REVIEW COMMUNICATIONS

CELL BIOLOGY

Osteogenic differentiation: a universal cell program of heterogeneous mesenchymal cells or a similar extracellular matrix mineralizing phenotype?

Arseniy Lobov and Anna Malashicheva

Laboratory of Regenerative Biomedicine, Institute of Cytology, Russian Academy of Sciences, Tikhoretskiy pr., 4, Saint Petersburg, 194064, Russian Federation

Address correspondence and requests for materials to Arseniy Lobov, arseniylobov@gmail.com

Abstract

Despite the popularity of mesenchymal stem cells (MSCs), many fundamental aspects of their physiology still have not been understood. The information accumulated to date argues that MSCs from different sources vary in their differentiation potential and, probably, in molecular mechanisms of trilineage differentiation. Therefore, this review consists of two parts. Firstly, we focus on the data on inter- and intra-source variation of MSCs. We discuss in detail MSC variation at the single-cell level and direct omics comparison of MSCs from four main tissue sources: bone marrow, adipose tissue, umbilical cord and tooth. MSCs from all tissues represent heterogeneous populations in vivo with sub-populational structures reflecting their functional role in the tissue. After in vitro cultivation MSCs lose their natural heterogeneity, but obtain a new one, which might be regarded as a cultivation artifact. Nevertheless, MSCs from various sources still keep their functional differences after in vitro cultivation. In the second part of the review, we discuss how these differences influence molecular mechanisms of osteogenic differentiation. We highlight at least one subtype of mesenchymal cells differentiation with matrix mineralization — odontoblastic differentiation. We also discuss differences in molecular mechanisms of pathological heterotopic osteogenic differentiation of valve interstitial and tumor cells, but these assumptions need additional empirical confirmation. Finally, we observe differences in osteogenic differentiation molecular mechanisms of several MSC types and argue that this differentiation might be influenced by the cell context. Nevertheless, bone marrow and adipose MSCs seem to undergo osteogenic differentiation similarly, by the same mechanisms.

Keywords: mesenchymal stem cells, osteogenic differentiation, MSCs, MSC heterogeneity, scRNA-seq, cell differentiation, systems biology

Ethics statement: This paper does not contain any studies involving human participants or animals performed by any of the authors.

Competing interests: The authors have declared that no competing interests exist.

Abbreviations:

MSCs — mesenchymal stem cells;

HSCs — hematopoietic stem cells;

ECM — extracellular matrix;

ALP — alkaline phosphatase;

Pi — inorganic phosphate;

scRNA-seq — single-cell RNA-sequencing;

BM — bone marrow;

Adipo-/Osteo-CAR — Cxcl12-abundant-reticular (CAR) cells commitment to adipo- and osteogenic lineages respectively;

ADSCs — adipose-derived stem cells;

DPSCs — dental pulp derived stem cells;

BM-MSCs — bone marrow derived mesenchymal stem cells;

WJ-MSCs — mesenchymal stem cells isolated from Wharton's jelly;

UC-MSCs — umbilical cord MSCs;

PB-MSCs — peripheral blood MSCs;

AM-MSCs — mesenchymal stem cells isolated from amniotic membrane;

CM-MSCs — mesenchymal stem cells isolated from chorionic membrane;

DC-MSCs — mesenchymal stem cells isolated from decidua;

Pl-MSCs — mesenchymal stem cells isolated from a placenta;

CAVD — calcific aortic valve disease;

VICs — valve interstitial cells;

dFBs — dermal fibroblasts;

UCPVCs — umbilical cord perivascular cells;

EMT — epithelial-to-mesenchymal transition.

Introduction

The term MSCs might be deciphered from its original name Mesenchymal Stem Cells (Caplan, 1991) to a wide range of other more specialized terms: Multipotent Stromal Cells, Marrow Stromal Cells, Mesenchymal Stromal Cell, Mesodermal Stem Cells, Medicinal Signaling Cells, etc. (Caplan, 2017a,b). Each of the terms focuses on one of the MSC features which made them the most popular cell types for fundamental cell biology and clinical studies. For example, Mesenchymal Stem Cells emphasizes their mesenchymal origin (though some MSCs come from the neural crest) and stem-cell properties, while Medicinal Signaling Cells emphasizes the potential of biomedical applications of their secretome. Nevertheless, all of these terms define the same cells (Caplan, 2017a,b; Mastrolia et al., 2019).

The minimal criteria of MSCs is rather simple: they must be plastic-adherent; express CD105, CD73, CD90, lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19, HLA-DR; and must be able to differentiate to osteoblast, adipocyte, and chondroblast lineages in vitro (Dominici et al., 2006). The main purpose of these criteria is to separate MSCs from other cell types usually co-isolated with MSCs such as endothelial cells, hematopoietic progenitors and hematopoietic cells, monocytes, and macrophages.

All MSCs might undergo trilineage differentiation, but the biological meaning of this phenomenon in vivo is still debatable. For example, despite the osteogenic potential, the contribution of osteogenic differentiation of bone marrow MSCs (BM-MSCs) to fracture healing seems to be minimal (Bragdon and Bahney, 2018). Moreover, trilineage differentiation is usually observed at the phenotypic level — in the vast majority of research articles, the authors do not go beyond specific staining (e.g., Alizarin Red stain for matrix mineralization) and qPCR or western-blotting for key markers of specific differentiation. Nevertheless, phenotypic similarity might mask actual physiological differences of these processes between different MSCs. For example, Runx2 is involved in both osteogenic and odontogenic differentiation, which have differences in molecular mechanisms and phenotype of differentiated cells (see the detailed discussion of these differentiation types below). Thus, a comparison of the physiology of trilineage differentiation of different types of MSCs is necessary. In this review, we focused on a discussion of osteogenic differentiation in the context of functional differences of MSCs from various tissues and their subpopulations.

A brief overview of osteogenic differentiation

Osteogenic differentiation is the process of differentiation of mesenchymal cells into osteoblast-like cells able to secrete and mineralize extracellular matrix (ECM).

In vivo osteogenic differentiation occurs by endochondral or intramembranous ossification. The molecular mechanisms and cellular regulation of these two types of bone formation were systematically observed by C. Hartmann and Y. Yang (2020), and we will provide only a brief description. Both types of ossification start from the condensation of mesenchymal cells of various origins. During intramembranous ossification mesenchymal cells directly differentiate into osteoblasts, start to produce osteoid (bone ECM) and form an ossification center. The ossification center grows by the differentiation of newly mesenchymal progenitors which form a periosteum. During its expansion, the ossification center starts to surround the capillaries, which form bone marrow and support the process of bone formation. Endochondral ossification, on the contrary, starts with the chondrogenic differentiation of condensed mesenchymal cells. As a result, the cartilaginous template of future bone surrounded by MSCs (perichondrium) is formed. Then the chondrocytes in the diaphysis region become hypertrophic. Hypertrophic chondrocytes attract vessels by vascular endothelial growth factor (VEGF) production. In this zone, osteogenic differentiation of mesenchymal cells and trans-differentiation of hypertrophic chondrocytes to osteoblasts occur. Thus, a primary ossification center is formed and the bone begins to take its final shape — the bone marrow cavity and two grow plates are formed in the primary ossification center. Finally, in some bones, the formation of a secondary ossification center occurs.

Both in vivo types of ossification described above have complicated regulation by systemic and paracrine signaling, cellular interactions, ECM, and even include epigenetic mechanisms (Hartmann and Yang, 2020; Salhotra et al., 2020). There are several signaling pathways

CELL BIOLOGY

involved in osteoblast differentiation and ossification: TGF-beta (transforming growth factor-beta; mainly bone morphogenic proteins; Rahman et al., 2015), Wnt (Kobayashi et al., 2016), FGF (fibroblast growth factors; Marie, Miraoui and Sévère, 2012) and Notch. Notch signaling has ambiguous effects on ossification — there is data about both inhibitory and pro-osteogenic effects of Notch on osteoblast differentiation and bone development. We argue that Notch signaling has a dose-dependent effect on ossification and osteogenic differentiation of mesenchymal cells (Semenova et al., 2020). Both intramembranous and endochondral ossifications are controlled by expression of Runx2 — the most upstream transcriptional factor of osteogenic differentiation regulating expression of several downstream genes such as collagen I, osterix and alkaline phosphatase (ALP; Hartmann and Yang, 2020).

The process of osteogenic differentiation of MSCs in vitro might be divided into three phases: proliferation, ECM maturation, and ECM mineralization (Pitkänen, 2020). One of the key markers of an early proliferation phase is the expression of collagen type I and osteopontin. The contact of extracellular matrix with collagen type I is known to stimulate osteogenic differentiation itself through integrin-dependent activation of Runx2 (runt-related transcription factor 2) through the MAPK (mitogen-activated protein kinase) signaling cascade (Salasznyk et al., 2004; Langenbach and Handschel, 2013; Arai et al., 2021). In the second stage of osteogenic differentiation, the cell proliferation fades while secretion of ECM continues and grows with the upregulation of alkaline phosphatase (ALP). The first stage is initiated with the expression of Runx2, which induces the expression of osterix necessary for the second stage associated with ECM maturation. Runx2 is essential for the early stages of osteoblast maturation from mesenchymal stem cells — Runx2$^{-/-}$ mice have no osteoblast marker gene expression (Komori et al., 1997). At the third stage, besides matrix mineralization, secretion of collagenase and many bone ECM proteins such as bone gamma-carboxyglutamic acid-containing protein (BGLAP) and sialoprotein also occur.

In most in vitro studies of MSCs, osteogenic differentiation is induced by a specific osteogenic medium. Classically, the osteogenic medium is similar to simple cultivation media supplemented with 100 nM of dexamethasone, 50 μM of ascorbic acid-2-phosphate, and 10 mM of β-glycerophosphate (Jaiswal et al., 1997; Langenbach and Handschel, 2013). This cocktail is assumed to partly reflect physiological inducers of osteogenic differentiation and each component has its function (Chang et al., 2006). Ascorbic acid is a cofactor of enzymes that hydroxylate pro-collagen, so it enhances the secretion of collagen type I to the extracellular matrix. β-Glycerophosphate mainly acts as a source of inorganic phosphate (Pi) re-

quired for extracellular matrix mineralization. Pi also might regulate the mineralization process by induction of expression of pro-osteogenic genes (bone morphogenic protein-2 (BMP-2) and osteopontin) by ERK signaling and cyclic-AMP/protein-kinase-A pathways (Tada et al., 2011; Langenbach and Handschel, 2013); induction of intracellular ROS production which enhances mineralization in committed osteogenic cells (Khalid et al., 2020). Finally, dexamethasone might directly induce Runx2 expression in MSCs through several signaling pathways (Langenbach and Handschel, 2013). Nevertheless, dental follicle cells might also be differentiated into mineralizing cells with dexamethasone, but in this case, it acts through PLZF (promyelocytic leukemia zinc finger protein), but not Runx2 (Felthaus et al., 2014; see more detailed discussion in the "odontoblastic differentiation" section). Moreover, it has been demonstrated that an osteogenic medium with 100 nM dexamethasone might promote not only osteogenic but also adipogenic differentiation of mouse BM-MSCs (Ghali et al., 2015). This ambiguous effect of dexamethasone on MSC differentiation may come from the observation that it might regulate expression of Runx2 not directly but through downregulation of SOX9 (SRY-Box Transcription Factor 9), which might inhibit Runx2 (Della Bella et al., 2021). Moreover, dexamethasone is also upregulating PPARG (peroxisome proliferator-activated receptor gamma), the high expression of which is assumed to switch MSC adipogenic differentiation direction in osteogenic conditions (Della Bella et al., 2021). Thus, dexamethasone has a complicated and unclear effect on cell fate and it might have different effects on cells in the same culture which might be demonstrated by the presence of cells in both osteo- and adipocyte differentiation state after dexamethasone treatment (Della Bella et al., 2021).

Apart from the described inducers of osteogenic differentiation, basal media is also an important component that has a high impact on osteogenic differentiation. For example, porcine BM-MSCs cultured in αMEM/aDMEM showed the highest ALP activity and, after induction of osteogenic differentiation, the highest calcium deposition, while cells culturing with αMEM/M199 showed a significant number of undifferentiated cells and lower calcium deposition (Kannan et al., 2020).

It is generally accepted that in vitro osteogenic differentiation of MSCs in 2D culture resulted in the formation of osteoblast-like cells. The further transition between osteoblast and osteocyte might occur in vitro by variation of matrix spatial structure. During the cultivation of osteoblast in 3D-culture (collagen gel), they might transform to osteocytes. Then, if osteocytes were transferred to a 2D culture they transform back to osteoblasts (Sawa et al., 2019).

Thus, osteogenic differentiation in vitro at least partly reflects the physiology of MSCs in vivo. Never-

theless, induction of osteogenic differentiation might be controlled by differential signal cascades in different mesenchymal cells. Moreover, while most of the studies focus only on measurements of ALP activity and matrix mineralization (Alizarin red stain), there is evidence of the differences between osteoblast-like cells, differentiated from different MSC types (e.g., Jääger et al., 2012; Dadras et al., 2020; Nantavisai et al., 2020).

That is why we sequentially discuss several questions further: "Are MSCs from different compartments physiologically similar?" and "How the differences of MSC influence molecular mechanisms of osteogenic differentiation and phenotype of various MSCs after osteogenic differentiation?"

MSC heterogeneity

There are several levels of MSC heterogeneity: at donor and tissue levels (Phinney, 2012; Zha et al., 2021). Inter-donor variation (age, genotype, and life history) is known to have a high impact on MSC physiology, especially in the case of biomedical applications (Phinney, 2012; Zha et al., 2021). Nevertheless, this level of variation is out of the scope of this review — we focus only on systematic intra-donor variation between MSCs from different tissue sources and between MSC functional subpopulations within one tissue. Thus, we discuss the MSCs from the main tissue sources and their intra-population variability in vivo and in vitro.

Pericytes

Pericyte definition. Pericytes are a heterogeneous group of mesenchymal cells from a perivascular niche. There are no specific pericyte markers, but they usually express PDGFRβ (platelet-derived growth factor receptor beta) and NG2 (neural/glial antigen 2) and are localized in a perivascular niche (Yamazaki and Mukouyama, 2018). Pericytes have heterogeneous embryonic origin: neural crest, mesothelium, and endocardium (Prazeres et al., 2017; Yamazaki and Mukouyama, 2018). Pericytes from the same tissue might also have a different origin (Prazeres et al., 2017). Moreover, the formation of pericytes from tissue mesenchymal stem cells in an adult organism has been demonstrated: BM-MSCs might differentiate to pericytes after stereotactic body radiation therapy and promote tumor recurrence and neovascularisation (Wang et al., 2016).

Pericytes are considered to be a source of tissue-resident MSCs in an adult organism in vivo (Caplan, 2017a,b; Supakul et al., 2019; Yianni and Sharpe, 2019). Caplan has assumed that the "in vivo progenitor of MSCs are pericytes and certainly not components of the connective tissue (stromal)" (Caplan, 1991, 2017a,b). The pericytes, thus, might be regarded as specialized MSCs

in a perivascular niche which is in a strong connection to tissue MSCs. For example, a bone marrow niche consists of all forms of pericytes, MSCs, and differentiated chondro-, osteo- and adipocytes (see further). Nevertheless, a perivascular niche is much more complex, and not all pericytes should be regarded as MSCs. Thus, we assume that a perivascular niche might differ in various tissues and consist of perivascular-specific and tissue-specific parts of pericytes. While pericyte-specific cells are necessary for perivascular niche maintaining, tissue-specific pericytes are part of the tissue-specific MSC pool. Nevertheless, the data about functional heterogeneity and a definition of pericytes is debatable and should be the subject of a specialized review. Here we will focus on the MSCs from the four main sources as in the most studied ones (Fig. 1).

MSCs in bone marrow

BM-MSC definition. Bone tissue was the first source for MSC isolation and it is still the most investigated model. BM-MSCs have all the classic MSC features: they are plastic-adherent, have fibroblast-like morphology, and are potent to trilineage differentiation. Naïve BM-MSCs express CD73 and CD90, and approximately half of their population express CD105. Due to high vascularization, part of the BM-MSC population expresses CD146 and PDGFRα (Kozlowska et al., 2019).

BM-MSC variation at single-cell level in vivo. Bone marrow (BM) consists of stromal cells involved in blood production (hematopoietic stem cells, HSCs) and BM homeostasis (BM-MSCs). Despite a complicated spatial structure, a BM niche is well characterized by the single-cell methodology. C. Baccin et al. (2020) performed droplet-based single-cell RNA-seq (scRNA-seq) combined with spatially resolved transcriptomics of murine BM, extracted from femurs, tibiae, hips, and spines with additional depletion or enrichment of abundant or rare cell populations subsequently. Authors described 32 clusters, 11 of which corresponded to mesenchymal lineage and contained chondrocytes, osteoblasts, myofibroblasts, three types of fibroblasts (endosteal, stromal, and arteriolar), fibroblast-chondrocyte progenitors, Ng2+ MSC, and two types of Cxcl12-abundant reticular (CAR) cells: adipo-CAR and osteo-CAR. All these cells have different spatial localizations. The authors define three niches in the BM: (1) a niche associated with arteriolar and included arteriolar fibroblasts, osteo-CAR, stromal fibroblasts, smooth muscles; (2) a sinusoidal compartment with adipo-CAR; (3) an endosteal niche that consists of endosteal fibroblasts, osteoblasts and chondrocytes. Ng2+ MSC was not associated with any of the defined niches.

Adipo-CAR and osteo-CAR are known to be the main cytokine-producing cells named CXCL12-abun-
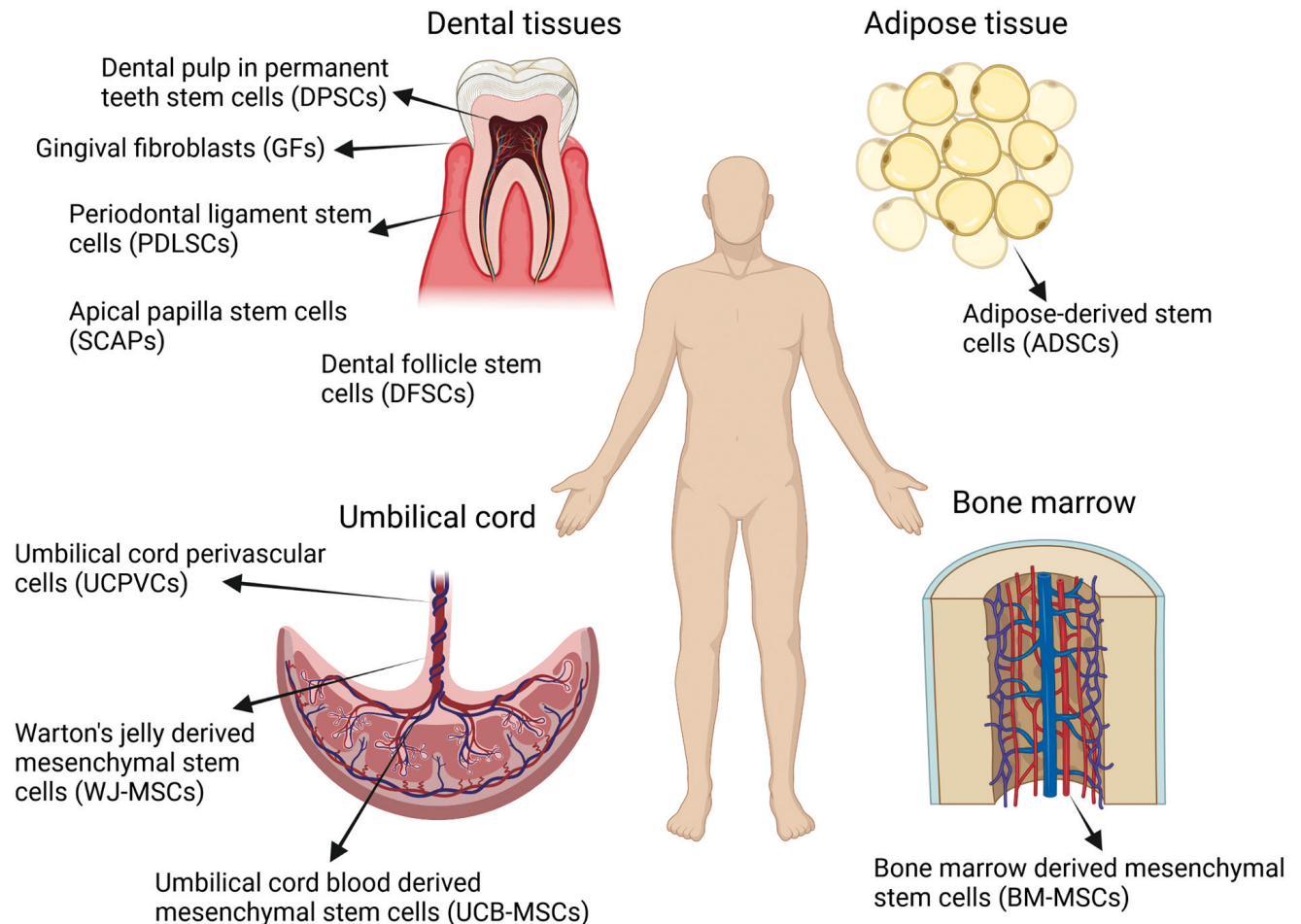
Fig. 1. Diversity of MSC types which might be isolated from the four most popular sources. Figure created with BioRender.com

dant reticular (CAR) cells (Sugiyama et al., 2006). The function of CAR-cells is maintaining the quiescent HSCs pool (Sugiyama et al., 2006; Omatsu et al., 2010).

Nevertheless, CAR-cells might have a higher level of heterogeneity than presented by the spatial data. Addo et al. (2019) performed scRNA-seq analysis of sorted VCAM-1+CD45-Ter119-CD31- cell fraction isolated from murine BM. The majority of isolated cells express Cxcl12 and LepR (leptin receptor). Thus, these cells are generally analogous to the adipo-CAR cells from Baccin et al. (2020). Nevertheless, some osteo-CAR cells might express LepR at a lower level, so part of their population might be also included (Baccin et al., 2020).

Addo et al. (2019) identified three subpopulations of stromal cells according to the expression level of Cxcl12: Cxcl12-low (12 % of cells), Cxcl12-intermediate (8 % of cells), and Cxcl12-high (80 %). Cxcl12-high cells have a high level of *adipoq* and *lepr* with a low level of *bglap*. Cxcl12-low, in contrast, has a low level of *adipoq* and *lepr*, but a high level of *bglap*. Thus, summarizing the data from Baccin et al. (2020), we might conclude that 80 % of cells in the work of Addo et al. (2019) corresponds to adipo-CAR, while ~12 % represent part of osteo-CAR heterogeneity.

This data is in good accordance with Tikhonova et al. (2019), who performed scRNA-seq of VE-Cad+, LEPR+, and COL2.3+ cells. LEPR+ cells were localized in the sinusoidal capillaries. Nevertheless, they identified four clusters of LEPR+ cells. Only two of these clusters covered the sinusoidal capillaries — Mgp-high and Lpl-high. Two other clusters — Wif1-high and Spp1-high/Ibsp-high — have an expression of osteogenic markers and were regarded as osteogenic-primed. Wif1-high and Spp1-high/Ibsp-high clusters are localized mainly in the trabecular part of the bone. Thus, Mgp-high and Lpl-high clusters correspond to adipo-CAR while Wif1-high and Spp1-high/Ibsp-high correspond to part of osteo-CAR population. Mgp-high and Lpl-high also have higher expression of Cxcl12 and SCF (stem cell factor). They also found an actively proliferative population of LEPR+ cells. It has been previously shown that these cells give rise to adipocytes and osteoblasts (Zhou et al., 2014).

The role of Ng2+Nestin+ MSC in the BM is not fully understood. It is known that these cells as the adipo- and osteo-CAR cells localize in the perivascular niche, show physical association with HSCs, and secrete factors essential for HSCs functioning, including Cxcl12.
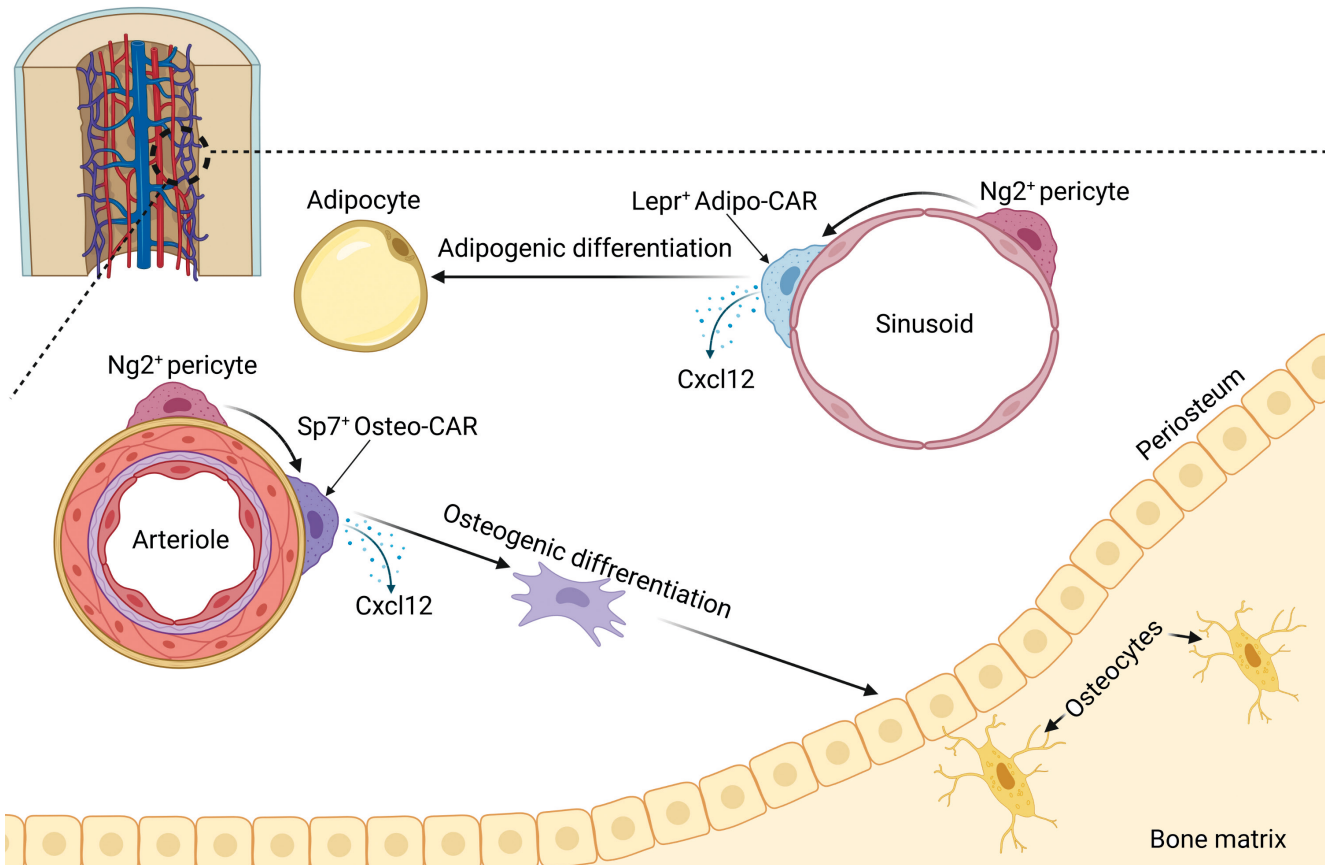
Fig. 2. Schematic representation of the role of mesenchymal cells heterogeneity in the bone marrow niche and the main directions of their differentiation from pericytes (see the detailed discussion in the text). Figure created with BioRender.com

Moreover, these cells are required for HSC/progenitor homing, and in vivo depletion of Nestin+ MSC leads to a 90 % decrease of hematopoietic progenitors. Nevertheless, Nestin+ MSCs are also known to be the progenitors of cells of osteochondral lineages and might form osteoblasts, osteocytes, chondrocytes, and probably osteo- and adipo-CAR (Méndez-Ferrer et al., 2010). Moreover, the Nestin+ MSCs are known to react to parathormone (Méndez-Ferrer et al., 2010). Thus, Nestin+ MSC might be assumed to be the main regulators of BM-niche and progenitors of other osteochondral cells, while CAR cells are assumed to be the main producers of SCF and CXCL12 (Méndez-Ferrer et al., 2010; Omatsu et al., 2010; Baccin et al., 2020).

Other researchers performed scRNA-seq of non-hematopoietic, non-endothelial CD45⁻ Ter119⁻ CD31⁻ cells from murine bone marrow (Wolock et al., 2019). They identified 7 clusters representing the (1) MSC, (2) adipocyte progenitor, (3) pre-adipocyte, (4) osteoblast/chondrocyte progenitor, (5) pre-osteoblast/chondrocyte, (6) pre-osteoblast, (7) pre-chondrocyte. MSC, adipocyte progenitors, pre-adipocyte, and osteoblast/chondrocyte progenitors have high levels of *cxcl12*, *adipoq*, *scf* expression. Gradually, the expression of *cxcl12*, *adipoq*, *scf* fades while the expression of the corresponding adipo- or osteogenic differentiation genes rise.

Thus, BM has several MSC populations, which are clearly explained in vivo by scRNA-seq data. Based on the data discussed above we can hypothesize a "waterfall mechanism" of BM maintenance (Fig. 2).

First of all, Ng2+ Nestin+ population of MSCs is induced to proliferation and Cxcl12 factor production by hormones (such as parathormone). The proliferation of Ng2+ MSC leads to a formation of adipo- and osteo-CAR cells — professional producers of paracrine factors (e.g., cxcl12) which produce most of the factors supporting the unique niche for HSC development. Nevertheless, CAR cells do not proliferate and start slowly differentiating into osteogenic and adipogenic lines consequently. During this differentiation, the production of BM niche paracrine factors fades and differentiated osteoblast and adipocytes have no expression factors necessary for the maintenance of the BM niche. The production of paracrine factors for HSC quiescence in their BM niche maintenance requires consequent proliferation of Ng2+Nestin+ cells. Accordingly, depletion of CAR cells will lead to disruption of the BM niche. Omatsu et al. (2010) performed selective ablation of CAR cells — they inserted Diphtheria toxin (DT) receptor-green fluorescent protein into *Cxcl12* locus with further system administration with diphtheria toxin. Depletion of *Cxcl12*-producing cells leads to the reduction of HSCs.

CELL BIOLOGY

From this point of view, osteogenic differentiation of osteo-CAR cells might be considered as cell aging, but not as reparative osteogenesis. Accordingly, in vivo pericyte-lineage-tracing studies demonstrate that endogenous pericytes might contribute to bone fracture healing as a source of osteogenic cells (Supakul et al., 2019). Nevertheless, BM-MSCs are not the primary source of cells for bone healing in vivo. Most of the osteochondroprogenitor cells migrate from the endosteum and periosteum, while the main function of BM-MSCs is trophic factor secretion, which supports the healing process (Bragdon and Bahney, 2018).

BM-MSC variation on single-cell level in vitro. Thus, BM-MSCs have a complicated subpopulational structure according to their functions in maintaining the BM-niche. Therefore, a high level of BM-MSC heterogeneity in vitro is well known and was described at phenotypic and molecular levels (e.g., Whitfield et al., 2013; Freeman et al., 2015). One of the most important levels of in vitro phenotypic heterogeneity is the size of a cell: at lower passages most BM-MSCs are small and spindle-shaped, but during long-term cultivation, the proportion of large rounded cells increases (Whitfield et al., 2013). Experiments with cell division-tracking of human BM-MSCs in vitro at the fifth passage revealed that small cells actively divided and half of all progeny arose from the 15 most proliferative small cells, while half of the initial cells never divided at all. While actively dividing cells remain small, non-dividing cells become larger in the course of time. Thus, the proportion of active and non-dividing cells is shifting to large senescent cells during the in vitro culture. According to phenotypic data, scRNA-seq of 16 mouse BM-MSCs revealed at least five physiological clusters: two clusters of dividing cells and three clusters of cells commitment to differentiation (Freeman et al., 2015). Thus, during in vitro cultivation, BM-MSCs remain heterogenous, but this heterogeneity is much less complex and has no evident functional subpopulations observed in vivo.

MSC in adipose tissue

ADSCs definition. Adipose tissues are easy to isolate, therefore adipose-derived stem cells (ADSCs) have become one of the most popular cell types for therapy. Naïve ADSCs have properties and immunophenotype similar to BM-MSCs. Adipose tissue is also vascularized, so pericyte subpopulation is also present after isolation. Nevertheless, unlike BM-MSCs, a percentage of ADSCs with CD146 (vascular marker) dramatically decreases up to a tenth passage during cultivation (Kozlowska et al., 2019).

ADSCs variation at single-cell level. Opposite to bone marrow, ADSCs are more homogenous at the single-cell level (Acosta et al., 2017; Zhou et al., 2019), but adipose

progenitor cells from subcutaneous and visceral adipose tissue form distinct clusters at the scRNA-seq level (Vijay et al., 2020). Both sources of ADSCs consist of four groups of adipose progenitors varying in CFD (Complement Factor D) expression and probably representing different stages of adipose progenitor cell differentiation (Vijay et al., 2020). By the less sensitive approach, ADSCs consist of an evenly distributed continuum (Hardy et al., 2017). Nonetheless, their actual heterogeneity might be expanded by more sensitive methods (Brooks et al., 2020).

Therefore, ADSCs should be preferred in the case of standardized biomedical applications (Zhou et al., 2019). Nevertheless, it should be emphasized that adipose tissue is highly vascularized, and isolation of ADCS in vitro usually leads to a mixed culture of fibroblast-like, endothelial-like subpopulations of cells and perivascular MSCs (Cheng et al., 2011; Zuttion et al., 2019).

Dental MSCs

Dental MSC definition. Teeth have several types of mesenchymal cells which form pulp, dentin, and cementum. MSCs can be isolated from most of the tooth tissues: dental pulp in permanent teeth (DPSCs), immature dental pulp in deciduous teeth (SHEDs), apical papilla of growing tooth roots (SCAPs), periodontal ligament (PDLSCs), a dental follicle (DFSCs), gingiva (GFSCs/GMSCs; Nagata, Ono and Ono, 2021). Unlike the MSCs from most other sources and despite their conformity to MSC criteria, the vast majority of dental MSCs are derived from the neural crest — ectoderm (Janowicz et al., 2019; Nagata, Ono and Ono, 2021).

DPSCs variation at single-cell level in vivo. Some data of scRNA-seq of mouse incisors revealed only two main mesenchymal clusters: odontoblast (Dmp1^{+} cells) and mesenchymal cells (Chiba et al., 2020). Nevertheless, scRNA-seq of mouse incisors with higher resolution revealed more clusters (Krivanek et al., 2020). Two subtypes of the dental follicular Aldh1a2^{+} cells surround the tooth encapsulated in the alveolar bone with alveolar osteocytes. The mesenchymal compartment inside the incisor (pulp) consists of a transient continuum of differentiated odontoblasts in several differentiation stages and two distinct pulp mesenchymal cell subtypes: apical Smoc2^{+} Sfrp2+ self-renewal subtype and Igfbp5^{+} Syt6^{+} differentiating distal subtype. An apical subpopulation is also heterogeneous and includes Foxd1^{+} mitotically active progenitor pool localized near the labial cervical loop (Krivanek et al., 2020).

Krivanek et al. (2020) also performed single-cell profiling of mouse molars and growing and nongrowing human teeth. As expected, nongrowing mouse molar mesenchyme forms a single cluster analogous to the distal differentiated subtype of incisor pulp. Hu-

man non-growing molars have much more heterogeny compared to mice — human pulp has a subpopulation spatially localized in the periodontoblastic layer. Similar to a mouse incisor, apical papilla in a growing human tooth has Smoc2$^+$ positive subtype of pulp mesenchyme. Smoc2$^-$ subpopulation was also detected in the growing pulp and might be regarded as mature pulp mesenchyme analogous to incisor dental pulp.

DPSCs variation at single-cell level in vitro. Dental pulp stem cells (DPSCs) have a relatively high proliferation rate and are actively used in biomedicine, e.g., in neurodegenerative disease and regenerative dentistry, while most other dental MSC types are less popular (Janowicz et al., 2019; Nagata, Ono and Ono, 2021). In vitro, there are two main subpopulations of DPSCs: highly proliferative/multipotent and low proliferative/unipotent. For example, Alraies et al. (2017, 2019) described high and low-proliferative populations of DPSCs also discriminated by single-cell Raman spectroscopy. Moreover, the authors revealed that only a high-proliferative population was able to undergo adipo- chondro- and osteogenic differentiation while low-proliferative subpopulations were lineage-restricted to osteogenic differentiation. The authors argue that these differences are associated with telomere lengths — high-proliferative DPSCs have longer telomeres (Alraies et al., 2017). Similar results were obtained by Harrington, Sloan and Waddington (2014) — among three investigated rat DPSC clones, all of them were capable of osteogenic differentiation, but only single clones were also able to undergo adipo- and chondrogenic differentiation. Some differences persist in the cultivation in the 3D collagen gels — both subpopulations have a similar proliferative ability, but they differ in gel contraction and matrix metalloproteinase activity (Alraies et al., 2020).

Nevertheless, Kobayashi et al. (2020) found that at early stages of a culture of human DPSCs, besides pro-odontogenic (14 % of total cells) and multipotent (29 %) subpopulations there are pro-adipogenic subpopulation (36 %) and clones which do not demonstrate any differentiation potential (26 %).

Umbilical cord mesenchymal stem cells

UC-MSC definition. The umbilical cord (*funiculus umbilicalis*) connects a developing embryo and placenta. An umbilical cord has several sources for MSC isolation such as sub-amniotic lining membrane (Kita et al., 2010), but only several sources are actively used: Wharton's jelly (WJ-MSCs), umbilical cord blood, and umbilical cord perivascular cells (UCPVCs; Carvalho et al., 2011; Lyons and Mattei, 2019).

MSCs from these two sources have different potency in various biomedicine applications, e.g., umbilical cord blood seems to be more effective for scarless wound healing (Doi et al., 2016; Lyons and Mattei, 2019). Nev-

ertheless, WJ-MSCs are easier to expand, might be cultured in higher passages without phenotype changes, and have a higher differentiating potential (Subramanian et al., 2015). All UC-MSCs should meet the minimal MSC criterion: express CD105, CD73, CD90; not express CD45 and CD34; be plastic-adherent; have fibroblast-like morphology; be able to differentiate to adipo-, chondro- and osteogenic directions (Carvalho et al., 2011; Lyons and Mattei, 2019).

WJ-MSC variation on single-cell level. scRNA-seq analysis of freshly isolated WJ-MSCs reveals two distinct subpopulations of MSCs (Zhang et al., 2021). Functional differences of these cells in vivo are not yet understood, but based on the gene expression profile one of these subpopulations was considered as more differentiated.

After in vitro culture at least six probable subpopulations of WJ-MSCs might be identified (Sun et al., 2020). Two of them have a relatively higher level of MKI67, a marker of cell proliferation. Three other populations correspond to cells with classic MSC properties, among which two subpopulations with different biological features might be isolated: the one with a higher level of expression of extracellular matrix and the other one with a higher level of chemokines expression. Finally, there was a small subpopulation of aging MSCs in G1 phase. This subpopulation is much less abundant and might be considered as an artifact of in vitro cultivation (e.g., cells after senescence).

Direct omics comparison of different MSC populations

Above we demonstrated that there are differences between MSCs from various tissues. Nevertheless, the fact that different research groups used various methodologies in the articles discussed above, direct comparison of MSCs from various sources would be incorrect. Fortunately, there are a number of articles with the direct comparison of MSCs from different sources by omics methodology, and we might discuss the comparison of major MSC types described above.

ADSCs and BM-MSCs have similar immunophenotypes and morphology, but ADSCs have higher proliferative potential (Mohamed-Ahmed et al., 2018). W. Zhou et al. (2019) performed a direct comparison of cultured BM-MSCs and ADSCs by scRNA-seq. According to in vivo data discussed below, ADSCs have much less heterogeneity than BM-MSCs — cell-to-cell distances based on scRNA-seq data of BM-MSCs were larger than that of ADSCs. These two cell types formed two distinct clusters on PCA with 4 033 differentially expressed genes between them ($|logFC| > 1.5$).

Comparison of umbilical cord MSCs (UC-MSCs) and BM-MSCs revealed similar results. There are data of comparative analysis of proteomes, DNA methylomes,

and transcriptomes of cultured porcine BM-MSCs and UC-MSCs (Huang et al., 2015; Yang et al., 2019, preprint). In summary, the authors found 587 genes differentially methylated, 1979 differentially expressed transcripts and 95 differentially expressed proteins between BM-MSCs and UC-MSCs. The enrichment analysis of the differentially expressed genes showed that UC-MSCs have more active genes involved in proliferation, cell motility, growth, and immunogenicity properties, while BM-MSCs have higher osteogenic potential.

Harman et al. (2020) compared three cultures of horse donor-matched adipose tissue, bone marrow, and peripheral blood MSCs (PB-MSCs) by scRNA-seq. All three types of cells formed distinct clusters on UMAP plot. They found differences in cellular motility, immune regulatory function, and chemoattractive capacity. Corresponding to other studies discussed below, in comparison to intragroup heterogeneity authors demonstrated that ADSCs were the most homogeneous cell population.

Summary on MSC heterogeneity

Summarizing information from the four most popular tissues for MSC isolation, we conclude that MSCs from various sources have functional differences which still persist after cultivation in vitro. These functional differences most probably come from the specificity of roles of these MSC types in the tissues from which they were isolated.

All MSC types discussed above represent a number of related subpopulations in vivo, reflecting a functionality of MSCs in a specific tissue. Nevertheless, we see much less variability after isolation of these MSCs and culture in vitro. Obviously, some subpopulations might be lost during cultivation while others might be more adherent to plastic and intensively proliferate.

It has been demonstrated that after isolation of MSCs from bone marrow and adipose tissues there is a fraction of non-adherent mesenchymal progenitors (NAMP; Di Maggio et al., 2012; Mehrkens et al., 2014). NAMP from both sources can be expanded in suspension. These cells are less committed and have higher proliferative potential than adherent MSCs. Moreover, during cultivation in suspension, they proliferate and also produce adherent progeny, which is similar to classically isolated adherent MSCs. The presence of an adherent MSC fraction supports NAMP maintenance through FGF-2 signaling (Di Maggio et al., 2012). We assume that NAMP might be associated with a population of MSC precursors from a perivascular compartment, but this assumption was not been experimentally tested yet.

Thus, isolation of MSCs into culture leads to a loss of some natural heterogeneity. Then, the cultivation in vitro causes the formation of other subpopulations, mainly as-

sociated with aging of culture, proliferative senescence of some cells, and spontaneous commitment to differentiation of the others. We might expect that all MSCs would form similar subpopulations during cultivation in vitro. According to this, W. Hou et al. (2021, preprint) compared four types of MSCs cultured in vitro by scRNA-seq: BM-MSCs, WJ-MSCs, ADSCs, and MSCs from synovial tissue. In all types of cells, they found three major clusters, corresponding to MSCs committed to osteo-, adipo- and chondrogenic differentiation. Nevertheless, MSCs in culture might still reflect some functional specificity of their in vivo progenitors and a comparison of different MSCs might be informative.

If we conclude that MSCs from different sources are not physiologically similar we should then assume that they have differences in molecular mechanisms of trilineage differentiation and, probably, in the resulting phenotype of differentiated cells. Osteogenic differentiation is one of the most described ones. Moreover, heterotopic osteogenic differentiation of mesenchymal cells involved in several pathologies, e.g., calcific aortic valve disease (CAVD) and heterotopic ossification, also might take place in various cancers, for example, in rectal cancer (Dukes, 1939; Rutkovskiy et al., 2017; Xu, Zhou and Yang, 2018). Thus, further we will discuss the data about a comparison of molecular mechanisms of osteogenic differentiation of various mesenchymal cells trying to describe functional subtypes of osteogenic differentiations.

MSC heterogeneity in the context of osteogenic differentiation

Pathological heterotopic ossification of non-osseous cells

Not only MSCs, but some other, normally non-osseous, cells might undergo differentiation with matrix mineralization in pathological conditions. Such pathological differentiation is usually considered as "osteogenic" differentiation, but the similarity of molecular mechanisms of these processes has not been confirmed yet.

Aortic valve interstitial cells. Calcific aortic valve disease (CAVD) is a slowly progressive disease developing from aortic valve thickening to valve calcification. Valve calcification is assumed to be similar to endochondral ossification and is also strongly regulated by BMP-signaling (Gomez-Stallons et al., 2016, 2020). One of the central processes in CAVD progression is osteogenic differentiation of valve interstitial cells (VICs; Rutkovskiy et al., 2017; Bogdanova et al., 2019).

Despite an overall similarity of matrix mineralization between VICs and osteoblasts, differences in molecular mechanisms of osteogenic differentiation of these cells were assumed (Monzack and Masters, 2011).

Omics comparison of VICs and osteoblast/MSCs during osteogenic differentiation is only yet to be made. We compared osteogenic differentiation of VICs with ADSCs in the context of dose-dependent activation of Notch signaling with qPCR analysis of several proosteogenic markers and untargeted metabolomic profiling (Semenova et al., 2020). VICs and ADSCs have different patterns of expression of proosteogenic markers during differentiation. Moreover, after different types of osteogenic differentiation (with or without Notch activation) these cells formed distinct clusters on clusterization (nMDS and PLS-DA) by metabolomics data. Thus, we argue that the mechanisms of osteogenic differentiation of VICs and ADSCs are different, but clarification of the exact differences has yet to be done.

Dermal fibroblasts. Dermal fibroblasts might undergo osteogenic differentiation in a similar manner to MSCs (Christy et al., 2019). Jääger et al. (2012) founded that osteogenic differentiation of fibroblasts is rather similar to MSCs. The authors performed a transcriptomics comparison of human donor-matched dermal fibroblasts (dFBs) and ADSCs during trilineage differentiation. Principal component analysis revealed that undifferentiated cells form distinct clusters, but after osteogenic differentiation they form overlapping clusters.

Heterotopic calcification in tumors. Heterotopic calcification in cancers is relatively rare but known for many tumor types. Despite many published cases, the molecular mechanisms of such ossification are not yet clearly understood. Nagata, Ono and Ono (2021) discussed the case of heterotopic ossification in a metachronous metastatic lymph node in an 83-year-old Japanese woman. Metastatic tumor cells were positive for many proosteogenic markers involved in normal ossification: BMP-2, TGF-β, osteocalcin, osteonectin, phosphorylated Smad2/3 (pSmad2/3) in the nucleus. Based on the comparison of the clinical case and literature data Nagata, Ono and Ono (2021) emphasized two principal sources of heterotopic ossification in the present case: (1) epithelial-to-mesenchymal transition (EMT) associated osteogenic-like differentiation of tumor cells and (2) induction of osteogenic differentiation of resident MSCs by BMP-2 and other proosteogenic factors secretion by tumor cells.

The second mechanism seems to be the major one, and similar data was also obtained in several other clinical cases. For example, Suzuki et al. (2019) presented a case of a 43-year-old woman with calcified adenocarcinoma. The ossification zone was surrounded by tumor cells secreting BMP-2 and osteopontin. Another example is the case of a 73-year-old male with rectal adenocarcinoma, presented by Katono et al. (2021). The heterotopic ossification zone was also surrounded by tumor cells and the expression of BMP-2 and EMT-associated factors (*Snail* and *Slug*) expression was also founded to be specifically increased in the area of heterotopic ossification.

Thus, it is still questionable whether tumor cells are able to differentiate in an osteogenic direction, nevertheless, they are able to obtain an osteogenic phenotype and to express some proosteogenic factors such as BMP-2 and osteopontin, which might induce heterotopic ossification with osteogenic differentiation of resident mesenchymal cells.

Odontoblastic differentiation as a distinct subtype of osteogenic differentiation

Odontoblastic differentiation. Besides pathological ossification, there is at least one clear subtype of normal osteogenic differentiation — dentinogenic differentiation. Production of extracellular matrix and its mineralization occurs in both osteogenic and dentinogenic differentiations. The main differences are in the repertoire of producing ECM proteins: extracellular DSPP and DMP-1 proteins are the main markers of odontogenic differentiation. Molecular mechanisms of odontogenic differentiation are largely unknown, but the primary transcriptional factors involved in odontoblastic differentiation are not the same as in osteogenic differentiation: Msx1, Msx2, Lhx7, Pax9 (Rosa et al., 2012; Dong et al, 2019). Expectedly, dental-derived stem cells are the main cell type able to differentiate to odontoblast-like cells. We assume that in most of the articles where dental-derived stem cell differentiation was demonstrated, the authors actually worked with odontoblastic differentiation. The assay on matrix mineralization and Runx2 expression should be supplemented by data on an expression of DSPP and DMP-1 in the case of dental-derived stem cells.

Osteogenic and odontoblast differentiation as two separate cell fates. A proteomics comparison of canine DPSCs and other BM-MSCs performed by S. Nantavisai et al. (2020) revealed that most of the differentially expressed proteins involved in osteogenic-like differentiation were unique for cell types. Among 571 total differentially expressed proteins (adj. P. val < 0.05), 163 and 58 were specific for the 7th and 14th days of osteogenic differentiation of BM-MSCs, respectively, and 47 and 86 for the 7th and 14th days of osteogenic differentiation of DPSCs. Based on obtained data, the authors indicated the differences in proteins involved in the key pro-osteogenic signaling cascades: Wnt, Notch, and BMP. Then they compared the effect of different inhibitors, which confirmed these differences. Only two signaling pathways have similar effects on both cell types: Notch signaling has an inhibitory effect while BMP has a positive effect on osteogenic differentiation of both cell types. In the case of BM-MSCs, TGF-beta and Wnt have a pro-osteogenic effect (which is considered their classic effect on osteogenic differentiation), while in DPSCs they have inhibitory effects on differentiation.

CELL BIOLOGY

The existence of two types of differentiation with matrix mineralization was demonstrated using several models where dental-derived stem cells were specifically stimulated to one of the differentiations. Induction of differentiation of dental follicle cells (DFCs) by classic osteogenic media described above and by BMP-2 or IGF-2 leads to different transcription patterns. Both inductors lead to high ALP activity and ECM matrix mineralization. But only induction of differentiation by BMP-2 or IGF-2 leads to a pattern similar to osteogenic differentiation with the high Runx2 expression. Induction of differentiation by osteogenic medium leads to an expression of late-osteogenic genes (Saugspier et al., 2010). Moreover, Runx2 independent osteogenic-like differentiation of DFCs was demonstrated (Felthaus et al., 2014). After induction of osteogenic differentiation by osteogenic media, the inhibition of Runx2 did not suppress differentiation, ALP activity, or matrix mineralization. At the same time, differentiation was decreased or enhanced by overexpression or inhibition of Zbtb16 (Plzf), respectively (Felthaus et al., 2014). In contrast, inhibition of Runx2 had a suppressive effect on BMP-2 induced differentiation of DFCs.

Extracellular matrix as important inductor of odontoblastic differentiation. Similarly, different differentiations of dental cells might be achieved by variation in the extracellular matrix. H. Xie et al. (2017) compared the differentiation of DPSCs on graphene or glass. Graphene matrix promotes osteogenic differentiation of DPSCs itself, without additional inductors. Moreover, expressions of MSX-1, PAX-6, and DMP-1 (odontoblastic genes) were downregulated while Runx2 and OCN (osteogenic genes) were upregulated. Thus, graphene specifically stimulates osteogenic, but not odontogenic differentiation.

Accordingly, dentine extracellular matrix components induce odontoblastic differentiation not only in DPSCs but also in BM-MSCs and ADSCs (Rosa et al., 2012; Davies et al., 2015; Dong et al., 2019). We might speculate that the differences in osteogenic/odontogenic potential of DPSCs in part may be the result of production by DPSCs of specific ECM proteins, which leads to odontogenic differentiation besides osteogenic. According to this, A. Kumar et al. (2018) described DSPP in the secretome of undifferentiated DPSCs. Nevertheless, dentine matrix proteins were not described in other secretome profiles of dental MSCs performed by S. Yu et al. (2016), so this hypothesis requires additional experimental confirmation. Nevertheless, transfection of ADSCs by DSPP-expressing adenovirus leads to an increase in ALP activity and matrix mineralization, and instead of an osteogenic phenotype, transfected ADSCs acquire an odontoblast-like phenotype with expression of Msx1, Msx2, Lhx7, and Pax9 (Wu et al., 2008).

Thus, while Runx2 is the master gene of osteogenic differentiation, DSPP is one of the central components of odontoblastic differentiation. Nevertheless, odontoblastic differentiation is also strongly dependent on Runx2. Runx2 deficiency leads to loss of ameloblast, overt odontoblast and causes abnormalities in dentin and enamel (D'Souza et al., 1999). Chen et al. (2005) analyzed a possible interaction of Runx2 and DSPP in the mouse model. They found three sites to which Runx2 might bind in promoters of a DSPP gene. Nevertheless, the effect of Runx2 on DSPP expression is controversial at different stages of odontoblast differentiation. Inhibition and overexpression of Runx2 in preodontoblast MD10-F2 cells decreases and increases DSPP expression, respectively. But these experiments cause an opposite effect on odontoblast MO6-G3 cells — inhibition of Runx2 causes an increase in DSPP promoter activity while its overexpression decreases DSPP expression. Thus, Runx2 is essential for early stages of odontoblast development, but its expression decreases in mature odontoblasts (D'Souza et al., 1999; Chen et al., 2005).

The molecular mechanism of switching between these programs is largely unknown. Probably, there are some undiscovered upstream components to Runx2 factors, involved in these processes, besides Runx2 and DSPP. For example, a recent study performed by Y. Lin et al. (2020) demonstrates the effect of SALL1, a transcription factor expressed in odontoblasts. ShRNA on SALL1 inhibits odontogenic differentiation by decreasing the accessibility of chromatin regions associated with odontoblast differentiation. Moreover, SALL1 might regulate Runx2 locus and physically interact with Runx2 protein.

Therefore, osteogenic and odontoblastic differentiation represent phenotypically similar, but different types of differentiation with crossing, but distinct molecular mechanisms need to be explored.

Comparison of molecular mechanisms of osteogenic differentiation of MSCs in vitro

Similar MSCs do not react identically to the differentiation stimuli. In contrast to tooth-derived MSCs, it is believed that MSCs from other sources undergo osteogenic differentiation in a similar manner. Nevertheless, even the comparison of MSCs from different compartments of one tissue might reveal significant differences in the effect of the same differentiation stimuli.

Comparison of osteogenic differentiation capacity of mesenchymal stem cells derived from placental tissues is a good example. C. Shen et al. (2019) isolated MSCs from a human amniotic membrane (AM), umbilical cord (UC), chorionic membrane (CM), and decidua (DC). Then the cells were induced to osteogenic differentiation by classic osteogenic medium with dexamethasone. Several key markers of osteogenic differentiation were measured on the null point, 7th, 14th, and 21st days.

AM-MSCs and UC-MSCs were similar and performed osteogenic differentiation — an expression of collagen I, BMP-6, and Runx2 were significantly increased on the 7th day. Then, on the 14th day, the expression of osteocalcin and sclerostin was increased. Finally, on the 21st day expression of sclerostin was maximal and expression of FGF23 increased. During osteogenic differentiation of CM-MSCs and DC-MSCs, sclerostin was not upregulated at all while significant upregulation of collagen I was found only on the 14th day. Accordingly, AM-MSCs and UC-MSCs displayed a much higher level of calcium deposition on the 21st day. The authors assumed that osteogenic differentiation of CM-MSCs and DC-MSCs are similar, but much slower than in AM-MSCs and UC-MSCs. One of the probable reasons for this phenomenon is differences in the extracellular matrix. Thus, the authors demonstrated that fibronectin increased calcium deposition in all MSC types used in this study, but their differences in osteogenic potential persisted (Shen et al., 2019). Similarly, Zajdel et al. (2017) compared osteogenic differentiation of ADSCs and WJ-MSCs. The overall phenotype was similar, but the expression levels of osteogenic markers were different. The same phenomenon was demonstrated for ADSCs and BM-MSCs. ADSCs have delayed osteogenic differentiation. Both cell types have increased ALP-activity, collagen I production and expression of Runx 2, and osteopontin and calcium deposition, but BM-MSCs have the greatest level of proosteogenic gene expression on the 14th day (Mohamed-Ahmed et al., 2018).

Thus, at the phenotypic level, the differences between various MSCs are in the osteogenic differentiation speed. Nevertheless, we still cannot answer the question: do these processes actually go by the same mechanisms in the case of all MSCs discussed above? There are only several articles with omics comparisons between different types of MSCs during osteogenic differentiation which might disclose the answer.

Omics comparison of BM-MSCs and ADSCs during osteogenic differentiation. Monaco et al. (2012) performed a microarray analysis of porcine BM-MSCs and ADSCs during osteo- and adipogenic differentiation. Only 64 genes were significantly differentially expressed between the cell types before differentiation. Several proteins probably influencing MSC differentiation were among them: periostin, secreted frizzled-related protein 2 (SFRP2), fibrulin-2, osteopontin, and fibronectin 1. During osteogenic differentiation they performed an analysis of differential expression on the 2nd, 7th, and 21st days of osteogenic differentiation. The overall number of DEGs between BM-MSCs and ADSCs decreased during differentiation from 106 on the 2nd day to 28 DEGS on the 21st day.

Similar results were obtained by Dadras et al. (2020), who performed a donor-matched analysis of BM-MSCs and ADSCs from 29 patients after 21 days of osteogenic differentiation by shotgun proteomics. A total of 2624 proteins with at least two unique peptides were quantified, and 427 and 102 were specific for BM-MSCs and ADSCs, respectively. Quantitative analysis revealed 75 differentially expressed proteins with fold change > |2|. Many of the cell-type differences were associated with the extracellular matrix, e.g., collagen alpha-1(VIII) chain, VCAM-1, EGF-like repeat, and discoidin I-like domain-containing protein 3 (EDIL-3) were unique for BM-MSCs, while 17 out of 75 differentially expressed proteins were associated with ECM with "integrin cell surface interaction" as one of the most significant pathways. Particularly, there were five alpha-integrins upregulated in BM-MSCs.

Therefore, BM-MSCs and ADSCs undergo osteogenic differentiation in a similar manner, and minor differences in this process might come from initial differences between these cells. Some of these initial differences are associated with ECM proteins. ECM plays an important role in the induction of osteogenic differentiation and, as we emphasized earlier, might influence osteogenic potential. For example, ECM derived from osteoblast-differentiated MSCs had increased osteogenic potential compared to ECM derived from undifferentiated MSCs (Baroncelli et al., 2018). In this case, differences in integrins between BM-MSCs and ADSCs described above might be associated with differences in ECM secreted by BM-MSCs and ADSCs, which might influence their differentiation.

MSCs from various sources have differences in ECM. Shin et al. (2021) performed comparative proteomic analysis of a secretome of WJ-MSCs, MSCs isolated from a placenta (Pl-MSCs), BM-MSCs, and ADSCs. They found 596 secreted proteins with many proteins specific for cell type: 8 for BM-MSCs, 13 for ADSCs, 44 for WJ-MSCs and 112 for Pl-MSCs. On the PCA, all four groups formed distinct clusters. Interestingly, that BM-MSCs are close to ADSCs while Pl-MSCs are closer to WJ-MSCs — fetal and adult MSCs form distinct groups. Based on the fact that MSCs both produce and receive signals from the extracellular matrix, we assume that differences in ECM production might be an important factor of differences in osteogenic potential of various MSCs. Nevertheless, as it was demonstrated, cultivation on the same ECM cannot erase differences in osteogenic potential of various MSCs and other factors such as epigenetic ones should be explored in more detail in the future.

Conclusion

MSCs are heterogeneous in vivo (Table 1). This heterogeneity reflects the functionality of MSCs in different tissues. During isolation and in vitro cultivation, we lose

CELL BIOLOGY

Table 1. The presence of heterogeneity and the ability to undergo several subtypes of osteogenic differentiation by four main MSC types

Cell type	Used in clinical study*	Might be induced to osteogenic differentiation	Might be induced to odontogenic differentiation	In vivo heterogeneity demonstrated	In vitro heterogeneity demonstrated	Pericyte markers may be detected in culture	References
BM-MSCs	Yes	Yes	Yes	Yes	Yes	Yes	Shi and Gronthos, 2003; Davies et al., 2015; Freeman, Jung and Olge, 2015; Baccin et al., 2020
ADSCs	Yes	Yes	Yes	Yes, but relatively low	Yes	Yes	Davies et al., 2015; Kozlowska et al., 2019; Zuttion et al., 2019; Vijay et al., 2020
DPSCs	Yes	Yes	Yes	Yes	Yes	Yes	Shi and Gronthos, 2003; Davies et al., 2015; Xie et al., 2017; Alraies et al., 2017, 2019; Krivanek et al., 2020
WJ-MSCs	Yes	Yes	Yes	Yes, but relatively low	Yes	Yes	Li et al., 2013; Subramanian et al., 2015; Sun et al., 2020; Zhang et al., 2021

* The data from the clinical study was verified by information from the U. S. National Library of Medicine (ClinicalTrials.gov; accessed 28.08.21).

most subpopulations due to their differences in proliferative capacity and in adhesion, e.g., some of the subpopulations of MSCs might be non-adherent to plastic.

MSCs are also heterogeneous in vitro, but this heterogeneity mainly comes from cultivation artifacts such as culture aging and spontaneous differentiation, and does not fully reflect natural functional subpopulations. Nevertheless, MSCs from various sources still preserve their functional features in vitro. We assume that at least part of their physiological specificity comes from the differences in ECM production, which partially restores their native niche. Besides ECM, there are many other stable differences between MSCs from various sources.

Functional differences between MSCs from various sources should be considered in their clinical applications. For example, ADSCs and WJ-MSCs seem to be the most homogeneous in vivo and in vitro, and they are one of the least immunogenic and have a high immunomodulatory ability (Table 1).

The natural heterogeneity of mesenchymal cells leads to differences in their osteogenic potential and molecular mechanisms of osteogenic differentiation. ADSCs and BM-MSCs undergo osteogenic differentiation in a similar manner despite their functional differences in vivo and in vitro discussed above. Nevertheless, other mesenchymal cells, e.g., VICs and fibroblast, are assumed to undergo osteogenic differentiation in a different way than the ADSCs. Finally, dental MSCs are prone to undergo odontoblastic differentiation which should be clearly separated from osteogenic differentiation by the presence of DSPP and DMP-1 expression. Moreover, all four types of MSCs discussed in this re-

view might undergo odontoblastic differentiation in a specific conditions (Table 1).

The data on omics comparison of osteogenic differentiation of MSCs from various sources are still limited. We consider that increasing the number of such studies might allow us to highlight more distinct subtypes of osteogenic differentiation.

Acknowledgments

References

Acosta, J. R., Joost, S., Karlsson, K., Ehrlund, A., Li, X., Aouadi, M., Kasper, M., Arner, P., Rydén, M., and Laurencikiene, J. 2017. Single cell transcriptomics suggest that human adipocyte progenitor cells constitute a homogeneous cell population. *Stem Cell Research & Therapy* 8(1):1–6. https://doi.org/10.1186/s13287-017-0701-4

Addo, R. K., Heinrich, F., Heinz, G. A., Schulz, D., Sercan-Alp, Ö., Lehmann, K., Tran, C. L., Bardua, M., Matz, M., Löhning, M., and Hauser, A. E. 2019. Single-cell transcriptomes of murine bone marrow stromal cells reveal niche-associated heterogeneity. *European Journal of Immunology* 49(9):1372–1379. https://doi.org/10.1002/eji.201848053

Alraies, A., Alaidaroos, N. Y., Waddington, R. J., Moseley, R., and Sloan, A. J. 2017. Variation in human dental pulp stem cell ageing profiles reflect contrasting proliferative and regenerative capabilities. *BMC Cell Biology* 18(1):1–4. https://doi.org/10.1186/s12860-017-0128-x

Alraies, A., Canetta, E., Waddington, R. J., Moseley, R., and Sloan, A. J. 2019. Discrimination of dental pulp stem cell regenerative heterogeneity by single-cell Raman spectroscopy. *Tissue Engineering Part C: Methods* 25(8):489–499. https://doi.org/10.1089/ten.TEC. 2019.0129

Alraies, A., Waddington, R. J., Sloan, A. J., and Moseley, R. 2020. Evaluation of dental pulp stem cell heterogeneity and behaviour in 3D type I collagen gels. *BioMed Research International* 2020:3034727. https://doi.org/10.1155/2020/3034727

Arai, Y., Choi, B., Kim, B. J., Park, S., Park, H., Moon, J. J., and Lee, S. H. 2021. Cryptic ligand on collagen matrix unveiled by MMP13 accelerates bone tissue regeneration via MMP13/Integrin α3/RUNX2 feedback loop. *Acta Biomaterialia* 125:219–230. https://doi.org/10.1016/j.actbio.2021.02.042

Baccin, C., Al-Sabah, J., Velten, L., Helbling, P. M., Grünschläger, F., Hernández-Malmierca, P., Nombela-Arrieta, C., Steinmetz, L. M., Trumpp, A., and Haas, S. 2020. Combined single-cell and spatial transcriptomics reveal the molecular, cellular and spatial bone marrow niche organization. *Nature Cell Biology* 22(1):38–48. https://doi.org/10.1038/s41556-019-0439-6

Baroncelli, M., van der Eerden, B. C., Kan, Y. Y., Alves, R. D., Demmers, J. A., van de Peppel, J., and van Leeuwen, J. P. 2018. Comparative proteomic profiling of human osteoblast-derived extracellular matrices identifies proteins involved in mesenchymal stromal cell osteogenic differentiation and mineralization. *Journal of Cellular Physiology* 233(1):387–395. https://doi.org/10.1002/jcp.25898

Bogdanova, M., Zabirnyk, A., Malashicheva, A., Enayati, K. Z., Karlsen, T. A., Kaljusto, M. L., Kvitting, J. P., Dissen, E., Sullivan, G. J., Kostareva, A., and Stensløkken, K. O. 2019. Interstitial cells in calcified aortic valves have reduced differentiation potential and stem cell-like properties. *Scientific Reports* 9(1):1–3. https://doi.org/10.1038/s41598-019-49016-0

Bragdon, B. C. and Bahney, C. S. 2018. Origin of reparative stem cells in fracture healing. *Current Osteoporosis Reports* 16(4):490–503. https://doi.org/10.1007/s11914-018-0458-4

Brooks, A. E., Boss, A. L., Lehnert, K., and Dunbar, P. R. 2020. Dissecting adipose tissue derived mesenchymal stromal cell heterogeneity using single cell rna sequencing and multiparameter spectral flow cytometry. *Cytotherapy* 22(5):S70–S71. https://doi.org/10.1016/j.jcyt.2020.03.110

Caplan, A. I. 1991. Mesenchymal stem cells. *Journal of Orthopaedic Research* 9(5):641–650. https://doi.org/10.1002/jor.1100090504

Caplan, A. I. 2017a. New MSC: MSCs as pericytes are sentinels and gatekeepers. *Journal of Orthopaedic Research* 35(6):1151–1159. https://doi.org/10.1002/jor.23560

Caplan, A. I. 2017b. Mesenchymal stem cells: time to change the name! *Stem Cells Translational Medicine* 6(6):1445–1451. https://doi.org/10.1002/sctm.17-0051

Carvalho, M. M., Teixeira, F. G., Reis, R. L., Sousa, N., and Salgado, A. J. 2011. Mesenchymal stem cells in the umbilical cord: phenotypic characterization, secretome and applications in central nervous system regenerative medicine. *Current Stem Cell Research & Therapy* 6(3):221–228. https://doi.org/10.2174/157488811796575332

Chang, P. L., Blair, H. C., Zhao, X., Chien, Y. W., Chen, D., Tilden, A. B., Chang, Z., Cao, X., Faye-Petersen, O. M., and Hicks, P. 2006. Comparison of fetal and adult marrow stromal cells in osteogenesis with and without glucocorticoids. *Connective Tissue Research* 47(2):67–76. https://doi.org/10.1080/03008200600584074

Chen, S., Rani, S., Wu, Y., Unterbrink, A., Gu, T. T., Gluhak-Heinrich, J., Chuang, H. H., and MacDougall, M. 2005. Differential regulation of dentin sialophosphoprotein expression by Runx2 during odontoblast cytodifferentiation. *Journal of Biological Chemistry* 280(33):29717–29727. https://doi.org/10.1074/jbc.M502929200

Cheng, K. H., Kuo, T. L., Kuo, K. K., and Hsiao, C. C. 2011. Human adipose-derived stem cells: Isolation, characterization and current application in regeneration medicine. *Genomic Medicine, Biomarkers, and Health Sciences* 3(2):53–62. https://doi.org/10.1016/j.gmbhs.2011.08.003

Chiba, Y., Saito, K., Martin, D., Boger, E. T., Rhodes, C., Yoshizaki, K., Nakamura, T., Yamada, A., Morell, R. J., Yamada, Y., and Fukumoto, S. 2020. Single-cell RNA-sequencing from mouse incisor reveals dental epithelial cell-type specific genes. *Frontiers in Cell and Developmental Biology* 8:841. https://doi.org/10.3389/fcell.2020.00841

Christy, B. A., Herzig, M. C., Delavan, C., Cantu, C., Salgado, C., Bynum, J. A., and Cap, A. P. 2019. Human primary fibroblasts perform similarly to MSCs in assays used to evaluate MSC safety and potency. *Transfusion* 59(S2):1593–1600. https://doi.org/10.1111/trf.15187

Dadras, M., May, C., Wagner, J. M., Wallner, C., Becerikli, M., Dittfeld, S., Serschnitzki, B., Schilde, L., Guntermann, A., Sengstock, C., and Köller, M. 2020. Comparative proteomic analysis of osteogenic differentiated human adipose tissue and bone marrow-derived stromal cells. *Journal of Cellular and Molecular Medicine* 24(20):11814–11827. https://doi.org/10.1111/jcmm.15797

Davies, O. G., Cooper, P. R., Shelton, R. M., Smith, A. J., and Scheven, B. A. A 2015. Comparison of the in vitro mineralisation and dentinogenic potential of mesenchymal stem cells derived from adipose tissue, bone marrow and dental pulp. *Journal of Bone and Mineral Metabolism* 33(4):371–382. https://doi.org/10.1007/s00774-014-0601-y

Della Bella, E., Buetti-Dinh, A., Licandro, G., Ahmad, P., Basoli, V., Alini, M., and Stoddart, M. J. 2021. Dexamethasone induces changes in osteogenic differentiation of human mesenchymal stromal cells via SOX9 and PPARG, but not RUNX2. *International Journal of Molecular Sciences* 22(9):4785. https://doi.org/10.3390/ijms22094785

Di Maggio, N., Mehrkens, A., Papadimitropoulos, A., Schaeren, S., Heberer, M., Banfi, A., and Martin, I. 2012. Fibroblast growth factor-2 maintains a niche-dependent population of self-renewing highly potent non-adherent mesenchymal progenitors through FGFR2c. *Stem Cells* 30(7):1455–1464. https://doi.org/10.1002/stem.1106

Doi, H., Kitajima, Y., Luo, L., Yan, C., Tateishi, S., Ono, Y., Urata, Y., Goto, S., Mori, R., Masuzaki, H., and Shimokawa, I. 2016. Potency of umbilical cord blood-and Wharton's jelly-derived mesenchymal stem cells for scarless wound healing. *Scientific Reports* 6(1):1–10. https://doi.org/10.1038/srep18844

Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F. C., Krause, D. S., Deans, R. J., Keating, A., Prockop, D. J., and Horwitz, E. M. 2006. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8(4):315–317. https://doi.org/10.1080/14653240600855905

Dong, Q., Wang, Y., Mohabatpour, F., Zheng, L., Papagerakis, S., Chen, D., and Papagerakis, P. 2019. Dental pulp stem cells: isolation, characterization, expansion, and odontoblast differentiation for tissue engineering; pp. 91–101 in Odontogenesis, Humana Press, New York. https://doi.org/10.1007/978-1-4939-9012-2_9

D'Souza, R. N., Aberg, T., Gaikwad, J., Cavender, A., Owen, M., Karsenty, G., and Thesleff, I. 1999. Cbfa1 is required for epithelial-mesenchymal interactions regulating tooth development in mice. *Development* 126(13):2911–2920. https://doi.org/10.1242/dev.126.13.2911

Dukes, C. E. 1939. Ossification in rectal cancer. *Proceedings of the Royal Society of Medicine* 32(11):1489–1494. https://doi.org/10.1177/003591573903201153

CELL
BIOLOGY

Felthaus, O., Gosau, M., Klein, S., Prantl, L., Reichert, T. E., Schmalz, G., and Morsczeck, C. 2014. Dexamethasone-related osteogenic differentiation of dental follicle cells depends on ZBTB16 but not Runx2. *Cell and Tissue Research* 357(3):695–705. https://doi.org/10.1007/s00441-014-1891-z

Freeman, B. T., Jung, J. P., and Ogle, B. M. 2015. Single-cell RNA-Seq of bone marrow-derived mesenchymal stem cells reveals unique profiles of lineage priming. *PLoS One* 10(9):e0136199. https://doi.org/10.1371/journal.pone.0136199

Ghali, O., Broux, O., Falgayrac, G., Haren, N., van Leeuwen, J. P., Penel, G., Hardouin, P., and Chauveau, C. 2015. Dexamethasone in osteogenic medium strongly induces adipocyte differentiation of mouse bone marrow stromal cells and increases osteoblast differentiation. *BMC Cell Biology* 16(1):1–5. https://doi.org/10.1186/s12860-015-0056-6

Gomez-Stallons, M. V., Hassel, K., and Yutzey, K. E. 2020. Developmental pathways and aortic valve calcification; pp. 47–71 in Aikawa, E., Hutcheson, J. D. (eds), *Cardiovascular Calcification and Bone Mineralization*, Humana Press, Cham. https://doi.org/10.1007/978-3-030-46725-8_3

Gomez-Stallons, M. V., Wirrig-Schwendeman, E. E., Hassel, K. R., Conway, S. J., and Yutzey, K. E. 2016. Bone morphogenetic protein signaling is required for aortic valve calcification. *Arteriosclerosis, Thrombosis, and Vascular Biology* 36(7):1398–1405. https://doi.org/10.1161/ATVBAHA. 116.307526

Hardy, W. R., Moldovan, N. I., Moldovan, L., Livak, K. J., Datta, K., Goswami, C., Corselli, M., Traktuev, D. O, Murray, I. R., Péault, B., and March, K. 2017. Transcriptional networks in single perivascular cells sorted from human adipose tissue reveal a hierarchy of mesenchymal stem cells. *Stem Cells* 35(5):1273–1289. https://doi.org/10.1002/stem.2599

Harman, R. M., Patel, R. S., Fan, J. C., Park, J. E., Rosenberg, B. R., and Van de Walle, G. R. 2020. Single-cell RNA sequencing of equine mesenchymal stromal cells from primary donor-matched tissue sources reveals functional heterogeneity in immune modulation and cell motility. *Stem Cell Research & Therapy* 11(1):1–5. https://doi.org/10.1186/s13287-020-02043-5

Harrington, J., Sloan, A. J., and Waddington, R. J. 2014. Quantification of clonal heterogeneity of mesenchymal progenitor cells in dental pulp and bone marrow. *Connective Tissue Research* 55(sup1):62–67. https://doi.org/10.3109/03008207.2014.923859

Hartmann, C. and Yang, Y. 2020. Molecular and cellular regulation of intramembranous and endochondral bone formation during embryogenesis; pp. 5–44 in Bilezikian, J. P., Martin, T. J., Clemens, Th. L., and Rosen, C. J. (eds), Principles of Bone Biology, Academic Press. https://doi.org/10.1016/B978-0-12-814841-9.00001-4

Hou, W., Duan, L., Huang, C., Li, X., Xu, X., Qin, P., Hong, N., Wang, D., and Jin, W. 2021. Mesenchymal stem cell subpopulations and their heterogeneity of response to inductions revealed by single-cell RNA-seq. *bioRxiv*. Jan 1. https://doi.org/10.1101/2021.05.07.443197

Huang, L., Niu, C., Willard, B., Zhao, W., Liu, L., He, W., Wu, T., Yang, S., Feng, S., Mu, Y., Zheng, L., and Li, K. 2015. Proteomic analysis of porcine mesenchymal stem cells derived from bone marrow and umbilical cord: implication of the proteins involved in the higher migration capability of bone marrow mesenchymal stem cells. *Stem Cell Research & Therapy* 6(1):1–8. https://doi.org/10.1186/s13287-015-0061-x

Jääger, K., Islam, S., Zajac, P., Linnarsson, S., and Neuman, T. 2012. RNA-seq analysis reveals different dynamics of differentiation of human dermis-and adipose-derived stromal stem cells. *PLoS One* 7(6):e38833. https://doi.org/10.1371/journal.pone.0038833

Jaiswal, N., Haynesworth, S. E., Caplan, A. I., and Bruder, S. P. 1997. Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. *Journal of Cellular Biochemistry* 64(2):295–312. https://doi.org/10.1002/(SICI)1097-4644(199702)64:2<295::AID-JCB12>3.0.CO;2-I

Janowicz, K., Mozdziak, P., Bryja, A., Kempisty, B., and Dyszkiewicz-Konwińska, M. 2019. Human dental pulp stem cells: recent findings and current research. *Medical Journal of Cell Biology* 7(3):119–124. https://doi.org/10.2478/acb-2019-0016

Kannan, S., Ghosh, J., and Dhara, S. K. 2020. Osteogenic differentiation potential of porcine bone marrow mesenchymal stem cell subpopulations selected in different basal media. *Biology Open* 9(10). https://doi.org/10.1242/bio.053280

Katono, N., Tsuda, M., Suzuka, J., Oda, Y., Wang, L., Tanei, Z. I., Tanino, M., Ohata, T., Nagabuchi, E., Ishida, Y., and Kimura, S. 2021. Involvement of BMP and Wnt Signals Leadingto Epithelial-Mesenchymal Transition in Colon Adenocarcinoma with Heterotopic Ossification. *Annals of Clinical & Laboratory Science* 51(2):271–276.

Khalid, S., Yamazaki, H., Socorro, M., Monier, D., Beniash, E., and Napierala, D. 2020. Reactive oxygen species (ROS) generation as an underlying mechanism of inorganic phosphate (Pi)-induced mineralization of osteogenic cells. *Free Radical Biology and Medicine* 153:103–111. https://doi.org/10.1016/j.freeradbiomed.2020.04.008

Kita, K., Gauglitz, G. G., Phan, T. T., Herndon, D. N., and Jeschke, M. G. 2010. Isolation and characterization of mesenchymal stem cells from the sub-amniotic human umbilical cord lining membrane. *Stem Cells and Development* 19(4):491–502. https://doi.org/10.1089/scd.2009.0192

Kobayashi, T., Torii, D., Iwata, T., Izumi, Y., Nasu, M., and Tsutsui, T. W. 2020. Characterization of proliferation, differentiation potential, and gene expression among clonal cultures of human dental pulp cells. *Human Cell* 33:490–501. https://doi.org/10.1007/s13577-020-00327-9

Kobayashi, Y., Uehara, S., Udagawa, N., and Takahashi, N. 2016. Regulation of bone metabolism by Wnt signals. *The Journal of Biochemistry* 159(4):387–392. https://doi.org/10.1093/jb/mvv124

Komori, T., Yagi, H., Nomura, S., Yamaguchi, A., Sasaki, K., Deguchi, K., Shimizu, Y., Bronson, R. T., Gao, Y. H., Inada, M., and Sato, M. 1997. Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. *Cell* 89(5):755–764. https://doi.org/10.1016/s0092-8674(00)80258-5

Kozlowska, U., Krawczenko, A., Futoma, K., Jurek, T., Rorat, M., Patrzalek, D., and Klimczak, A. 2019. Similarities and differences between mesenchymal stem/progenitor cells derived from various human tissues. *World Journal of Stem Cells* 11(6):347–374. https://doi.org/10.4252/wjsc.v11.i6.347

Krivanek, J., Soldatov, R. A., Kastriti, M. E., Chontorotzea, T., Herdina, A. N., Petersen, J., Szarowska, B., Landova, M., Matejova, V. K., Holla, L. I., and Kuchler, U. 2020. Dental cell type atlas reveals stem and differentiated cell types in mouse and human teeth. *Nature Communications* 11(1):1–8. https://doi.org/10.1038/s41467-020-18512-7

Kumar, A., Kumar, V., Rattan, V., Jha, V., and Bhattacharyya, S. 2018. Secretome proteins regulate comparative osteogenic and adipogenic potential in bone marrow and dental stem cells. *Biochimie* 155:129–139. https://doi.org/10.1016/j.biochi.2018.10.014

Langenbach, F. and Handschel, J. 2013. Effects of dexamethasone, ascorbic acid and β-glycerophosphate on the os-

teogenic differentiation of stem cells in vitro. *Stem Cell Research & Therapy* 4(5):1–7. https://doi.org/10.1186/scrt328

Li, T. X., Yuan, J., Chen, Y., Pan, L. J., Song, C., Bi, L. J., and Jiao, X. H. 2013. Differentiation of mesenchymal stem cells from human umbilical cord tissue into odontoblast-like cells using the conditioned medium of tooth germ cells in vitro. *BioMed Research International* 218543. https://doi.org/10.1155/2013/218543

Lin, Y., Xiao, Y., Lin, C., Zhang, Q., Zhang, S., Pei, F., Liu, H., and Chen, Z. 2020. SALL1 regulates commitment of odontoblast lineages by interacting with RUNX2 to remodel open chromatin regions. *Stem Cells* 39(2):196–209. https://doi.org/10.1002/stem.3298

Lyons, F. G. and Mattei, T. A. 2019. Sources, identification, and clinical implications of heterogeneity in human umbilical cord stem cells; pp. 243–256 in *Stem Cells Heterogeneity in Different Organs*. Springer, Cham. https://doi.org/10.1007/978-3-030-24108-7_13

Marie, P. J., Miraoui, H., and Sévère, N. 2012. FGF/FGFR signaling in bone formation: progress and perspectives. *Growth Factors* 30(2):117–123. https://doi.org/10.3109/08977194.2012.656761

Mastrolia, I., Foppiani, E. M., Murgia, A., Candini, O., Samarelli, A. V., Grisendi, G., Veronesi, E., Horwitz, E. M., and Dominici, M. 2019. Challenges in clinical development of mesenchymal stromal/stem cells: concise review. *Stem Cells Translational Medicine* 8(11):1135–1148. https://doi.org/10.1002/sctm.19-0044

Mehrkens, A., Di Maggio, N., Gueven, S., Schaefer, D., Scherberich, A., Banfi, A., and Martin, I. 2014. Non-adherent mesenchymal progenitors from adipose tissue stromal vascular fraction. *Tissue Engineering Part A* 20(5–6):1081–1088. https://doi.org/10.1089/ten.TEA. 2013.0273

Méndez-Ferrer, S., Michurina, T. V., Ferraro, F., Mazloom, A. R., MacArthur, B. D., Lira, S. A., Scadden, D. T., Ma'ayan, A., Enikolopov, G. N., and Frenette, P. S. 2010. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature* 466(7308):829–834. https://doi.org/10.1038/nature09262

Mohamed-Ahmed, S., Fristad, I., Lie, S. A., Suliman, S., Mustafa, K., Vindenes, H., and Idris, S. B. 2018. Adipose-derived and bone marrow mesenchymal stem cells: a donor-matched comparison. *Stem Cell Research & Therapy* 9(1):1–5. https://doi.org/10.1186/s13287-018-0914-1

Monaco, E., Bionaz, M., Rodriguez-Zas, S., Hurley, W. L., and Wheeler, M. B. 2012. Transcriptomics comparison between porcine adipose and bone marrow mesenchymal stem cells during in vitro osteogenic and adipogenic differentiation. *PLoS One* 7(3):e32481. https://doi.org/10.1371/journal.pone.0032481

Monzack, E. L. and Masters, K. S. 2011. Can valvular interstitial cells become true osteoblasts? A side-by-side comparison. *The Journal of heart Valve Disease* 20(4):449.

Nagata, M., Ono, N., and Ono, W. 2021. Unveiling diversity of stem cells in dental pulp and apical papilla using mouse genetic models: a literature review. *Cell and Tissue Research* 383:603–616. https://doi.org/10.1007/s00441-020-03271-0

Nantavisai, S., Pisitkun, T., Osathanon, T., Pavasant, P., Kalpravidh, C., Dhitavat, S., Makjaroen, J., and Sawangmake, C. 2020. Systems biology analysis of osteogenic differentiation behavior by canine mesenchymal stem cells derived from bone marrow and dental pulp. *Scientific Reports* 10(1):1–8. https://doi.org/10.1038/s41598-020-77656-0

Omatsu, Y., Sugiyama, T., Kohara, H., Kondoh, G., Fujii, N., Kohno, K., and Nagasawa, T. 2010. The essential functions of adipo-osteogenic progenitors as the hematopoietic stem and progenitor cell niche. *Immunity* 33(3):387–399. https://doi.org/10.1016/j.immuni.2010.08.017

Phinney, D. G. 2012. Functional heterogeneity of mesenchymal stem cells: implications for cell therapy. *Journal of Cellular Biochemistry* 113(9):2806–2812. https://doi.org/10.1002/jcb.24166

Pitkänen, S. 2020. In vitro and in vivo osteogenesis and vasculogenesis in synthetic bone grafts. PhD thesis.

Prazeres, P. H., Sena, I. F., da Terra Borges, I., de Azevedo, P. O., Andreotti, J. P., de Paiva, A. E., de Almeida, V. M., de Paula Guerra, D. A., Dos Santos, G. S., Mintz, A., and Delbono, O. 2017. Pericytes are heterogeneous in their origin within the same tissue. *Developmental Biology* 427(1):6–11. https://doi.org/10.1016/j.ydbio.2017.05.001

Rahman, M. S., Akhtar, N., Jamil, H. M., Banik, R. S., and Asaduzzaman, S. M. 2015. TGF-β/BMP signaling and other molecular events: regulation of osteoblastogenesis and bone formation. *Bone Research* 3(1):1–20. https://doi.org/10.1038/boneres.2015.5

Rosa, V., Della Bona, A., Cavalcanti, B. N., and Nör, J. E. 2012. Tissue engineering: from research to dental clinics. *Dental Materials* 28(4):341–348. https://doi.org/10.1016/j.dental.2011.11.025

Rutkovskiy, A., Malashicheva, A., Sullivan, G., Bogdanova, M., Kostareva, A., Stensløkken, K. O., Fiane, A., and Vaage, J. 2017. Valve interstitial cells: the key to understanding the pathophysiology of heart valve calcification. *Journal of the American Heart Association* 6(9):e006339. https://doi.org/10.1161/JAHA. 117.006339

Salasznyk, R. M., Williams, W. A., Boskey, A., Batorsky, A., and Plopper, G. E. 2004. Adhesion to vitronectin and collagen I promotes osteogenic differentiation of human mesenchymal stem cells. *Journal of Biomedicine and Biotechnology* 2004(1):24–34. https://doi.org/10.1155/S1110724304306017

Salhotra, A., Shah, H. N., Levi, B., and Longaker, M. T. 2020. Mechanisms of bone development and repair. *Nature Reviews Molecular Cell Biology* 21:696–711. https://doi.org/10.1038/s41580-020-00279-w

Saugspier, M., Felthaus, O., Viale-Bouroncle, S., Driemel, O., Reichert, T. E., Schmalz, G., and Morsczeck, C. 2010. The differentiation and gene expression profile of human dental follicle cells. *Stem Cells and Development* 19(5):707–717. https://doi.org/10.1089/scd.2010.0027

Sawa, N., Fujimoto, H., Sawa, Y., and Yamashita, J. 2019. Alternating differentiation and dedifferentiation between mature osteoblasts and osteocytes. *Scientific Reports* 9(1):1–9. https://doi.org/10.1038/s41598-019-50236-7

Semenova, D., Bogdanova, M., Kostina, A., Golovkin, A., Kostareva, A., and Malashicheva, A. 2020. Dose-dependent mechanism of Notch action in promoting osteogenic differentiation of mesenchymal stem cells. *Cell and Tissue Research* 379(1):169–179. https://doi.org/10.1007/s00441-019-03130-7

Shen, C., Yang, C., Xu, S., and Zhao, H. 2019. Comparison of osteogenic differentiation capacity in mesenchymal stem cells derived from human amniotic membrane (AM), umbilical cord (UC), chorionic membrane (CM), and decidua (DC). *Cell & Bioscience* 9(1):1–11. https://doi.org/10.1186/s13578-019-0281-3

Shi, S. and Gronthos, S. 2003. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. *Journal of Bone and Mineral Research* 18(4):696–704. https://doi.org/10.1359/jbmr.2003.18.4.696

Shin, S., Lee, J., Kwon, Y., Park, K. S., Jeong, J. H., Choi, S. J., Bang, S. I., Chang, J. W., and Lee, C. 2021. Comparative proteomic analysis of the mesenchymal stem cells secretome from adipose, bone marrow, placenta and Wharton's jelly. *International Journal of Molecular Sciences* 22(2):845. https://doi.org/10.3390/ijms22020845

CELL BIOLOGY

Subramanian, A., Fong, C. Y., Biswas, A., and Bongso, A. 2015. Comparative characterization of cells from the various compartments of the human umbilical cord shows that the Wharton's jelly compartment provides the best source of clinically utilizable mesenchymal stem cells. *PLoS One* 10(6):e0127992. https://doi.org/10.1371/journal.pone.0127992

Sugiyama, T., Kohara, H., Noda, M., and Nagasawa, T. 2006. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. *Immunity* 25(6):977–988. https://doi.org/10.1016/j.immuni.2006.10.016

Sun, C., Wang, L., Wang, H., Huang, T., Yao, W., Li, J., and Zhang, X. 2020. Single-cell RNA-seq highlights heterogeneity in human primary Wharton's jelly mesenchymal stem/stromal cells cultured in vitro. *Stem Cell Research & Therapy* 11:1–6. https://doi.org/10.1186/s13287-020-01660-4

Supakul, S., Yao, K., Ochi, H., Shimada, T., Hashimoto, K., Sunamura, S., Mabuchi, Y., Tanaka, M., Akazawa, C., Nakamura, T., and Okawa, A. 2019. Pericytes as a source of osteogenic cells in bone fracture healing. *International Journal of Molecular Sciences* 20(5):1079. https://doi.org/10.3390/ijms20051079

Suzuki, J., Kanauchi, N., Endo, M., Hamada, A., and Watanabe, H. 2019. Pulmonary adenocarcinoma with heterotopic ossification. *The Japanese Journal of Thoracic Surgery* 72(5):363–366.

Tada, H., Nemoto, E., Foster, B. L., Somerman, M. J., and Shimauchi, H. 2011. Phosphate increases bone morphogenetic protein-2 expression through cAMP-dependent protein kinase and ERK1/2 pathways in human dental pulp cells. *Bone* 48(6):1409–1416. https://doi.org/10.1016/j.bone.2011.03.675

Tikhonova, A. N., Dolgalev, I., Hu, H., Sivaraj, K. K., Hoxha, E., Cuesta-Domínguez, Á., Pinho, S., Akhmetzyanova, I., Gao, J., Witkowski, M., and Guillamot, M. 2019. The bone marrow microenvironment at single-cell resolution. *Nature* 569(7755):222–228. https://doi.org/10.1038/s41586-019-1104-8

Vijay, J., Gauthier, M. F., Biswell, R. L., Louiselle, D. A., Johnston, J. J., Cheung, W. A., Belden, B., Pramatarova, A., Biertho, L., Gibson, M., Simon, M.-M., Djambazian, H., Staffa, A., Bourque, G., Laitinen, A., Nystedt, J., Vohl, M.-C., Fraser, J. D., Pastinen, T., Tchernof, A., and Grundberg, E. 2020. Single-cell analysis of human adipose tissue identifies depot-and disease-specific cell types. *Nature Metabolism* 2(1):97–109. https://doi.org/10.1038/s42255-019-0152-6

Wang, H. H., Cui, Y. L., Zaorsky, N. G., Lan, J., Deng, L., Zeng, X. L., Wu, Z. Q., Tao, Z., Guo, W. H., Wang, Q. X., and Zhao, L. J. 2016. Mesenchymal stem cells generate pericytes to promote tumor recurrence via vasculogenesis after stereotactic body radiation therapy. *Cancer Letters* 375(2):349–359. https://doi.org/10.1016/j.canlet.2016.02.033

Whitfield, M. J., Lee, W. C., and Van Vliet, K. J. 2013. Onset of heterogeneity in culture-expanded bone marrow stromal cells. *Stem Cell Research* 11(3):1365–1377. https://doi.org/10.1016/j.scr.2013.09.004

Wolock, S. L., Krishnan, I., Tenen, D. E., Matkins, V., Camacho, V., Patel, S., Agarwal, P., Bhatia, R., Tenen, D. G., Klein, A. M., and Welner, R. S. 2019. Mapping distinct bone marrow niche populations and their differen-

tiation paths. *Cell Reports* 28(2):302–311. https://doi.org/10.1016/j.celrep.2019.06.031

Wu, L., Zhu, F., Wu, Y., Lin, Y., Nie, X., Jing, W., Qiao, J., Liu, L., Tang, W., Zheng, X., and Tian, W. 2008. Dentin sialophosphoprotein-promoted mineralization and expression of odontogenic genes in adipose-derived stromal cells. *Cells Tissues Organs* 187(2):103–112. https://doi.org/10.1159/000110079

Xie, H., Chua, M., Islam, I., Bentini, R., Cao, T., Viana-Gomes, J. C., Neto, A. H., and Rosa, V. 2017. CVD-grown monolayer graphene induces osteogenic but not odontoblastic differentiation of dental pulp stem cells. *Dental Materials* 33(1):e13–21. https://doi.org/10.1016/j.dental.2016.09.030

Xu, R., Hu, J., Zhou, X., and Yang, Y. 2018. Heterotopic ossification: mechanistic insights and clinical challenges. *Bone* 109:134–142. https://doi.org/10.1016/j.bone.2017.08.025

Yamazaki, T. and Mukouyama, Y. S. 2018. Tissue specific origin, development, and pathological perspectives of pericytes. *Frontiers in Cardiovascular Medicine* 5:78. https://doi.org/10.3389/fcvm.2018.00078

Yang, Y., Liu, Z., Zhao, W., Huang, L., Wu, T., Mu, Y., and Li, K. 2019. Integrated analysis of DNA methylome and transcriptome reveals the differences in biological characteristics of porcine mesenchymal stem cells from bone marrow and umbilical cord. *Research Square* https://doi.org/10.21203/rs.2.9875/v1

Yianni, V. and Sharpe, P. T. 2019. Perivascular-derived mesenchymal stem cells. *Journal of Dental Research* 98(10):1066–1072. https://doi.org/10.1177/0022034519862258

Yu, S., Zhao, Y., Ma, Y., and Ge, L. 2016. Profiling the secretome of human stem cells from dental apical papilla. *Stem Cells and Development* 25(6):499–508. https://doi.org/10.1089/scd.2015.0298

Zajdel, A., Kałucka, M., Kokoszka-Mikołaj, E., and Wilczok, A. 2017. Osteogenic differentiation of human mesenchymal stem cells from adipose tissue and Wharton's jelly of the umbilical cord. *Acta Biochimica Polonica* 64(2):365–369. https://doi.org/10.18388/abp.2016_1488

Zha, K., Li, X., Yang, Z., Tian, G., Sun, Z., Sui, X., Dai, Y., Liu, S., and Guo, Q. 2021. Heterogeneity of mesenchymal stem cells in cartilage regeneration: from characterization to application. *NPJ Regenerative Medicine* 6(1):1–5. https://doi.org/10.1038/s41536-021-00122-6

Zhang, S., Wang, J. Y., Li, B., Yin, F., and Liu, H. 2021. Single-cell transcriptome analysis of uncultured human umbilical cord mesenchymal stem cells. *Stem Cell Research & Therapy* 12(1):1–5. https://doi.org/10.1186/s13287-020-02055-1

Zhou, W., Lin, J., Zhao, K., Jin, K., He, Q., Hu, Y., Feng, G., Cai, Y., Xia, C., Liu, H., and Shen, W. 2019. Single-cell profiles and clinically useful properties of human mesenchymal stem cells of adipose and bone marrow origin. *The American Journal of Sports Medicine*. 47(7):1722–1733. https://doi.org/10.1177/0363546519848678

Zhou, B. O., Yue, R., Murphy, M. M., Peyer, J. G., and Morrison, S. J. 2014. Leptin-receptor expressing mesenchymal stromal cells represent the main source of bone formed by adult bone marrow. *Cell Stem Cell* 15(2):154–168. https://doi.org/10.1016/j.stem.2014.06.008

Zuttion, M. S., Câmara, D. A., Dariolli, R., Takimura, C., Wenceslau, C., and Kerkis, I. 2019. In vitro heterogeneity of porcine adipose tissue-derived stem cells. *Tissue and Cell* 58:51–60. https://doi.org/10.1016/j.tice.2019.04.001