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Determinants of stem cell lineage differentiation toward chondrogenesis versus adipogenesis

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Abstract

Adult stem cells, also termed as somatic stem cells, are undifferentiated cells, detected among differentiated cells in a tissue or an organ. Adult stem cells can differentiate toward lineage specific cell types of the tissue or organ in which they reside. They also have the ability to differentiate into mature cells of mesenchymal tissues, such as cartilage, fat and bone. Despite the fact that the balance has been comprehensively scrutinized between adipogenesis and osteogenesis and between chondrogenesis and osteogenesis, few reviews discuss the relationship between chondrogenesis and adipogenesis. In this review, the developmental and transcriptional crosstalk of chondrogenic and adipogenic lineages are briefly explored, followed by elucidation of signaling pathways and external factors guiding lineage determination between chondrogenic and adipogenic differentiation. An in-depth understanding of overlap and discrepancy between these two mesenchymal tissues in lineage differentiation would benefit regeneration of high-quality cartilage tissues and adipose tissues for clinical applications.

1. Introduction

Stem cells are gaining importance due to their potential to regenerate damaged tissues [1,2]. Adult stem cells, which exist in the postnatal organism, have been identified to have multi-lineage or unilineage differentiation capacity toward which they are committed to differentiate. Mesenchymal stem cells (MSCs), as part of the multi-lineage differentiation of adult stem cells, have the ability to form articular cartilage, fat and bone [3]. The balances

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Conflict of Interest

The authors declare no conflict of interest.

between adipogenesis and osteogenesis and between chondrogenesis and osteogenesis have been comprehensively reviewed [4,5]; however, few reviews explore the crosstalk between chondrogenesis and adipogenesis.

There is a strong and close relationship between chondrogenesis and adipogenesis. For example, a high concentration of dexamethasone could induce adipogenic differentiation even during chondrogenic induction of human synovium-derived stem cell (SDSC) pellets [6]. Pericytes in pellet cultures in chondrogenic medium also underwent adipogenic differentiation, as evidenced by the fact that some cells within the pellets displayed a signet-ring adipocyte-like morphology [7]. Interestingly, depletion of *RUNX2* (Runt-related transcription factor 2), a typical osteogenic marker, resulted in the loss of chondrocyte phenotype and induced adipogenic differentiation in primary chondrocytes *in vitro* [8]. Furthermore, Qu *et al.* found that genetic deletion of Vav1, a guanine exchange factor for Rho GTP highly expressed in murine bone marrow-derived MSCs (BMSCs), led to spontaneous adipogenesis but disabled chondrogenic differentiation [9]. They also found that overexpression reversed this phenotype, resulting in increased chondrogenesis but decreased adipogenesis [9].

The transcription factors of chondrogenesis and adipogenesis are interrelated, which can influence stem cell fate. Down-regulation of Sox9 (SRY-Box 9), a classical transcription factor for chondrogenesis, seems to be required for adipocyte differentiation since Sox9 can bind to and suppress the major adipogenic transcription factors CCAAT/enhancer-binding protein beta (C/EBP β) and C/EBP δ promoter activity directly [10]. On the contrary, Sox9 directly regulates *COL2A1* (type II collagen) but it binds to the element overlapping with C/EBP motif in RCS (rat chondrosarcoma) cells [11]; thereby, C/EBP β and C/EBP δ may participate in interleukin 1 β (IL-1 β)-induced repression of *COL2A1* expression. Furthermore, chondrogenic marker genes *COL2A1*, *ACAN* (aggrecan) and *SOX9* are reported to be suppressed by C/EBP α , C/EBP β and C/EBP δ in ATDC5 cells (derived from mouse teratocarcinoma cells and characterized as a chondrogenic cell line) [12,13]. These findings imply negative regulation between C/EBP family members and Sox9. However, other reports indicate that Sox9 is imperative for adipogenic differentiation by stabilizing C/EBP β mRNA in rat adult BMSCs [14] and C/EBP family members show potent transactivation of *SOX9* in both ATDC5 and Hela cells [15]. Therefore, the interaction of transcription factors between chondrogenesis and adipogenesis is complicated. The in-depth investigation is still in its infancy.

In this review, for the first time, we briefly discuss developmental origins of articular cartilage and adipose tissue, followed by signaling pathways guiding chondrogenic and adipogenic differentiation of stem cells as well as regulators controlling the crosstalk of chondrogenesis and adipogenesis. Further investigations of lineage-specific differentiation may lead to promising applications of MSCs in tissue engineering and regeneration.

2. Developmental origins of articular cartilage and adipose tissue

MSCs developing from the mesoderm commit to chondrogenic and adipogenic differentiation (brown, brite/beige and white adipocytes) (Figure 1) and other lineages.

Transcription factors promote the differentiation of chondroblasts and preadipocytes to acquire their specific functions.

In the chondrogenic lineage, Sox9 is necessary for induction and maintenance of chondrocytic phenotypes in concert with Sox5 and Sox6 [16]. Transforming growth factor beta (TGF β), bone morphogenetic protein (BMP), GLI-Kruppel family member 3 (Gli3) and Runx2 also promote chondrogenic differentiation [17]. Cartilage developmental stages can be divided into three phases: mesenchymal condensation, interzone formation and cavitation and stabilization of articular cartilage [18]. During mesenchymal condensation, chondroblasts migrate from the lateral plate of the mesoderm followed by an interruption of continuous cartilage anlagen by interzone formation. The interzone is composed of three layers: two chondrogenic layers and one intermediate layer. The former covers the cartilage while the latter aids intra-articular structure formation [19]. At early stages of joint morphogenesis, *GDF5* (growth differentiation factor 5) mRNA is highly expressed in regions flanking future joint sites, within the flattened intermediate interzone [20]. Cells with a GDF5-expressing lineage actively take part in joint tissue formation, and constitute a progenitor cell cohort endowed with joint-formation capacity [21].

Brown adipocytes arise from precursors that express myogenic factor 5 (Myf5), a gene that was also expressed in the myogenic lineage with transcriptional co-factor PRDM16 (PRD1-BF-1-RIZ1 homologous domain-containing protein-16) as a dominant regulator [22,23]. Despite the fact that some white fat cells arise from Myf5+ precursors, most evidence suggests that white and brown adipocytes take different developmental paths [24]. Adipocytes in the cephalic region are ectodermal due to the mesenchyme in this part of the body deriving from the neuroectoderm [25]. Multiple signaling pathways are involved in the fate of adipocyte lineage. For example, Wingless/int (Wnt) and Hedgehog proteins are important for MSC myogenic lineage commitment but prevent MSCs from proceeding toward an adipogenic lineage [26]. In the adipogenic lineage, peroxisome proliferator-activated receptor gamma (PPAR γ) is necessary and sufficient for adipogenesis [27]. C/EBP α is required for the differentiation of white but not brown adipocytes [28]. With BMP2 and BMP4 as white adipogenic factors, BMP7 serves as the unique brown fat inducer [29]. Forced expression of PRDM16 in a white preadipocyte cell line or white adipocytes *in vivo* promotes a robust brown adipocyte phenotype [22].

3. Signaling pathways guiding chondrogenic and adipogenic differentiation of stem cells

Recent studies have demonstrated that multiple signaling pathways are involved in determining stem cell fate, including TGF β /BMP signaling, Hedgehog, Wnt and Notch signaling (Figure 2).

TGF β

TGF β 1 has the ability to induce chondrogenic differentiation in chick periosteum-derived mesenchymal cells [30] and in human and animal MSCs but in a dose-dependent manner [31–33]. For example, 10 ng/mL of TGF β 1 induced human BMSC differentiation into

chondrocytes while lower doses, such as 0.01–0.1 ng/mL and 0.5–1.0 ng/mL, did not work in monolayer culture [34,35]. TGF β 2 and TGF β 3 were more effective than TGF β 1 in promoting chondrogenic differentiation in human BMSCs [32]. Interestingly, TGF β 1 and TGF β 3 exerted an inhibitory effect on adipogenesis. TGF β 1 induced dominant chondrogenesis while suppressed adipogenic differentiation of CL-1 cells (a cell line that can spontaneously differentiate into chondrocytes and adipocytes) in the presence of fetal calf serum (FCS) [36] or human BMSCs [37]. *In vitro* treatment of 100 pM TGF β 1 also inhibited 3T3–L1 (mouse cells that resemble preadipocytes) differentiation into mature adipocytes [38] and 1 ng/mL TGF β 1 hampered lipid accumulation in mouse embryonic fibroblasts [39]. TGF β 3 (10 ng/mL) suppressed the induction of adipogenesis-associated genes, such as *PPARG* (PPAR γ), *FABP4* (fatty acid binding protein 4), even in a two-dimensional micromass chondrogenic culture of human SDSCs [6].

Mechanically, TGF β in chondrogenic differentiation can signal through the canonical Smad2 (small mothers against decapentaplegic homolog 2) or Smad3-mediated pathway or non-canonical p38 mitogen-activated protein kinase (MAPK) pathway [40–43]. However, MAPK kinase (MEK)/extracellular signal-regulated protein kinase (Erk) signaling pathway in TGF β -induced chondrogenesis remains controversial and complex [43]. In adipogenesis, however, overexpression of Smad2 or Smad3 inhibited lipid accumulation of 3T3–F442A preadipocytes, with Smad3 exerting a stronger effect [44]. A dominant-negative form of Smad3 is able to suppress the inhibitory function of TGF β signaling on adipogenesis while adipogenesis proceeds normally in the presence of the dominant-negative form of Smad2, supporting Smad3 as a TGF β signaling component in inhibiting adipogenic differentiation [44]. Moreover, Smad3, along with Smad4, associated with C/EBP β and C/EBP δ resulted in decreased PPAR γ expression in NIH3T3 cells [45]. The above evidence indicates that TGF β may exhibit opposite effects on chondrogenic and adipogenic lineage differentiation *via* Smad3.

BMP

More than 20 different BMP isoforms have now been identified. Among them, BMP2, BMP4 and BMP7 are the well-established determinants of chondrogenic and/or adipogenic differentiation of MSCs.

BMP2 is a positive regulator in chondrogenic differentiation. In human BMSCs, BMP2 is the most effective in promoting chondrogenic differentiation as compared to BMP4 and BMP6 [46]. BMP2 (500 ng/mL), associated with a thiazolidinedione activator of PPAR γ , also stimulated adipogenesis in 3T3–L1 cells and rat BMSCs [47]. BMP2 (50 ng/mL) could even induce adipogenesis on a two-dimensional micromass chondrogenic culture of human SDSCs [6]. In fact, the concentration of BMP2 strongly influences mesenchymal cell differentiation. For example, differentiation of C3H10T1/2 mouse embryonic stem cells (ESCs) into adipocytes occurred at lower concentrations (such as 10 ng/mL) of BMP2, while chondrocyte differentiation was prevalent at higher concentrations (such as 1000 ng/mL) [48]. However, BMP2 (50 ng/mL) was also reported to increase proteoglycan and type II collagen expression but decrease the level of adipocyte-specific *aP2* (adipocyte protein-2) expression in human BMSCs [49].

BMP4 is a useful agent for stimulating chondrogenic differentiation both *in vitro* and *in vivo* [50–52]. Nakayama *et al.* demonstrated that BMP4 promotes chondrogenesis of ESC-derived mesodermal cells in a dose-dependent manner. They found that 50 ng/mL of BMP4 enhanced more cartilage formation compared to 20 ng/mL, while 5 ng/mL was not effective [53]. BMP4 also promotes stem cell commitment to the adipocyte lineage. Taha *et al.* found that 100 ng/mL of BMP4 induced more adipocyte clusters in ESC-derived embryoid body outgrowth compared to 50 and 10 ng/mL, suggesting BMP4 induces adipogenesis in a dose-dependent manner [54].

BMP7 alone or with TGF β increased chondrogenesis in bovine synovium explants, human ESCs, SDSCs and BMSCs [55–59]. BMP7 also augments phosphorylation of Smad1/5/8 in white and brown preadipocytes. However, *via* p38 MAPK, BMP7 initiated a full program of brown adipogenesis including increased expression of *UCP1* (uncoupling protein 1), *CEBPs* and *PPARG* and blockade of adipogenic inhibitors such as *NDN* (Necdin), *PREF1* (preadipocyte factor 1) and *WNT10A* (a canonical Wnt signaling molecule) [60]. Interestingly, BMP7 favors adipogenic differentiation over chondrogenic differentiation. For example, BMP7 dose-dependently decreased the level of aggrecan assessed by Alcian blue staining and increased the number of lipid-filled cells in human BMSCs [61]. Moreover, BMP7 (50 ng/mL) initiated adipogenesis instead of chondrogenesis of human BMSCs even in micromass cultures, which usually favors chondrogenic differentiation [61]. In addition, treatment with 100 ng/mL BMP7 alone did not increase chondrogenic gene expression, such as proteoglycan, *COL2A1* and *SOX9*, after a 21-day chondrogenic induction of human BMSCs [62]. Interestingly, even in osteogenic induction, BMP7 elevated the expression of adipogenic genes *PPARG*, *ADIPOQ* (adiponectin) and *LPL* (lipoprotein lipase) of human BMSCs [62].

Hedgehog

Extracellular ligands of the hedgehog protein family are modulators of stem cell chondrogenesis along with potential interactions on adipocyte differentiation pathways [34,63,64]. The interaction of Hedgehogs with receptors of the patched (PTCH) family, a conserved transmembrane protein receptor that negatively regulates of the Hedgehog signaling pathway, ultimately rescues Ci (in *Drosophila*)/Gli (in vertebrates) from proteolytic degradation and then promotes their nuclear localization.

Hedgehog signaling is well known to stimulate MSC chondrogenic differentiation. Indian Hedgehog (IHH), expressed and secreted by pre-hypertrophic and early hypertrophic cells, is a key regulator of endochondral ossification [65]. IHH-deficient mice displayed a markedly reduced chondrocyte proliferation and premature chondrocyte hypertrophy [66]. Generally, chondrogenic differentiation of human BMSCs was characterized by an increase of IHH expression [34]. Knockdown of IHH or pharmacological inhibition of Hedgehog signaling with cyclopamine or HhAntag could completely block TGF β 1 or BMP2-induced chondrogenesis in mesenchymal cells [34,67]. Furthermore, overexpression of IHH was sufficient to drive chondrogenesis, even when TGF β signaling was inhibited [34]. Likewise, treatment with IHH or the recombinant amino half of SHH (recombinant N-terminal portion of Sonic Hedgehog) induced chondrocyte differentiation in clonal pre-chondrogenic RMD-1

and ATDC5 cells [68]. SHH is also a critical moderator of cell differentiation due to its anti-adipogenic and pro-chondrogenic properties in mouse adipose-derived stem cells (ADSCs) [69].

The anti-adipogenic potential of Hedgehog signaling has been observed in a variety of multipotent cell lineages. Generally, adipogenic differentiation of human ADSCs was characterized by a decrease in *Gli1*, *Gli2*, *Gli3* and *PTCH* expression [64]. A dominant negative form of *Gli2* was reported to promote adipogenesis of 3T3–L1 cells [70]. Conversely, treatment with purmorphamine, a Hedgehog agonist, decreased adipocyte-specific markers, such as *FABP*, *CFD* (complement factor D, Adipsin), *CD36*, *ADIPOQ* and *LEP* (leptin) in human ADSCs [64]. Likewise, SHH resulted in suppression of pro-adipogenic effects of BMP2 in multipotent C3H10T1/2 cells and transgenic activation of Hedgehog signaling in both *Drosophila* and mammalian models impaired fat formation [70–72]. In summary, current data suggest that Hedgehog signaling promotes stem cell chondrogenic differentiation over adipogenic differentiation, primarily *via* Gli transcription factor activity.

Wnt

Over the course of several decades, Wnt signaling has been identified as playing an essential role in cell fate determination and differentiation [73,74]. Collectively, canonical Wnt signaling has demonstrated both pro-chondrogenic and anti-adipogenic activities while the non-canonical pathways promote both of these differentiations.

Many studies showed that Wnt proteins, such as Wnt3a, have been reported to promote chondrogenic differentiation [75]. Moreover, secreted frizzled-related protein 1 (sFRP1) and Dickkopf-related protein 1 (Dkk1) enhanced glycosaminoglycan (GAG) synthesis, *SOX9* and *COL2A1* expression only in the early chondrogenesis of human BMSC pellet cultures [76]. However, several members of the Wnt signaling family have been identified to inhibit early stage adipogenic differentiation [77]. Wnt1, Wnt6, Wnt10a and Wnt10b have been shown to maintain 3T3–L1 cells in an undifferentiated state *via* inhibition of PPAR γ and C/EBP α [77–81]. Further studies also suggest that canonical ligand Wnt3a inhibits activation of both PPAR γ and C/EBP α in order to elicit its anti-adipogenic effects [82]. Additionally, during adipogenic differentiation of human ADSCs, the mRNA levels of *SFRP4* and *DKK1*, two Wnt antagonists, were higher than in the undifferentiated state [83]. Also, a 48-hour treatment with sFRP1 and sFRP4 up-regulated adiponectin secretion in human ADSCs [84]. Accordingly, inhibition of Wnt/ β -catenin signaling *via* treatment with Sclerostin or Dkk family proteins positively regulated adipogenesis [77,78,85,86]. A higher level of sFRP2 enhanced adipogenic differentiation and a decrease of sFRP2 suppressed adipogenesis in mouse BMSCs [87]. Interestingly, Kirton *et al.* found that Wnt/ β -catenin signaling enhanced chondrogenesis while attenuating adipogenic differentiation of pericytes in both monolayer and pellet cultures [7].

The non-canonical signaling pathway has also been implicated as a modulator of chondrogenic and adipogenic differentiation of MSCs. To date, the β -catenin independent pathway has been reported to be a determinant of stem cell chondrogenesis. For example, TGF β up-regulated the level of Wnt5a, which promoted chondrogenesis of chick wing bud

mesenchymal cells [88]. Overexpression of Wnt11 promoted chondrogenesis in rat BMSCs [89]. Moreover, hyaluronan-grafted chitosan promoted chondrogenesis by Wnt5a-mediated non-canonical Wnt signaling in rat ADSCs [90]. Furthermore, Wnt5a and Wnt11 mediated non-canonical Wnt signals were actively involved in the enhanced chondrogenesis of decellularized extracellular matrix (ECM) expanded human SDSCs [91]. These results indicated that non-canonical Wnt signaling plays a positive role in promoting the chondrogenic differentiation response. Non-canonical Wnt ligands, Wnt4 and Wnt5a, promoted the adipogenesis of 3T3–L1 cells through the Protein Kinase C (PKC)-CamKII pathway [92]. These data indicated that, different from canonical Wnt signals, the non-canonical Wnt pathway was a positive regulator in adipogenic differentiation.

Notch

Notch signaling has been shown to be active in undifferentiated stem cells and in the early phase of chondrogenic differentiation. Notch signaling was down-regulated when chondrocyte differentiation ensued in pellet cultures of human BMSCs [93]. Correspondingly, overexpression of *NICD* (Notch intracellular domain), *HES1* (Hairy and enhancer of split-1) and *HEY1* (Hairy/enhancer-of-split related with YRPW motif protein 1) prevented chondrogenesis of human BMSCs [93]. Further studies found that inhibition of Notch signaling on chondrogenesis of murine limb bud mesenchymal progenitor cells was markedly reduced by knockdown of *TWIST1* (Twist-related protein 1) [94]. These results suggest Notch signaling plays a negative role during chondrogenic differentiation.

Notch receptor mRNA expression, such as *NOTCH1*, *2*, *3* and *4*, decreased as adipogenic differentiation of human ADSC clones [95]. However, only Notch1 and 4 increased during adipogenic differentiation of 3T3–L1 cells [96]. Existing evidence indicates Notch signaling plays an inhibitory and obligatory role in adipogenesis [97,98]. Exposure to ligand jagged1 in 3T3–L1 cells or jagged1 transgene expression in human BMSCs blocked PPAR γ and C/EBP α induction and inhibited adipocyte formation in response to adipogenic induction [98,99]. N-[N-(3,5-difluorophenacetyl-L-alanyl)]-S-phenylglycine t-butyl ester (DAPT, γ -secretase inhibitor) inhibited Notch signaling, induced autophagy and promoted adipogenesis of human BMSCs through the Phosphatase and tensin homolog (PTEN)-phosphatidylinositol-3 kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) pathway [100]. However, exposure of jagged1 to mouse ADSCs stimulated adipogenesis by promoting PPAR γ expression [101]. Active form Notch4 promoted adipogenic differentiation of 3T3–L1 cells [96]. Moreover, reduction of *HES1* using siRNA or impaired Notch1 expression by antisense constructs was associated with inhibition of adipogenic differentiation in 3T3–L1 cells, which may involve modulation of DLK1 (Delta-like 1 homolog)/Pref1 [97,98]. Thus, the Notch signaling pathway inhibits or promotes adipogenesis in a complex manner through multiple intracellular signaling pathways.

4. Regulators controlling the balance of chondrogenic and adipogenic differentiation of stem cells

The crosstalk between chondrogenic and adipogenic differentiation is important and accumulating evidence shows that a multitude of cues direct the lineage commitment. Here,

we will discuss the cues controlling the crosstalk between chondrogenic and adipogenic differentiation of MSCs, including biochemical, biophysical and biological factors.

4.1. Biochemical factors

Culture conditions such as culture medium and growth factor supplements are crucial for MSC differentiation toward a specific lineage. To induce chondrogenesis and adipogenesis of MSCs, various combinations have been included such as induction reagents and growth factors (Figure 3) (Table 1). For example, typical chondrogenic factors, including 40 µg/mL proline, 100 nM dexamethasone, 0.1 mM ascorbic acid-2-phosphate, 1×ITS Premix and TGFβ3, could promote chondrogenic differentiation as the induction medium [91]. Moreover, stem cells cultured in medium supplemented with 1 mM dexamethasone, 0.5 mM IBMX (isobutyl-1-methylxanthine), 200 mM indomethacin and 10 mM insulin supported adipogenic differentiation [102].

Dexamethasone—Dexamethasone typically included in the cocktail of both chondrogenic and adipogenic media is to stimulate chondrogenesis and adipogenesis, indicating that it is a crucial component in differentiation induction. Interestingly, low dose (10 nM) dexamethasone treatment during the expansion period increased chondrogenic and adipogenic potential in human BMSCs [103]. Unexpectedly, adipocyte-like oil droplets were recognized in a three-dimensional micromass aggregate culture of human SDSCs with dexamethasone (100 or 1000 nM) plus BMP2 (50 ng/mL), which indicates that high concentration of dexamethasone could cause adipogenesis in chondrogenic culture of human SDSCs [6].

Many studies found that dexamethasone increased the aggrecan or proteoglycan synthesis rates in human ADSCs, human adult trabecular bone mesenchymal progenitor cells (MPCs) and equine BMSCs [33,104,105]. However, dexamethasone (1 µM) inhibited insulin-induced chondrogenesis and decreased chondrogenic potential in ATDC5 cells [106]. In combination with TGFβ1 or TGFβ3, dexamethasone augments the levels of *ACAN*, *COL2A1* and *COMP* (cartilage oligomeric matrix protein) in human ADSCs and trabecular bone MPCs, and bovine BMSCs [33,104,107]. However, Kurth *et al.* found that the TGFβ1-mediated expression of *ACAN* and *COL2A1* mRNAs was not enhanced in the presence of 100 nM dexamethasone, whereas the BMP2-induced expression of these markers was markedly suppressed in human SDSCs [108]. In fact, tissue sources of MSCs, dosages and growth factors combined have an impact on the role of dexamethasone in chondrogenesis. In aggregates of bovine BMSCs, 100 nM dexamethasone enhanced TGFβ1-induced chondrogenesis, but had little influence on BMP2-induced response [109]. In human ADSCs, dexamethasone (10 or 100 nM) significantly reduced TGFβ1-mediated increases in proteoglycan synthesis rates on days 1 and 5, but notably increased the rates on day 9 [33]. In aggregates of bovine SDSCs, 100 nM dexamethasone exerted no remarkable effect on either TGFβ1- or BMP2-induced chondrogenesis [109]. Dexamethasone concentration also affects chondrogenic differentiation of adult stem cells [6,105]. For example, less than 10 nM dexamethasone combined with 10 ng/mL TGFβ1 or less than 1 nM dexamethasone with 50 ng/mL BMP2 enhanced the synthesis of proteoglycan and type II collagen in human SDSCs [6]. However, a higher concentration of dexamethasone (100 nM) with TGFβ1 or

more than 10 nM dexamethasone with BMP2 disturbed cartilaginous tissue formation [6]. These results indicate that the influence of dexamethasone on chondrogenesis is context dependent.

Studies also found that dexamethasone could increase adipogenic potential in human BMSCs, ROB-C26 (the clonal rat mesenchymal progenitor cell line) and 3T3–L1 cells [110–112]. A high concentration of dexamethasone (100 nM) favors adipogenic differentiation over osteogenic differentiation of human BMSCs or mouse pluripotent mesenchymal cells [113]. Combined with BMP2, dexamethasone treatment (10 nM) increased the early phase of differentiation of adipocytes in ROB-C26 [114].

A glucocorticoid receptor (GR) is required for dexamethasone-mediated modulation of chondrogenesis. Dexamethasone could promote chondrogenic differentiation of MSCs through enhancing TGFβ3-induced phosphorylation of Smads [115]. However, the mechanisms of dexamethasone in chondrogenic studies are not clear. There is more information about how dexamethasone induces adipogenesis. Type I Runx2 kept 3T3–L1 cells in a growth-arrested state and was significantly down-regulated during adipocyte differentiation [116]. Knockdown of *RUNX2* stimulated adipogenesis of 3T3–L1 cells; dexamethasone repressed type I Runx2 through direct binding of GR in the *RUNX2* P2 promoter at the transcriptional level [116]. These results indicate that Runx2 may be a downstream target of dexamethasone in the adipogenic differentiation of 3T3–L1 cells. Moreover, C/EBPα was significantly up-regulated in dexamethasone-induced osteoporotic BMSCs by a mechanism that involved inhibited DNA hypermethylation of its promoter [117].

FGF—In mammals, fibroblast growth factors (FGFs) are heparin-binding growth factors which contain 23 members. In general, FGF2, FGF9 and FGF18 have been primarily studied in chondrogenic differentiation while FGF1 and FGF2 are most investigated in adipogenic differentiation [118,119].

Many studies showed that FGF2 has a positive role in chondrogenic differentiation in human SDSCs and BMSCs, and equine synovial fluid chondroprogenitor cells (SFCPCs) [120–122]. FGF2 has been shown to retain chondrogenic potential when supplemented during expansion [121,123–125]. Our study discovered that 10 ng/mL FGF2 treatment during expansion, but not differentiation, increased GAG deposition, pellet size and chondrogenic gene expression during chondrogenic induction in human SDSCs [120]. However, another study found that treatment with 100 ng/ml FGF2 during the expansion period significantly accelerated cell expansion without affecting subsequent chondrogenic capacity in equine SFCPCs [126]. Similarly, supplementation of 1 ng/mL FGF2 during the expansion period increased adipogenic rather than chondrogenic differentiation in human BMSCs [127]. Moreover, supplementation with 10 ng/mL FGF2 alone or combined with 10 ng/mL TGFβ3 during the differentiation period decreased *COL2A1* in human BMSCs [128]. Hildner *et al.* also found that the addition of 10 ng/mL FGF2 during the differentiation period abolished the chondrogenic effect of combined 10 ng/mL BMP6 and 10 ng/mL TGFβ3 in human ADSCs [129]. However, 10 ng/mL FGF2 and 10 ng/mL TGFβ2 have a synergistic effect in chondrogenic differentiation of human BMSCs [130].

FGFs, such as FGF1 and FGF2, have been demonstrated to have strong adipogenic effects in the presence of adipocyte differentiation stimuli [131–133]. FGF2 seems to enhance adipogenic potential of human ADSCs when present (1000 ng/mL) during expansion [133]. However, addition of FGF2 in the differentiation phase only was not effective for adipogenesis of human ADSCs (at 10 ng/mL) [134] and even inhibited adipogenic differentiation of human BMSCs (at 25 ng/mL) in collagen gels in the presence of heparin (25 µg/mL) [119]. FGF1 has been shown to promote adipogenic differentiation for preadipocytes. Hutley *et al.* demonstrated that FGF1 treatment (1 ng/ml) only during the expansion phase or a continuous treatment with FGF1 in the expansion and differentiation periods enhanced primary human preadipocyte adipogenic differentiation [131]. However, FGF1 treatment (25 ng/mL) in the presence of heparin (25 µg/mL) only during differentiation induction had a tendency to inhibit adipogenic differentiation of human BMSCs [119].

Exposure of FGF2 to MSCs during expansion up-regulated *SOX9* [118]. In addition, Pizzute *et al.* found FGF2 pretreatment significantly up-regulated both p-p38 and p-Jnk (Jun N-terminal kinase) signals in human SDSCs; total Erk1/2 was markedly reduced, while p-Erk1/2 was moderately suppressed [120]. Similarly, short-term treatment of FGF2 to 3T3–L1 cells promoted adipogenesis by phosphorylation of Erk1/2 and increased the expression of *PPARG* and *CEBPA* [135]. Another study showed that the inhibitory effect of FGF2 on adipogenesis of human BMSCs is dependent on high mobility group A-2 (HMGA2) [136]. Moreover, FGF1 down-regulates BAMBI (BMP and activin membrane-bound inhibitor homolog) in a PI3K-dependent manner to induce adipogenic differentiation [137].

IGFI—Insulin-like growth factor I (IGFI) binding with IGF receptor (IGFR), which belongs to the family of receptor tyrosine kinases [138], has been shown to stimulate chondrogenic and adipogenic differentiation [36,139,140]. Treatment using 100 ng/mL IGFI alone for three weeks did not induce dominant chondrogenesis in CL-1 cells; however, in the presence of 10% FCS, IGFI increased both Alcian blue staining intensity and fractional Oil Red O positive area [36]. Furthermore, Frisch *et al.* utilized recombinant adeno-associated virus (rAAV) vectors to overexpress IGFI and found that application of IGFI vector significantly increased pellet diameters, proteoglycan and type II collagen in the aggregates and the intensities of Oil Red O staining in human BMSCs and bone marrow aspirates [139,140]. These data indicate that IGFR signaling is involved in chondrogenesis and adipogenesis.

MAPK pathways are a downstream signal of the IGFR pathway [141]. A marked reduction of IGFI-mediated Erk1/2 activation has been demonstrated to occur during chondrogenesis [142]. Moreover, ATDC5 cells continually exposed to PD98059, a selective inhibitor of MAPK kinases, plus IGFI showed a greater degree of chondrogenic differentiation, as demonstrated by both Alcian blue staining and *COL10A1* (type × collagen) expression, than cells exposed to IGFI alone [142]. Moreover, an alginate-chondrocyte system with IGFI treatment inhibited Erk1/2 and resulted in an increase in proteoglycan synthesis [143]. Similarly, there was a dramatic decrease in IGFI-stimulated MAPK activity during early differentiation of 3T3–L1 cells, which was permissive for IGFI-mediated adipogenic differentiation [144]. PD98059 enhanced adipogenic markers, such as *PPARG*, *aP2* and *LPL*, suggesting that inhibition of MAPK in subconfluent, proliferating 3T3–L1 cells

accelerates adipogenic differentiation [145]. These data indicate that down-regulation of the Erk1/2 pathway is indispensable for IGFI-stimulated chondrogenesis and adipogenesis.

IGFI has also been shown to stimulate chondrogenic and adipogenic differentiation through the PI3K pathway. In the absence of serum, 100 ng/mL IGFI activated PI3K and its downstream signaling molecule, Akt (protein kinase B), in chick limb bud mesenchymal cells. Moreover, inhibition with LY294002, a selective PI3K inhibitor, blocked the ability of IGFI to stimulate the accumulation of proteoglycan in chick mesenchymal cells, human ankle articular chondrocytes and rat BMSCs, implying that IGFI induces chondrogenesis of mesenchymal cells *via* the PI3K/Akt pathway [143,146,147]. Similarly, LY294002 abolished the *LPL* expression, which functions as a terminal marker of adipogenesis in human orbital preadipocytes [148]. Moreover, activation of insulin receptor substrate 1 (IRS1) and IRS2 and associated PI3K pathways led to activation of PPAR γ and C/EBP α and thereby resulted in the induction of adipogenic differentiation [149]. Thus, IGFI signaling is involved in both chondrogenic and adipogenic differentiation.

Calcium ions—Calcium ions (Ca^{2+}) play a pivotal role in regulating cell differentiation potential [150,151]. Calcium homeostasis during chondrogenesis is complicated. Ca^{2+} concentrations influenced the response of MSCs to chondrogenic induction. A low concentration (1.8 mM) showed no effect for chondrogenic differentiation while a higher one (5 or 8 mM) showed no difference or negative influence [152,153]. Moreover, transient Ca^{2+} exposure (5 mM) enhanced chondrogenesis while subsequent exposures to elevated Ca^{2+} (5 mM) suppressed chondrogenic differentiation [154]. Calcium channels, which are regulated by physical stimuli, such as hydrostatic pressure, electrical stimulation and pulse electromagnetic fields, seemed to play a crucial role in chondrogenic differentiation of MSCs [154–156]. Voltage operated calcium channels, transient receptor potential channels and purinergic receptors have been reported to be regulated by physical stimuli [150,151,157,158]. Modulating these channels or receptors can influence the concentration of intracellular Ca^{2+} , which may have an impact on chondrogenesis. For example, transient receptor potential channel antagonists could effectively block chondrogenesis of the first exposition to pulse electromagnetic fields [154].

As for adipogenesis, increasing Ca^{2+} inhibited the early stages while promoting the late stage of differentiation, thus exerting a biphasic regulatory role [159]. What is more, continuous high concentrations of Ca^{2+} inhibited adipogenic differentiation of 3T3–L1 preadipocytes [160]. Calcium ions have been reported to play a positive role in adipogenic differentiation of porcine SDSCs and BMSCs, mouse BMSCs and human umbilical cord blood-derived MSCs through different pathways [152,161–165].

4.2. Biophysical factors

Given physical interaction with elements in the microenvironment, the shape of a stem cell is one of the biophysical factors implicated in cell fate decision. Cell shape can be influenced through micropatterned substrates [166], chitosan/polycaprolactone blended materials [167], gelatin/hyaluronic acid cryogels [168], ECM composition and mechanical properties [169] and chemicals [169,170], indicating that spherical morphology encourages

chondrogenic and adipogenic differentiation. There is increasing evidence showing that stem cell fate is also influenced by macro-mechanical stimulation, such as compression and shear forces, and by micro-mechanical stress, such as substrate stiffness [171]. (Figure 4) (Table 2)

Micro-mechanical stress—As an external signal, substrate stiffness of ECM, usually represented by elastic modulus or Young's modulus, is a determinant of stem cell lineage differentiation [172]. Many studies show that, compared to stiffer ones, softer substrates, such as hydrogel, porous/fibrous scaffold and decellularized ECM (dECM), were more likely to support MSC chondrogenesis [171]. Moreover, less stiff acellular ECM scaffolds, such as cartilage-derived dECMs and cell-derived dECMs, also enhanced chondrogenic differentiation by increasing chondrogenic gene expression compared to stiffer scaffolds [91,102,173,174]. Similarly, softer matrix also promotes stem cell differentiation into adipogenic lineage. For instance, Park *et al.* showed that, compared to stiffer substrates (3, 15 kPa), human BMSCs seeded onto a soft substrate (1 kPa) had a higher expression of the adipogenic marker *LPL* as well as chondrogenic marker *COL2A1* expression [175]. Moreover, MSCs on softer substrates, such as adipose matrix-coated polyacrylamide gel and hydrogel substrates, exhibited more adipogenic markers and fat droplets compared to a stiffer matrix [176–178].

Direct evidence showed that dECM deposited by fetal SDSCs with lower elasticity promoted chondrogenic and adipogenic differentiation [102]. Generally, substrate stiffness may likely guide MSC differentiation down corresponding tissue lineages of similar stiffness. For example, substrates approximating the elastic moduli of cartilage (0.4–0.8 MPa) may be more likely to enhance stem cells toward chondrogenesis [179–181], while scaffolds closely mimicking that of adipose tissue (2–8 kPa) might promote adipogenesis [176,182–184].

The exact mechanisms of substrate stiffness underlying chondrogenesis and adipogenesis are unknown, but recent studies indicate that actin and ROCK (Rho-associated protein kinase)/RhoA might be involved. Cytochalasin D disrupted the actin cytoskeleton and promoted chondrogenic and adipogenic differentiation [170,185]; treatment with Y27632, a selective inhibitor of ROCK, increased GAG production and decreased the number of actin fibers [186]. Inhibiting both ROCK and RhoA also promoted MSCs toward adipogenic differentiation [170]. These studies indicate that MSCs with a less organized and less stiff actin cytoskeleton organization are more prone to differentiate into chondrocytes and adipocytes.

Macro-mechanical stress—Many studies showed that direct compression promoted chondrogenesis [187–189]. For instance, human BMSCs were seeded in either chitosan-coated poly L-lactide-co-ε-caprolactone scaffolds [190] or hyaluronic acid hydrogel [191] with dynamic compression (5 or 10% strain, 1 Hz) enhanced cartilage formation and suppressed chondrocyte hypertrophy. However, the effects of mechanical stimuli on adipogenic differentiation are not well known. One study subjected SGBS (a human preadipocyte cell line) to a compressive force of 226 Pa for 12 hours [192]. They found that compressive force immediately after adipogenic induction did not affect adipogenic differentiation; however, compressive force before adipogenic induction significantly inhibited PPARγ and C/EBPα through the up-regulation of cyclooxygenase-2.

Oscillatory fluid flow (1 Hz, peak shear stresses of 1.0 Pa, 1 hour) has also been shown to induce the up-regulation of *SOX9* and *PPARG* in C3H10T1/2 progenitor cells and its promotion of chondrogenic and adipogenic differentiation depended on inhibiting tension within the actin cytoskeleton, indicating it has the potential to regulate stem cell fate [193]. However, with the increasing magnitude of fluid shear stimulation (from 0.009 to 1.089 dyne/cm²), higher YAP (Yes associated protein) decreased adipogenic differentiation and initiated dedifferentiation for chondrocytes [194]. Further research is needed to clarify the role of shear force in chondrogenic and adipogenic differentiation.

4.3. Biological factors

Biological factors, such as hypoxia and aging, provide a better way to understand MSC differentiation toward chondrogenesis versus adipogenesis due to pathophysiologic conditions. (Figure 5) (Table 3)

Hypoxia—In general, hypoxia promotes chondrogenic differentiation while suppressing adipogenic differentiation, although with some inconsistent results. Hypoxia during the differentiation period could enhance chondrogenic marker genes, transcription factors and ECM deposition in rat BMSCs and human infrapatellar fat pad stem cells and BMSCs [195–197]. Hypoxia (3% O₂) during the expansion and differentiation periods could enhance expression of *COL2A1*, *ACAN* and *SOX9* in human BMSCs [198]. However, results are inconsistent. For example, hypoxia during chondrogenic induction of human ADSCs did not significantly alter the levels of *COL2A1* and *ACAN* [199]. Cicone *et al.* even found that expression of *SOX9* and *ACAN* in human BMSCs decreased during chondrogenic induction under severe hypoxia (1% O₂) [200].

Many studies showed that adipogenic induction of stem cells under hypoxia resulted in attenuated adipogenic differentiation [201–203]. However, other studies show that hypoxic conditions in the myogenic cell lines, C2C12 and G8, increased adipocyte differentiation [204]; in addition, mild hypoxia (4% O₂) in 3T3–L1 cells or extreme hypoxia (0.2% O₂) in human BMSCs promoted adipogenesis [205,206]. These observations indicate that the effect of oxygen on adipogenic differentiation is extensive and cell-type specific. Interestingly, stem cells that had previously been cultured in hypoxia could subsequently be stimulated to exhibit normal or even significantly higher differentiation capacity, indicating that hypoxic preconditioning may represent a strategy to enhance MSC adipogenic differentiation [207–209].

In terms of mechanism, hypoxia inducible factors (HIFs) may play critical roles in stem cell chondrogenic and adipogenic differentiation under hypoxia. The expression level of HIF1 α was significantly increased on day 21 during chondrogenic differentiation of equine hypoxia-expanded BMSCs [210]. Besides, HIF1 α was found to be able to bind to a Sox9 promoter and up-regulate this key transcription factor [211]. Except for HIFs, hypoxia was demonstrated to enhance chondrogenic differentiation by inhibiting apoptosis *via* activating the PI3K/Akt/FoxO pathway [212]. During adipogenic differentiation, HIF1 was increased to regulate DEC1 (Differentiated embryo-chondrocyte expressed gene 1)/Stra13 (Stimulated with retinoic acid 13), thereby repressing PPAR γ 2 promoter activation [201]. This finding

indicates that HIF1 mediates hypoxia-induced inhibition of adipogenic differentiation. Moreover, hypoxia increased miR-27a and miR-27b, which strongly inhibited PPAR γ and C/EBP α in preadipocytes [213]. Interestingly, extreme hypoxia (0.2% O₂) induced adipogenic differentiation of human BMSCs through HIF1 α and C/EBPs [206].

Aging—Many studies showed that *in vitro* aging (passage number in culture) and *in vivo* aging (donor age) influenced the differentiation potential of adult stem cells. Several reports showed that aging impaired stem cell chondrogenic potential in porcine, bovine and human BMSCs, and rat tendon-derived stem cells [214–217]. Different from chondrogenic differentiation, which declines with age, adipogenic differentiation seems to accelerate with aging. Aging up-regulated adipocyte specific genes such as *PPARG* and *aP2* [218,219]. In line with these molecular changes, the number and size of adipocytes increased in late passage MSCs compared to early passage ones [217]. However, no difference or a general decrease has been reported in adipogenic differentiation capacity of aged stem cells [216,220–223].

Stable gene expression levels of cyclin-dependent kinase inhibitor 2A (CDKN2A) and CDKN2C, the senescence-associated marker genes, in early passages contributed to effective chondrogenic differentiation [217]. Human BMSCs preserved chondrogenic potential with low CDKN2C and stable CDKN2A expression level. Increased CDKN2A expression led to impaired chondrogenic potential [217]. Adipogenic differentiation was less affected by CDKN2A and CDKN2C expression, but higher expression of CDKN2A resulted in a more effective adipogenic differentiation [217].

As aging progresses, reactive oxygen species (ROS) and oxidative stress have been reported to increase and to play vital roles in stem cell differentiation. Suppression of Heme oxygenase-1 (HO-1), an agent known to neutralize oxidative stress [224], strongly increased PPAR γ expression and adipogenesis in human BMSCs [225]. Correspondingly, overexpressing HO-1 suppressed adipogenic differentiation in porcine ADSCs [226]. Adenovirus-mediated expression of HO-1 in human BMSCs slightly decreased adipogenic differentiation of MSCs, but did not affect chondrogenic differentiation [227]. The abovementioned reports indicate that increasing intracellular oxidative stress may be one of the major drivers of adipogenesis during the aging process.

5. Conclusions

Over decades of studies, the relationship between chondrogenesis and adipogenesis is still not clear. For example, TGF β and Hedgehog signaling pathways can promote chondrogenesis and inhibit adipogenesis; interestingly, BMP signaling can promote both chondrogenesis and adipogenesis simultaneously through the Smad1/5/8 pathway and the p38 pathway. These findings indicate that chondrogenic and adipogenic differentiations are competing and reciprocal. To make matters more complicated, some external chemical factors have differing roles in stem cell differentiation through different pathways. For example, dexamethasone can promote adipocyte differentiation through C/EBP α but inhibits adipogenesis *via* Runx2. Studies have demonstrated that biochemical, biophysical and biological cues can exert their effects on the crosstalk between chondrogenesis and

adipogenesis *via* a variety of signaling pathways. These signals approach at a controlled cascade of transcription events, including Sox9 for chondrogenesis and C/EBPs and PPAR γ for adipogenesis. Unlike the relationship between chondrogenesis and osteogenesis or adipogenesis and osteogenesis, studies on the decision between chondrogenesis and adipogenesis are few. Understanding the mechanisms underlying the balance between chondrogenic and adipogenic differentiation is more meaningful *via in vivo* studies and *in vitro* studies at a clonal level. These new data will be of great significance to identify the pathogenic causes of cartilage and fat related diseases and will lead to better clinical applications of adult stem cells in cartilage and fat tissue engineering.

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Figure 1. Developmental origins of articular cartilage and adipose tissue.

Adult stem cells develop from the mesoderm and then commit into different lineages, including but not limited to chondrogenic and non-skeletal adipogenic lineage (brown adipocyte, brite/beige adipocyte, white adipocyte). However, in the cephalic region, adipocytes have a neuroectodermal origin. Lineage determination is influenced by a number of transcription factors and growth factors in a spatiotemporal pattern (See text for details).

Figure 2. Signaling pathways involved in regulating chondrogenic and adipogenic differentiation of MSCs.

These signaling pathways maintain a delicate balance between chondrogenesis and adipogenesis through regulating *SOX9* or *CEBPβ*. For example, TGFβ binding with TGFβR results in further activation of p38 and Smad2/3. The p38 signaling and Smad2/3 promotes chondrogenesis while Smad2/3 inhibits adipogenesis. BMP2/4/7 enhance chondrogenic and adipogenic differentiation. Interestingly, BMP7 favors adipogenesis over chondrogenesis (indicated by the dotted line). Hedgehog signaling has pro-chondrogenic and anti-adipogenic properties, which is consistent with canonical Wnt (cWnt) signaling while non-canonical Wnt (ncWnt) pathways promote both differentiations. Notch signaling is a negative regulator of chondrogenesis but plays an inhibitory and obligatory role in adipogenesis.

Figure 3. Biochemical factors that regulate chondrogenic and adipogenic differentiation of MSCs.

During the period of expansion, FGF2 has no effect or pro-chondrogenic properties while plays a pro-adipogenic role. During the differentiation period, the pro- and anti-differentiation roles of FGF2 have been reported in chondrogenesis and adipogenesis. Moreover, no effect (indicated by dotted line) has been reported for the addition of FGF2 in adipogenic differentiation. FGF1 in both the expansion and differentiation periods enhances adipogenesis. However, during the differentiation period, FGF1 treatment has a tendency to inhibit adipogenesis. Low-dose dexamethasone (Dex) treatment during the period of expansion increases both chondrogenic and adipogenic potentials. However, Dex supplementation in the differentiation period can enhance, inhibit or have no effect on chondrogenesis, depending on the tissue sources of MSCs, dosages and growth factors combined. IGFI signaling plays a positive role in both chondrogenesis and adipogenesis. During the period of expansion, calcium plays a positive role in adipogenesis while has no effect for chondrogenesis. During the differentiation period, the pro- and anti-differentiation roles of calcium have been reported in chondrogenesis and adipogenesis.

Figure 4. Biophysical factors that regulate chondrogenic and adipogenic differentiation of MSCs. (A) Cell shape: round morphology encourages chondrogenic and adipogenic differentiation. (B) Substrate stiffness: soft stiffness guides MSC differentiation down corresponding tissue lineages of similar stiffness, such as both chondrogenesis and adipogenesis. (C) Compression: compression promoted chondrogenesis; however, compression immediately after adipogenic induction does not have an effect (indicated by dotted line) but before stimulation significantly inhibits adipogenesis. (D) Shear force: oscillatory fluid flow up-regulates chondrogenic and adipogenic differentiation while a higher magnitude resulted in decreased chondrogenic and adipogenic differentiation.

Figure 5. Biological factors that regulate chondrogenic and adipogenic differentiation of MSCs. Both hypoxia and aging have complex effects on chondrogenesis and adipogenesis. In general, hypoxia promotes chondrogenic differentiation while suppressing adipogenic differentiation, although differential roles in either differentiation have been reported. Interestingly, aging impairs chondrogenic potential of MSCs while it has complex roles in adipogenic differentiation.

Table 1.

Studies investigating biochemical factors guiding lineage determination between chondrogenic and adipogenic differentiation

Cell Type	Treatment	Chondrogenic Differentiation	Adipogenic Differentiation	Possible Mechanisms	Refs
<i>Dexamethasome</i>					
3T3-L1 preadipocyte	1 μ M Dex during differentiation induction	-	increased	by repressing the transcription of TAZ, a suppressor of PPAR γ	112
	Dex(10, 100, 1000 nM) during differentiation induction	-	increased	by repressing type I <i>RUNX2</i> , which blocked adipocyte differentiation	116
ADSC (human)	Dex(10, 100 nM) and TGF β 1 (1, 10ng/mL)in alginate beads during differentiation induction	accumulated sulfated GAG on day 9 by combining with TGF β 1	-	-	33
ATDC5	1 μ M Dex during differentiation induction	suppressed formation of cartilage nodule-like structures	-	by down-regulating Wnt/ β -catenin signaling	106
BMSC (human)	10 nM Dex during expansion phase	increased	increased	-	103
	100 nM Dex during expansion and differentiation phases (pellets or cells)	increased	increased	-	110
	100 nM Dex during differentiation induction	increased	-	by increasing TGF β 3-induced phosphorylation of Smad2 and Smad3	115
BMSC (bovine)	100 nM Dex and 10 ng/mL TGF β 1 in agarose gels during differentiation induction	increased	-	by positive regulation through Smad2/3	107
BMSC or SDSC (bovine)	100 nM Dex and 10 ng/mL TGF β 1 or 200 ng/mL BMP2 in micromass culture during differentiation induction	increased TGF β 1-induced chondrogenesis in BMSCs, while exerting no remarkable effect on either-induced chondrogenesis in SDSCs	-	-	109
BMSC (equine)	Dex (1, 100 nM) in agarose gel during differentiation induction	increased matrix accumulation while 100 nM Dex suppressed undesirable hypertrophy	-	-	105
BMSC (mouse) or C3H10T1/2	BMSCs isolated from Dex (50 mg/kg daily)-treated mice or C3H10T1/2 treated with 1 μ M Dex during differentiation induction	-	increased	by up-regulating C/EBP α expression through inhibiting its promoter methylation <i>via</i> down-regulating Wnt/ β -catenin signaling	117
D1 cells	Dex (10^{-9} to 10^{-7} M) during	-	increased	-	113

Cell Type	Treatment	Chondrogenic Differentiation	Adipogenic Differentiation	Possible Mechanisms	Refs
ROB-C26	differentiation induction				
	100 nM Dex during differentiation induction	-	increased	by inhibiting β -catenin expression	111
SDSC (human)	10 nM Dex and 100 ng/mL BMP2 during differentiation induction	-	increased	by inhibiting osterix expression	114
	100 nM Dex combined with 10 ng/mL TGF β 1 or 200 ng/mL BMP2 in alginate during differentiation induction	suppressed BMP2-induced increase of <i>ACAN</i> and <i>COL2A1</i> but did not affect TGF β 3-induced expression	-	-	108
TBMPC (human)	Dex (1 nM to 1 μ M) with or without 50 ng/mL BMP2 and 10 ng/mL TGF β 3 in micromass aggregates or 3D pellets during differentiation induction	1 or 10 nM Dex combined with TGF β 3 or less than 1 nM Dex with BMP2 increased proteoglycan synthesis and type II collagen. 100 nM Dex with TGF β 3 or more than 10 nM Dex with BMP2 disturbed cartilage formation.	oil droplets increased when 1 μ M Dex with BMP2 was used and TGF β 3 suppressed adipogenesis induced by Dex in micromass aggregates.	-	6
	100 nM Dex combined with 10 ng/mL TGF β 3 during differentiation induction	increased	-	G α is required for Dex-mediated modulation of chondrogenesis.	104
FGF					
3T3-L1 preadipocyte	1 nM FGF2 during differentiation induction	-	increased	by phosphorylation of Erk1/2	135
ADSC (human)	10 ng/mL FGF2 or 10 ng/mL BMP6 or 10 ng/mL TGF β 3 during differentiation induction	FGF2 abolished chondrogenic effect of combined BMP6 and TGF β 3.	-	-	129
BMSC (human)	FGF2(1, 10, 100, 1000, 10000 ng/mL) during expansion phase	-	the strongest enhancement in the group of 1000 ng/mL FGF2	-	133
	10 ng/mL FGF2 during differentiation induction	-	no effect	-	134
	10 ng/mL FGF2 during expansion phase	increased	-	-	121,123
	FGF2 (1, 10 ng/mL) during expansion phase	increased	-	-	125
	1 ng/mL FGF2 during expansion phase	no effect	increased	-	127
	10 ng/mL FGF2 or 10 ng/mL TGF β 3	FGF2 alone or combined with TGF β 3 decreasing <i>COL2A1</i>	-	-	128

Cell Type	Treatment	Chondrogenic Differentiation	Adipogenic Differentiation	Possible Mechanisms	Refs
	during differentiation induction				
	10 ng/mL FGF2 or 10 ng/mL TGFβ2 during differentiation induction	FGF2 alone or combined with TGFβ2 increasing chondrogenesis	-	-	130
	25 ng/mL FGF1 or FGF2 together with 25 μg/mL heparin in 3D collagen gels during differentiation induction	-	decreased	-	119
	10 ng/mL FGF2 during expansion phase	increased	-	by up-regulating <i>SOX9</i>	118
	10 ng/m LFGF2 during differentiation induction	-	decreased	by up-regulating HMGA2	136
BMSC (rat)	3 ng/mL FGF2 during the expansion or differentiation period	-	increased	-	132
IPFSC (porcine)	5 ng/mL FGF2 during expansion phase followed by encapsulation in agarose hydrogels	increased	-	-	124
Preadipocyte (human)	1 ng/mL FGF1 during only expansion or both expansion and differentiation phases	-	increased	-	131
	1 ng/mL FGF1 and 90 μg/mL heparin during expansion and differentiation phases	-	increased	by down-regulating BAMBI in a PI3K-dependent manner	137
SDSC (human)	10 ng/mL FGF2 during expansion or differentiation phase	increased for the group with treatment in expansion	-	by up-regulating both p-p38 and p-Jnk while p-Erk1/2 was moderately suppressed	120
	FGF2(0.1, 1, 10, 100 ng/mL) during expansion phase	increased	-	-	122
SFCPC (equine)	100 ng/mL FGF2 during expansion phase	accelerated cell expansion without affecting subsequent chondrogenic capacity	-	-	126
<i>IGFI</i>					
3T3–L1 preadipocyte	7 nM IGFI during expansion and differentiation phases	-	increased	-	144
	7 or 10 nM IGFI during expansion and differentiation phases	-	increased	-	145

Cell Type	Treatment	Chondrogenic Differentiation	Adipogenic Differentiation	Possible Mechanisms	Refs
AC (human)	50 ng/mL IGFI in alginate gel during differentiation induction	increased	-	by activating the PI3K/Akt and Erk/MAPK pathways	143
ATDC5	IGFI(1, 10, 50 nM) during expansion and differentiation phases	increased	-	-	142
BMA (human)	transduced with rAAV to overexpress IGFI	increased	increased	-	140
BMSC (human)	transduced with rAAV to overexpress IGFI	increased	increased	-	139
BMSC (rat)	100 ng/mL IGFI during differentiation induction	increased	-	by upregulating p38, Erk1/2 and PI3K signaling in monolayer culture while upregulating PI3K signaling in 3D culture	147
CL-1	100 ng/mL IGFI during differentiation induction with 10% FCS	increased	increased	-	36
LBMC (chick)	100 ng/mL IGFI during differentiation induction	increased	-	by activating PI3K	146
Preadipocyte (human)	50 ng/mL IGFI during expansion phase	-	increased	by activating PI3K1A and mTORC1 signaling	148
Calcium					
3T3-L1 preadipocyte	Ca ²⁺ (1.8, 2.5, 5, 10 mM) during expansion and differentiation phases	-	decreased with treatment of Ca ²⁺ higher than 2.5 mM	through a G-protein-coupled mechanism mediated by a novel Ca ²⁺ sensor or receptor	160
ADSC (human)	Ca ²⁺ (1.8 or 8mM)during differentiation induction	elevated calcium inhibiting chondrogenesis	-	-	153
BMSC (human)	5 mM Ca ²⁺ or PEMF during differentiation induction	transient calcium exposure increasing chondrogenesis while subsequent exposures to elevated calcium suppressing chondrogenic differentiation	-	through the TRP channel superfamily by activation of the SOX9 pathway	154
BMSC (mouse)	under electrical stimulation (0, 1, 5 or 25 V/cm, with a duration of 8 ms and a frequency of 5.0 Hz)	increased	-	by driving ATP/Ca ²⁺ oscillations	156
	9 mM CaCl ₂ during differentiation induction	-	increased	by suppressing Erk activity	161
		-	increased	through CaSR following by a decrease in cAMP	163
	CaCl ₂ (1.8, 5.4, 10.8 mM) during	-	increased	-	162

Cell Type	Treatment	Chondrogenic Differentiation	Adipogenic Differentiation	Possible Mechanisms	Refs
BMSC (porcine)	differentiation induction				
	chelating free Ca ²⁺	depleting intracellular calcium stores suppressing the beneficial effect of hydrostatic pressure on chondrogenesis	-	<i>via</i> vimentin adaptation to loading	155
preadipocyte (human)	4 mM Ca ²⁺ during differentiation induction	-	increased	by activation of the CaMKII and PI3K/Akt signaling pathway	164
	Ca ²⁺ agonists (30 nM thapsigargin and 2 μ M A23187)	-	increasing Ca ²⁺ inhibiting the early stage while promoting the late stage of differentiation	-	159
SDSC (porcine)	5 mM Ca ²⁺ during expansion phase	no effect	increased	-	152
UCB-MSC (human)	1.8 mM CaCl ₂ during expansion phase	no effect	increased	by negatively regulating the Wnt5a/ β -catenin pathway	165

Abbreviation: AC: articular chondrocyte; ACAN: aggrecan; ADSC: adipose-derived stem cell; Akt: protein kinase B; ATDC5: derived from mouse teratocarcinoma cells and characterized as a chondrogenic cell line; BAMBI: BMP and activin membrane-bound inhibitor; BMA: bone marrow aspirates; BMP: bone morphogenetic protein; BMSC: bone marrow-derived stem cell; C3H10T1/2: a mouse mesenchymal cell line; CaCl₂: calcium chloride; CaMKII: calmodulin-dependent protein kinase II; cAMP: cyclic adenosine 3',5'-monophosphate; CaSR: calcium-sensing receptor; CDM: cartilage-derived matrix; C/EBP: CCAAT/enhancer-binding protein; CL-1: a bipotent cell line established from tibia of normal adult mouse; CoCl₂: cobalt chloride; COL2A1: type II collagen; D1 cells: pluripotent mesenchymal cells, which were cloned from BALB/c mouse bone marrow cells; Dex: dexamethasone; Erk: extracellular signal-regulated protein kinase; FCS: fetal calf serum; FGF: fibroblast growth factors; GAG: glycosaminoglycan; GR: glucocorticoid receptor; HMGA2: high mobility group A-2; IGF1: Insulin-like growth factor I; IPFSC: infrapatellar fat pad derived stem cell; Jnk: Jun N-terminal kinase; LBMC: limb bud mesenchymal cell; MAPK: mitogen-activated protein kinase; mTORC: mammalian target of rapamycin complex; PEMF: pulse electromagnetic fields; PI3K: Phosphatidylinositol 3-kinase; PPAR γ : peroxisome proliferator-activated receptor gamma; rAAV: recombinant adeno-associated virus; ROB-C26: the clonal rat mesenchymal progenitor cell line; RUNX2: Runt-related transcription factor 2; SDSC: synovium-derived stem cell; SFCPC: synovial fluid chondroprogenitor cell; Smad: small mothers against decapentaplegic homolog; TAZ: transcriptional co-activator with PDZ binding motif; TBMP: Trabecular bone mesenchymal progenitor cell; TGF: transforming growth factor; TRP: transient receptor potential; UCB-MSCs: umbilical cord blood-derived MSCs; Wnt: Wingless/int

Table 2.

Studies investigating biophysical factors guiding lineage determination between chondrogenic and adipogenic differentiation

Cell Type	Treatment	Chondrogenic Differentiation	Adipogenic Differentiation	Possible Mechanisms	Refs
<i>Micro-mechanical stress</i>					
Articular chondrocyte (bovine)	seeded on 3D fiber deposition PEOT/PBT scaffold	Cartilage mechanically matching scaffolds ($E' = 10$ MPa) increased chondrogenesis.	-	-	179
ADSC (human)	CDM within multi-layer electrospun PCL versus PCL construct	increased	-	-	174
	cultured on adipose matrix-coated PA gel or tissue culture plastic	-	gels mimicked the stiffness of adipose tissue (2 kPa) increasing adipogenesis most	-	176
BMSC (human)	PA gels with varied stiffness (1, 3, 15 kPa)	soft substrates (1 kPa) increasing chondrogenesis	soft substrates (1 kPa) increasing adipogenesis	by weaker cell adhesion	175
	cultured on a 1.37 or 4.47 kPa hydrogel matrix	-	soft substrate increasing adipogenesis	-	177
	cross-linked hydrogel based on thiol modified HA and gelatin with varied stiffness (0.15, 1.5 or 4 kPa)	-	hydrogel substrates with 4 kPa increasing adipogenesis	-	183
MSC (neonatal rat)	cultured on PA, PDMS substrates or PA-PEG-RGD gels with varied stiffness (~130, ~3170 kPa)	-	soft substrate increasing adipogenesis	-	178
SDSC (human)	dECM deposited by human adult SDSCs during expansion phase	Increased differentiation potential	-	-	91
	dECM deposited by human fetal SDSCs versus human adult SDSCs	dECM deposited by human fetal SDSCs increasing chondrogenesis	dECM deposited by human fetal SDSCs increasing adipogenesis	-	102
<i>Macro-mechanical stress</i>					

Cell Type	Treatment	Chondrogenic Differentiation	Adipogenic Differentiation	Possible Mechanisms	Refs
BMSC (human)	dynamic compression (an intermittent regimen, a strain amplitude of 15% and frequency of 1 Hz)	increased	-	-	187
	dynamic compression (1 Hz with 10 static offset strain)	increased	-	-	188
	dynamic compression (1 Hz with 10% sinusoidal strain, superimposed on a 10% static offset strain)	increased	-	by up-regulating TGF β pathway	189
	under free swelling or dynamic compression conditions (5% of strain, 1 Hz of frequency)	increased chondrogenesis in chitosan-coated PLCL	-	through TGF(3/Smad and integrin signaling	190
	dynamic compressive loading (10% peak compressive sinusoidal strain at 1 Hz frequency, superimposed on a 5% compressive tare strain)	increased chondrogenesis in encapsulated HA hydrogels	-	-	191
BMSC and AC (rat)	fluid shear stimulation (from 0.009 to 1.089 dyne/cm ²)	an increase in magnitude of stimulation initiating dedifferentiation for chondrocytes	an increase in magnitude of stimulation decreasing adipogenic differentiation	by increasing YAP expression to regulate differentiation	194
C3H10T1/2	oscillatory fluid flow (1 Hz, peak shear stresses of 1.0 Pa, 1 h)	increased	increased	by inhibiting tension within the actin cytoskeleton	193
SGBS	a compressive force of 226 Pa for 12 h	-	compressive force before induction significantly inhibiting adipogenesis	by suppressing <i>PPARG2</i> and <i>CEBPA</i> in a COX-2-dependent manner	192

Abbreviation: AC: articular chondrocyte; ADSC: adipose-derived stem cell; BMSC: bone marrow-derived stem cell; C3H10T1/2: a mouse mesenchymal cell line; CDM: cartilage-derived matrix; CEBP: CCAAT/enhancer-binding protein; COX-2: cyclooxygenase 2; dECM: decellularized extracellular matrix; HA: hyaluronic acid; MAPK: mitogen-activated protein kinase; PA: polyacrylamide; PCL: poly(ϵ -caprolactone); PDMS: polydimethylsiloxane; PEG: poly(ethylene glycol); PEOT/PBT: poly(ethylene oxide-terephthalate)/poly(butylene terephthalate); PLCL: poly L-lactide-co- ϵ -caprolactone; PPARG: PPAR γ , peroxisome proliferator-activated receptor gamma; RGD: Arg-Gly-Asp;

SDSC: synovium-derived stem cell; SGBS: (Simpson–Golabi–Behmel syndrome) cells, a preadipocyte cell line derived from human adipose tissue; Smad: small mothers against decapentaplegic homolog; TGF: transforming growth factor; Wnt: Wingless/int; YAP: Yes-associated protein

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Table 3.

Studies investigating biological factors guiding lineage determination between chondrogenic and adipogenic differentiation

Cell Type	Treatment	Chondrogenic Differentiation	Adipogenic Differentiation	Possible Mechanisms	Refs
<i>Hypoxia</i>					
3T3-L1 preadipocyte	2% O ₂ during differentiation induction	-	decreased	by regulating DEC1/ Stra13 to repress <i>PPARG2</i> promoter	201
	chemical hypoxia (CoCl ₂) during differentiation induction	-	decreased	by inhibiting <i>PPARG</i> in a HDAC-independent manner	202
	1 or 4% O ₂ during differentiation induction	-	mild, but not severe, promoting differentiation	through excess of acetyl-CoA independently of HIF activation	205
	1% O ₂ during differentiation induction	-	decreased	by increasing miR-27a and miR-27b to inhibit PPAR γ and C/EBP α	213
ADSC (human)	5% O ₂ during differentiation induction	no effect	-	-	199
	2% O ₂ during expansion phase	-	increased	-	209
ADSC (murine)	2% O ₂ during expansion phase	-	increased	by increasing Sca-1/CD44	208
BMSC (equine)	5% O ₂ during expansion phase	increased	no effect	by up-regulating HIF1 α	210
BMSC (human)	5% O ₂ during differentiation induction	increased	-	-	197
	3% O ₂ during expansion and differentiation phases	increased	-	by up-regulating HIF2 α	198
	1% O ₂ during differentiation induction	decreased	decreased	-	200
	0.2% O ₂ during differentiation induction	-	increased	-	206
	5% O ₂ during differentiation induction	increased	-	by up-regulating HIF1 α	211
	1% O ₂ during differentiation induction	increased	-	by activating the PI3K/Akt/FoxO pathway	212
BMSC (rat)	2% O ₂ during differentiation induction	increased	-	by up-regulating HIF1 α	196

Cell Type	Treatment	Chondrogenic Differentiation	Adipogenic Differentiation	Possible Mechanisms	Refs
C2C12 and G8 (murine)	1% O ₂ during differentiation induction	-	increased	-	204
IPFP cells (human)	5% O ₂ during differentiation induction	increased	-	by up-regulating HIF2 α	195
MSC (human)	3% O ₂ during expansion phase	-	increased	-	207
Preadipocyte (human)	1% O ₂ or chemical hypoxia (DFO) during differentiation induction	-	decreased	-	204
<i>Aging</i>					
ADSC (bovine)	passages 2, 5	passage 2 > passage 5	passage 2 > passage 5	-	222
BMSC (porcine)	passage 5–15	early > late passage	-	-	214
BMSC (bovine)	fetal, juvenile and adult bovine donors	fetal > juvenile > adult donor	-	-	215
BMSC (human)	passages 3, 5, 6, 7, 9, 11	early and mid > late passage	early and mid < late passage	by increasing <i>CDKN2A</i> level	217
	80 patients (14–79 year-old)	-	young < old donor	-	218
	passage 1–10	-	differentiation potential 6th passage on	-	220
	passages 3, 5, 8, 14	-	early > late passage	-	221
	young (aged 18–29 years) and old (aged 68–81 years)	-	no effect	-	223
BMSC (murine)	adult (6–8 month-old) and old (20–26 month-old)	-	adult < old donor	by altering TGF β and BMP2/4 signaling pathways	219
TDSC (rat)	early (P5), mid (P10) and late passages (P20, P30) of TDSCs	early and mid > late passages	early and mid > late passages	-	216

Abbreviation: ADSC: adipose-derived stem cell; Akt: protein kinase B; BMSC: bone marrow-derived stem cell; CEBP: CCAAT/enhancer-binding protein; CDKN2A (cyclin-dependent kinase inhibitor 2A); CoCl₂: cobalt chloride; DEC1/Stra13: differentiated embryo-chondrocyte expressed gene 1/stimulated with retinoic acid 13; DFO: desferrioxamine; FoxO: forkhead box protein O; HDAC: histone deacetylase; HIF: hypoxia inducible factor; IPFP: infrapatellar fat pad; PI3K: Phosphatidylinositol 3-kinase; PPAR γ : PPAR γ , peroxisome proliferator-activated receptor gamma; TDSC: tendon-derived stem cells