: Future clinical applications. **Placenta.** 32(4): S311-S315.

Takeda, S., Bonnamy, J-P., Owen, M.J., Ducy, P., & Karsenty, G. (2001). Continuous expression of Cbfa1 in nonhypertrophic chondrocytes uncovers its ability to induce hypertrophic chondrocyte differentiation and partially rescues Cbfa1-deficient mice. **Genes Dev.** 15(4): 467-481.

Talens-Visconti, R., Bonora, A., Jover, R., Mirabet, V., Carbonell, F., Castell, J. V. & Go'mez-Lecho'n, M. J. (2006). Hepatogenic differentiation of human mesenchymal stem cells from adipose tissue in comparison with bone marrow mesenchymal stem cells. **World J. Gastroenterol.** 12(36): 5834-5845.

Teng, Y., Li, X., Chen, Y., Cai, H., Cao, W., Chen, X., Sun, Y., Liang, J., Fan, Y., & Zhang, X. (2017). Extracellular matrix powder from cultured cartilage-like tissue as cell carrier for cartilage repair. **J. Mater. Chem. B.** 5(18): 3283-3292.

Tessier, J. J., Bowyer, J., Brownrigg, N. J., Peers, I. S., Westwood, F. R., Waterton, J. C., & Maciewicz, R. A. (2003). Characterisation of the guinea pig model of osteoarthritis by *in vivo* three-dimensional magnetic resonance imaging. **Osteoarthritis Cartilage.** 11(12): 845-853.

Timothy, F. D., Xizhi, G., Lisa, G-B., & Yingzi, Y. (2005). Wnt/β-Catenin Signaling in Mesenchymal Progenitors Controls Osteoblast and Chondrocyte Differentiation during Vertebrate Skeletogenesis. **Dev. Cell.** 8(5): 739-750.

Tivig, I., Vallet, L., Moisescu, M. G., Fernandes, R., Andre, F. M., Mir, L. M., & Savapol, T. (2024). Early differentiation of mesenchymal stem cells is reflected in their dielectrophoretic behavior. **Sci. Rep.** 14: 4330.

Tondreau, T., Meuleman, N., Delforge, A., Dejeneffe, M., Leroy, R., Massy, M., Mortier, C., Bron, D., & Lagneaux, L. (2005). Mesenchymal stem cells derived from

CD133-positive cells in mobilized peripheral blood and cord blood: proliferation, Oct4 expression, and plasticity. **Stem Cells.** 23: 1105-1112.

Topol, L., Chen, W., Song, H., Day, T. F., & Yang, Y. (2009). Sox9 inhibits Wnt signaling by promoting β-catenin phosphorylation in the nucleus. **J. Biol. Chem.** 284(5): 3323-3333.

Troeberg, L., & Nagase, H. (2012). Proteases involved in cartilage matrix degradation in osteoarthritis. **Biochim. Biophys. Acta.** 1824(1): 133-45.

Troyer, D. L., & Weiss, M. L. (2008). Wharton's jelly-derived cells are a primitive stromal cell population. **Stem Cells.** 26(3): 591-599.

Tsai, M. S., Lee, J. L., Chang, Y. J. & Hwang, S. M. (2004). Isolation of human multipotent mesenchymal stem cells from second-trimester amniotic fluid using a novel two-stage culture protocol. **Hum. Reprod.** 19(6): 1450-1456.

Tufan, A. C., & Tuan, R. S. (2001). Wnt regulation of limb mesenchymal chondrogenesis is accompanied by altered N-cadherin-related functions. **FASEB. J.** 15: 1436-1438.

Tufan, A. C., Daumer, K. M., DeLise, A. M., & Tuan, R. S. (2002). AP-1 transcription factor complex is a target of signals from both WnT-7a and N-cadherin-dependent cell-cell adhesion complex during the regulation of limb mesenchymal chondrogenesis. **Exp. Cell Res.** 273: 197-203.

Tuli, R., Tuli, S., & Nandi, S. (2003). Transforming growth factor-beta-mediated chondrogenesis of human mesenchymal progenitor cells involves N-cadherin and mitogen-activated protein kinase and Wnt signaling cross-talk. **J. Biol. Chem.** 278: 41227-41236.

Urbani, S., Caporale, R., Lombardini, L., Bosi, A., & Saccardi, R. (2006). Use of CFDA-SE for evaluating the *in vitro* proliferation pattern of human mesenchymal stem cells. **Cytotherapy.** 8: 243 253.

Ushita, M., Saito, T., & Ikeda, T. (2009). Transcriptional induction of SOX9 by NF-kappaB family member RelA in chondrogenic cells. **Osteoarthritis Cartilage.** 17: 1065-1075.

Vasanthan, J., Gurusamy, N., Rajasingh, S., Sigamani, V., Kirankumar, S., Thomas, E. L., & Rajasingh. J. (2020). Role of Human Mesenchymal Stem Cells in Regenerative Therapy. **Cells.** 10(1): 54.

Veeman, M. T., Axelrod, J. D., & Moon, R. T. (2003). A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling. **Dev. Cell.** 5: 367-377.

Vicinanza, C., Lombardi, E., Da Ros, F., Marangon, M., Durante, C., Mazzucato, M., & Agostini, F. (2022). Modified mesenchymal stem cells in cancer therapy: A smart weapon requiring upgrades for wider clinical applications. **World J. Stem Cells.** 14(1): 54-75.

Vinod, E., Padmaja, K., Ramasamy, B., & Sathishkumar, S. (2023). Systematic review of articular cartilage derived chondroprogenitors for cartilage repair in animal models. **J. Orthop.** 35: 43-53.

Vlashi, R., Zhang, X., Wu, M., & Chen, G. (2023). Wnt signaling: Essential roles in osteoblast differentiation, bone metabolism and therapeutic implications for bone and skeletal disorders. **Genes & Diseases.** 10 (4):1291-1317.

Voisin, C., Cauchois, G., Reppel, L., Laroye, C., Louarn, L., Schenowitz, C., Sonon, P., Poras, I., Wang, V., Carosella. E., Benkirane-Jessel, N., Moreau, P., Rouas-Freiss, N., Bensoussan, D., & Huselstein, C. (2020). Are the Immune Properties of Mesenchymal Stem Cells from Wharton's Jelly Maintained during Chondrogenic Differentiation? **J. Clin. Med.** 9(2): 423.

Wang, W-J., Sun, C., Liu, Z., Sun, X., Zhu, F., Zhu, Z. Z., & Qiu, Y. (2014). Transcription factor Runx2 in the low bone mineral density of girls with adolescent idiopathic scoliosis. **Orthop. Surg.** 6(1): 8-14.

Wieland, H. A., Michaelis, M., Kirschbaum, B. J. & Rudolphi, K. A. (2005). Osteoarthritis-an untreatable disease? **Nat. Rev. Drug Discov.** 4: 331-344.

Wise, C. (2010). Osteoarthritis. In Dale, D. C., & Federman, D. D. (eds). *ACP Medicine* (section 15, chap. 10, pp. 1-12.) New York: WebMD.

Witkowska-Zimny, M., & Wrobel, E. (2011). Perinatal sources of mesenchymal stem cells: Wharton’s Jelly, amnion and chorion. **Cell Mol. Biol. Lett.** 16(3): 493-514.

Wolock, S. L., Krishnan, I., Tenen, D. E., Matkins, V., Camacho, V., Patel, S., Agarwal, P., Bhatia, R., Tenen, D. G., Klein, A. M., & Welner, R. S. (2019). Mapping Distinct Bone Marrow Niche Populations and Their Differentiation Paths. **Cell Rep.** 28: 302-311.

Wang, M., Wu, Y., Li, G., Lin, Q., Zhang, W., Liu, H., & Su, J. (2024). Articular cartilage repair biomaterials: strategies and applications. **Mater. Today Bio.** 24: 100948.

Wang, R. N., Green, J., Wang, Z., Deng, Y., Qiao, M., Peabody, M., Zhang, Q., Ye, J., Yan, Z., Denduluri, S., Idowu, O., Li, M., Shen, C., Hu, A., Haydon, R. C., Kang, R., Mok, J., Lee, M. J., Luu, H. L., & Shi, L. L. (2014). Bone Morphogenetic Protein (BMP) signaling in development and human diseases. **Genes Dis.** 1(1): 87-105.

Wang, X., Wei, L., Xue, Y., & Li, R-S. (2021). Experimental observation of the sequence of tibial plateau chondrocyte and matrix degeneration in spontaneous osteoarthritis in Guinea pigs. **BMC Musculoskelet. Disord.** 22: 395.

Wang, Y. & Sul, H. S. (2009). Pref-1 regulates mesenchymal cell commitment and differentiation through Sox9. **Cell Metab.** 9: 287-302.

Wong, C-C., Ou, K-L., Lin, Y-H., Lin, M-F., Yang, T-L., Chen, C-H., & Chan, W. P. (2020). Platelet-rich fibrin facilitates one-stage cartilage repair by promoting chondrocytes viability, migration, and matrix synthesis. **Int. J. Mol. Sci.** 21: 577.

Wu, M., Wu, S., Chen, W., & Li, Y-P. (2024). The roles and regulatory mechanisms of TGF-ß and BMP signaling in bone and cartilage development, homeostasis and disease. **Cell Res.** 34: 101-123.

Yamashita, S., Miyaki, S., & Kato, Y. (2012). L-Sox5 and Sox6 proteins enhance chondrogenic miR-140 microRNA expression by strengthening dimeric Sox9 activity. **J. Biol. Chem.** 287: 22206-22215.

Yan, J-Y., Tian, F-M., Wang, W-Y., Cheng, Y., Xu, H-F., Song, H-P., Zhang, Y-Z., & Zhang, L. (2014). Age dependent changes in cartilage matrix, subchondral bone mass, and estradiol levels in blood serum, in naturally occurring osteoarthritis in Guinea pigs. **Int. J. Mol. Sci.** 15(8): 13578-13595.

Yang, Y. H. K., Ogando, C. R., Wang See, C., Chang, T-Y., & Barabino, G. A. (2018). Changes in phenotype and differentiation potential of human mesenchymal stem cells aging *in vitro*. **Stem Cell Res. Ther.** 9: 131.

Yang, X., Tian, S., Fan, L. Niu, R, Yan, M., Chen, S., Zheng, M., & Zhang, S. (2022). Integrated regulation of chondrogenic differentiation in mesenchymal stem cells and differentiation of cancer cells. **Cancer Cell Int.** 22: 169.

Yano, F., Kugimiya, F., & Ohba, S. (2005). The canonical Wnt signalling pathway promotes chondrocyte differentiation in a Sox9- dependent manner. **Biochem. Biophys. Res. Commun.** 333: 1300-1308.

Yea, J. H, Kim, Y., & Jo, C. H. (2023). Comparison of mesenchymal stem cells from bone marrow, umbilical cord blood, and umbilical cord tissue in regeneration of a full-thickness tendon defect i*n vitro* and *in vivo*. **Biochem. Biophys. Rep.** 34: 101486.

Yoshida, C. A., Yamamoto, H., Fujita, T., Furuichi, T., Ito, K., Inoue, K., Yamana, K., Zanma, A., Takada, K., Ito, Y., & Komori, T. (2004). Runx2 and Runx3 are essential for chondrocyte maturation, and Runx2 regulates limb growth through induction of Indian hedgehog. **Genes Dev.** 18(8): 952-63.

Yoon, B. S., Ovchinnikov, D. A., & Yoshii, I. (2005). Bmpr1a and Bmpr1b have overlapping functions and are essential for chondrogenesis *in vivo*. **Proc. Natl. Acad. Sci. U. S. A.** 102: 5062-5067.

Zhang, L., Hu, J., & Athanasiou, K. A. (2009). The role of tissue engineering in articular cartilage repair and regeneration. **Crit. Rev. Biomed. Eng.** 37: 1-57.

Zhang, Q., Pan, Y., Ji, J., Xu, Y., Zhang, Q., & Qin, L. (2021). Roles and action mechanisms of WNT4 in cell differentiation and human diseases: a review. **Cell Death Discov.** 7: 287.

Zheng, Q., Zhou, G., & Morello, R. (2003). Type X collagen gene regulation by Runx2 contributes directly to its hypertrophic chondrocyte-specific expression *in vivo*. **J. Cell. Biol.** 162: 833-842.

Zhou, G., Zheng, Q., Engin, F., Munivez, E., Chen, Y., Sebald, E., Krakow, D., & Lee, B. (2006). Dominance of SOX9 function over RUNX2 during skeletogenesis. **Proc. Natl. Acad. Sci. U. S. A.** 103(50): 19004-19009.

Zhou, S., Eid, K., & Glowacki, J. (2004). Cooperation between TGF-ß and Wnt pathways during chondrocyte and adipocyte differentiation of human marrow stromal cells. **J. Bone Min. Res.** 19: 463-470.

Zhuang, W. Z., Lin, Y. H., Su, L. J., Su, L-J., Wu, M-S., Jeng, H-Y., Chang, H-C., Huang, Y-H., & Ling, T-Y. (2021). Mesenchymal stem/stromal cell-based therapy: mechanism, systemic safety and biodistribution for precision clinical applications. **J. Biomed. Sci.** 28: 28.

Zuk, P. A., Zhu, M., Mizuno, H., Huang, J., Futrell, J. W., Katz, A. J., Benhaim, P., Lorenz, H. P. & Hedrick, M. H. (2001). Multilineage cells from human adipose tissue: implications for cell-based therapies. **Tissue Eng.** 7(2): 211- 228.

Zuscik, M. J., Hilton, M. J., Zhang, X., Chen, D., & O'Keefe, R. J. (2008). Regulation of chondrogenesis and chondrocyte differentiation by stress. **J. Clin. Invest.** 118(2): 429-38.