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REVIEW

The tissue specific nature of mesenchymal stem/stromal cells: Gaining better understanding for improved clinical outcomes

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Mesenchymal stem/stromal cells (MSCs) are multipotent progenitors that are derived from most adult tissue as well as cord blood and placenta. MSCs are defined by their adherent nature, ability to propagate in culture and capacity to differentiate into bone, fat, and cartilage. However, many studies have shown that MSCs, derived from different tissues, differ both in their in situ and in vitro phenotypes. Despite abundance of MSCs studies, little is known about the molecular events that control their tissue specific nature. Two recent studies comparing MSCs derived from different tissues have now found clues to the molecular mechanisms that control the tissue specific nature of these cells. In the first, the superior genomic stability of adipose derived MSCs (ASCs), compared to bone marrow (BM) MSCs, was explained by reduced H19 long non-coding RNA expression, and increased p53 activity of ASCs. In the second, a comparison of abdominal and subcutaneous ASCs revealed poor propagation, differentiation and migration capacities of abdominal ASCs that is explained by their increased tendency to over-accumulate reactive oxygen species (ROS) in culture. ROS over production in abdominal ASCs was shown to be controlled by the NADPH oxidase NOX1. The unique features of MSCs derived from different tissues suggest a tissue specific molecular signature arising from the tissue of origin that is retained during culture. The implications of this phenomenon on our understanding of the role and function of MSCs *in situ* as well as on their clinical utilization, is discussed.

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What defines a Mesenchymal stem/stromal cells (MSCs)?

Mesenchymal cells from bone marrow (BM) and adipose tissues were isolated and propagated under tissue culture conditions since the 1960's [1-3]. The concept of multipotent mesenchymal cells that are responsible for the repair and turnover of adult tissue was later suggested and the term mesenchymal stem cells (MSCs) was coined to indicate their multipotent differentiation potential. Based on the MSC concept, a new field of cell based regenerative medicine has emerged [4]. MSCs have since been isolated from various adult tissues, as well as from cord blood and placenta [5], and their clinical potential as a regenerative or immunosuppressive tool was appreciated leading to a dramatic increase in MSCs clinical and pre-clinical research [6]. Given that an important clinical requirement for any pharmaceutical treatment, including cell therapy, is it's uniform and reproducible nature, a set of unifying characteristics for all MSCs was suggested at 2006 [7]. The characteristics in these guidelines were based on the common properties of MSCs that include their plastic adherent nature, their multipotent differentiation ability and a set of positive

surface markers that appear on all MSCs but are expressed also on other cell types.

Are in situ MSC progenitors and cultured MSCs that arise from different tissues the same?

In spite of the common properties of MSCs that were listed in the International Society for Cellular Therapy (ISCT) guidelines, significant differences between mesenchymal stem/stromal cells that were derived from different tissues were observed since their identification. Differences in the relative ease of propagation and differentiation spectrum of mesenchymal cells that were isolated from perirenal and epididymal fat were observed [8-10]. Following these early studies many subsequent ones have now reported differences in the propagation and differentiation abilities of MSCs that were isolated from different tissues, as was recently reviewed in [11]. The tissue specific nature of MSCs became most evident during the continuous search for a unique MSC surface marker that would allow their isolation and identification *in situ.* As was recently reviewed by LV *et al* [12] most of the suggested markers (Stro-1, CD27, SSEA-4 etc.) are heterogeneously expressed on MSCs derived from different tissues. This tissue specificity is also observed in the case of CD34, which is uniquely expressed in adipose derived MSCs (ASCs). CD34 expression on ASCs was reported to be transient (in early passages) or continuous (20 weeks) [13, 14]. The *in situ* origin of MSCs is also a matter of debate. The perivascular niche was proposed as an origin for MSCs of various tissues [15], but it was recently shown that CD34 positive ASCs (CD34+ cells were demonstrated to be responsible for the CFU-F capabilities of stromal fat cells) do not localize in proximity to blood vessels and are therefore not of perivascular origin [16]. Craniofacial-bone MSCs that were able to give rise to all craniofacial bones in the adult and were activated during injury repair were also not associated with vasculature [17].

Can the tissue specific signature of mesenchymal cells be maintained despite the culture adaptation of MSCs?

The tissue specific phenotype of MSCs is most likely controlled by a molecular signature that originates from their *in situ* environmental niche. MSCs undergo a drastic transformation/selection process during their culture cultivation starting from a heterogeneous population to become a relatively homogenous one in which the majority of cells express what is termed mesenchymal stem cells markers, such as CD90, CD29, CD105, CD73, among others. This homogenous expression is in sharp contrast to the heterogeneous marker expression in the original stromal vascular fraction (SVF)/BM populations that express CD45, CD34, CD31 as well as the mesenchymal markers but to a much lower extent. Given the drastic transition of MSCs during culture it is unclear whether the tissue specific signature would be maintained under culture conditions. Alternatively, the proposed signature may still be evident in the more heterogeneous but early progenitor population. In two recently published studies we therefore compared BM marrow derived MSCs to adipose derived MSCs (ASCs) [18] and abdominal ASCs (aASCs) to subcutaneous ASCs (scASCs) [19]. It was found that ASCs are more genetically stable than BM MSCs and demonstrate a more homogenous nature in their response to changing or stressful conditions. ASCs from abdominal and subcutaneous sources were found to differ in their propagation, fat differentiation and migration potentials. All of these tissue specific traits were reproducible in many repeats of MSC preparations and were demonstrated in cultured MSCs that were propagated under identical conditions. Importantly, it was shown that the phenotypic differences between MSCs from different tissues could be explained by the differential expression of specific genes that controlled their phenotype. It thus seems that the tissue specific signature of MSCs can be studied in cultured MSCs and not only in early progenitors immediately following their isolation.

Why are ASCs more gnomically stable and less tumorigenic than BM MSCs?

In a recent work [20] a clear correlation between the expression levels of H19, a long non-coding RNA, which also serves as a precursor for mir675, and the tendency of mouse BM MSCs to become polyploid in culture was demonstrated. More specifically, it was observed that BM MSCs rarely maintain a diploid genome in culture, as most populations become polyploid. In BM MSCs, polyploidy appeared to be a more stable state than diploidy as diploid BM MSCs were significantly more tumorigenic following subcutaneous administration, and expressed much higher levels of H19. The correlation between H19 expression and the tendency of BM MSCs to become polyploid was further substantiated in another recent publication that compared the genomic stability of BM MSCs to that of ASCs [18]. Altogether, it was found that 76% (13 out of 17 cell preparations) of mouse BM MSCs preparations, demonstrated a polyploid phenotype compared to only 9% polyploidy (3 out of 32 cell preparations) of ASCs. The reduced tendency of ASCs to become polyploid was demonstrated under various conditions that included hypoxic (3% oxygen) and normoxic (21% oxygen) conditions and a range of culture media. Importantly, under both hypoxic and normoxic conditions diploid ASCs demonstrated a dramatically reduced H19 expression compared to polyploid BM MSCs (43 and 59 fold respectively) further supporting

the idea that lower H19 expression is correlated with increased genomic stability and reduced tumorigenicity. The exact role of H19 in the maintenance of genomic stability and in the process of cellular transformation is unclear. However, previous studies showed that increased H19 expression levels can serve as a marker for tumor cells [21-24], which lead to its use as a clinical bio-marker in the treatment of various cancer types [25]. In the recent work [20] it was unveiled that H19 expression is higher in proliferating cells, and is also elevated dramatically in cells exposed to UV light. It is therefore possible that H19 expression facilitates the ability of normal cells to adjust to stressful conditions. In cancerous cells, however, unregulated expression of H19 promotes uncontrolled cell proliferation. The PI3K/Akt pathway is a critical regulator of the transition of diploid hepatocytes to a tetraploid state after weaning [26], and also plays a role in epithelial-mesenchymal transition (EMT), tumor metastasis and H19 expression [23]. PI3K/Akt may thus take part in both H19 regulation and the transition of BM MSCs from a diploid to a tetraploid state.

Is p53 activity involved in ASCs increased genomic stability?

It was previously proposed that tetraploidy leads to cell transformation and tumor formation and is therefore prevented by the tumor suppressor p53 [27]. The recent study [20] suggested that in some instances the opposite is true, namely tetraploidy is necessary for p53 wild type cells to remain non-tumorigenic. It was additionally found that the basal p53 activity, evident by the RNA expression of various p53 target genes, was significantly higher in ASCs compared to BM MSCs [18]. Interestingly, three different DNA damage stresses (UV eradiation, oxidative stress by H_20_2 and doxorubicin) all resulted in a significant p53 activation in both tetraploid BM MSCs and in ASCs demonstrating the presence of a wild type p53 in these cells. It thus seems that the increased basal p53 activity together with the reduced H19 expression contributes to the superior ability of ASCs to remain diploid [18]. Importantly, a non-transformed state is achieved in different ways in ASCs and BM MSCs, as the former maintain a diploid genome whereas the latter become tetraploid.

Do ASCs, derived from different adipose tissues, differ in fundamental properties such as their propagation, fat differentiation and migration potential?

The tissue specific nature of MSCs was further demonstrated in a comparison of aASCs and scASCs which revealed superior propagation, fat differentiation and migration capacities of the latter [19]. The poor expansion ability of aASCs, which was translated into cytostasis at early passages, was found to occur because of increased apoptosis. Importantly, cytostasis and apoptosis were correlated with ROS accumulation and increased NOX1 expression in aASCs that was not observed in scASCs. Inhibition of NOX1 by a specific inhibitor (ML171) reduced ROS accumulation and apoptosis of aASCs and allowed their long term expansion, substantiating the importance of NOX1 activity in aASCs cytostasis. NOX1 specific inhibition was able also to improve aASCs fat differentiation and migration capabilities reaffirming NOX1 role in aASCs tissue specific phenotype. As was previously described, MSCs undergo adaptation to tissue culture conditions following their isolation. One of this adaptations is the adjustment of ASCs to atmospheric oxygen level (21% oxygen) which is much higher than physiological oxygen levels within the tissue (3-6% oxygen in fat) [28]. The findings presented in the recent study indicate that NOX1 induced ROS accumulation resulted from the adaptation of aASCs to atmospheric oxygen conditions since aASCs that were cultured at 3% oxygen did not display increased NOX1 and ROS levels and were able to undergo long-term expansion in culture. In conclusion, ASCs derived from different tissues seem to harbor a tissue specific molecular signature, displayed by a discriminant gene expression profile that leads to tissue specific phenotypes.

Does the MSC in vitro phenotype reflect the physiological role of MSC progenitors within the tissue?

The function of MSCs *in vivo* is largely unknown, however, they are believed to play an important role in tissue homeostasis, protection and regeneration. In order to survive under and react to stressful and rapidly changing conditions, MSCs must retain a plastic nature. One of the manifestation of the plastic nature of MSCs is the heterogeneous phenotypes that are displayed by distinct BM MSC preparations in culture [29]. Indeed, in a study examining the reaction of MSCs to different toll-like receptor (TLR) ligands, it was found that distinct MSC preparations respond with great variability to different types of treatments [30]. A different aspect of the plasticity of MSCs was discovered in a recent study showing that a single cell isolation of MSCs triggered a spontaneous reprogramming event which allowed the cells to acquire new differentiation potentials [31]. This reprogramming event, however, occurred only in normoxic conditions, and not in cells cultured in 3% oxygen. Thus, it is possible that within the niche, MSCs are protected from changing oxygen levels, and other environmental cues (e.g. TLR ligands) and thus retain a stable phenotype. Once they migrate out of the niche towards damaged tissue sites and are exposed to new environments, their plastic nature allows them to survive and take part in recovery processes. In sharp contrast to BM MSCs, ASCs appear to be more homogenous, however, oxygen availability still plays an important role in their behavior [19].

Another indication for the possible *in vivo* functions of MSCs comes from the recent findings which demonstrate the tendency of aASCs to accumulate ROS in culture [19]. The role of increased ROS accumulation in adipose tissue during obesity and its involvement in the development of the metabolic syndrome is well established [32-35]. The involvement of NOX enzymes in ROS production in adipose tissue during obesity has also been demonstrated [33, 34, 36-38]. The development of the metabolic syndrome is accompanied by accumulation of pro-inflammatory cytokines. Importantly, obesity is attributed to abdominal/visceral fat rather than subcutaneous fat [39]. In light of the above, our recent findings regarding the increased tendency of aASCs and not of scASCs to express NOX enzymes, accumulate reactive oxygen species, accumulate pro-inflammatory cytokines and undergo extensive apoptosis, may provide novel mechanistic clues to the involvement of ASCs progenitors in the development of the metabolic syndrome [19]. This notion is further substantiated by the claim that MSCs are derived from a perivascular cells [15] and by the many evidences that connect ROS production by vascular cells to the development of obesity: NOX1 was shown to be expressed in vascular smooth muscle cells, and its mRNA expression was up-regulated and activated by vascular pathological stimuli [40, 41]. Overexpressing of ph22phox (a subunit of the NOX complex) in vascular cells was recently shown to play a causal role in development of the metabolic syndrome indicating that overexpression of ROS in vascular cells may precede obesity [42]. Perivascular adipose tissue (PVAT) was shown to express NOX and secrete ROS influencing hypertension and the metabolic syndrome [32, 38, 43, 44]. The stromal vascular fraction (SVF) from which ASCs are derived is composed largely by PVAT [13]. Further studies of the specific nature of abdominal ASCs may provide additional mechanistic indications to the genetic signature that promote ASCs phenotype and possibly ROS accumulation in obese individuals.

Does the epigenetic state of MSCs dictate their tissue specific traits?

The underlying basis for tissue specific cell phenotypes is most likely epigenetic. It was previously shown that induced pluripotent stem cells (iPSCs) carry an epigenetic memory from their tissue of origin [45]. It is therefore possible that the distinction between MSCs that are derived from different tissues could be made based on their epigenetic profile. Bisulfite sequencing revealed, however, that DNA methylation of lineage specific promoters of MSCs derived from different tissues is similar [46]. In contrast, our previous findings showed that H4K20me1 modulation is a key event in the reprogramming of BM MSCs [31]. Thus, it is possible that although tissue specific MSCs display similar DNA methylation marks they can still display an epigenetic profile with distinct features. To uncover this possibility, further studies comparing different histone marks between tissue specific MSCs are needed.

Conclusions

Although all MSCs are multipotent and plastic adherent, accumulating data now suggest that tissue specific MSCs differ in basic and fundamental properties that may have a critical effect on their clinical efficacy. This understanding should be harnessed to the development of distinct propagation and treatment protocols for MSCs that are isolated from different tissues. Gaining deeper understanding of the tissue specific traits of MSCs and their molecular signatures will not only assist in designing such protocols but may also shed light on the physiological properties of MSC progenitors *in situ*.

References

1. Friedenstein A, Chailakhjan R, Lalykina K. The development of fibroblast colonies in monolayer cultures of guinea−pig bone marrow and spleen cells. Cell Proliferation 1970;3:393-403.
2. Ng C, Poznanski W, Borowiecki M, Reimer G. Differences in growth in vitro of adipose cells from normal and obese patients. Nature 1971;231:445.
3. Rodbell M. The metabolism of isolated fat cells. J Biol Chem 1964;239:375-380.
4. Caplan AI. Mesenchymal stem cells. J Orthop Res 1991;9:641-650.
5. Hass R, Kasper C, Bohm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): a comparison of adult and neonatal tissue-derived MSC. Cell Commun Signal 2011;9:12.
6. Ren G, Chen X, Dong F, Li W, Ren X, Zhang Y, *et al*. Concise review: mesenchymal stem cells and translational medicine: emerging issues. Stem Cells Transl Med 2012;1:51-58.
7. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, *et al*. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315-317.
8. Djian P, Roncari A, Hollenberg C. Influence of anatomic site and age on the replication and differentiation of rat adipocyte precursors in culture. J Clin Invest 1983;72:1200.
9. Djian P, Roncari D, Hollenberg C. Adipocyte precursor clones vary in capacity for differentiation. Metabolism 1985;34:880-883.
10. Kirkland JL, Hollenberg CH, Gillon WS. Age, anatomic site, and the replication and differentiation of adipocyte precursors. Am J Physiol 1990;258:C206-C210.
11. Via AG, Frizziero A, Oliva F. Biological properties of mesenchymal Stem Cells from different sources. Muscles Ligaments Tendons J 2012;2:154.
12. Lv F-J, Tuan RS, Cheung K, Leung VY. Concise review: the

surface markers and identity of human mesenchymal stem cells. Stem cells 2014;32:1408-1419.

13. Bourin P, Bunnell BA, Casteilla L, Dominici M, Katz AJ, March KL, *et al*. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). Cytotherapy 2013;15:641-648.
14. Yoshimura K, Shigeura T, Matsumoto D, Sato T, Takaki Y, Aiba-Kojima E, *et al*. Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates. J Cell Physiol 2006;208:64-76.
15. Crisan M, Yap S, Casteilla L, Chen C-W, Corselli M, Park TS, *et al*. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell stem cell 2008;3:301-313.
16. Maumus M, Peyrafitte J-A, D'Angelo R, Fournier-Wirth C, Bouloumié A, Casteilla L, *et al*. Native human adipose stromal cells: localization, morphology and phenotype. Int J Obes 2011;35:1141-1153.
17. Zhao H, Feng J, Ho T-V, Grimes W, Urata M, Chai Y. The suture provides a niche for mesenchymal stem cells of craniofacial bones. Nat cell biology 2015;17:386–396.
18. Ravid O, Shoshani O, Sela M, Weinstock A, Sadan TW, Gur E, *et al*. Relative genomic stability of adipose tissue derived mesenchymal stem cells: analysis of ploidy, H19 long non-coding RNA and p53 activity. Stem Cell Res Ther 2014;5:139.
19. Sela M, Tirza G, Ravid O, Volovitz I, Solodeev I, Friedman O, *et al*. NOX1-induced accumulation of reactive oxygen species in abdominal fat derived mesenchymal stromal cells impinges on long-term proliferation. Cell Death Dis 2015;6:e1728.
20. Shoshani O, Massalha H, Shani N, Kagan S, Ravid O, Madar S, *et al*. Polyploidization of murine mesenchymal cells is associated with suppression of the long noncoding RNA H19 and reduced tumorigenicity. Cancer Res 2012;72:6403-6413.
21. Chan L, Wang W, Yeung W, Deng Y, Yuan P, Mak K. Hedgehog signaling induces osteosarcoma development through Yap1 and H19 overexpression. Oncogene 2013.
22. Matouk IJ, DeGroot N, Mezan S, Ayesh S, Abu-lail R, Hochberg A, *et al*. The H19 non-coding RNA is essential for human tumor growth. PloS one 2007;2:e845.
23. Matouk IJ, Raveh E, Abu-lail R, Mezan S, Gilon M, Gershtain E, *et al*. Oncofetal H19 RNA promotes tumor metastasis. Biochim Biophys Acta 2014;1843:1414-1426.
24. Shi Y, Wang Y, Luan W, Wang P, Tao T, Zhang J, *et al*. Long non-coding RNA H19 promotes glioma cell invasion by deriving miR-675. PloS one 2014;9:e86295.
25. Matouk I, Raveh E, Ohana P, Lail RA, Gershtain E, Gilon M, *et al*. The increasing complexity of the oncofetal h19 gene locus: functional dissection and therapeutic intervention. Int J Mol Sci 2013;14:4298-4316.
26. Celton-Morizur S, Merlen G, Couton D, Margall-Ducos G, Desdouets C. The insulin/Akt pathway controls a specific cell division program that leads to generation of binucleated tetraploid liver cells in rodents. J Clin Invest 2009;119:1880.
27. Aylon Y, Oren M. p53: guardian of ploidy. Mol Oncol 2011;5:315-323.
28. Trayhurn P. Hypoxia and adipose tissue function and dysfunction in obesity. Physiol Rev 2013;93:1-21.
29. Pevsner-Fischer M, Levin S, Zipori D. The origins of mesenchymal stromal cell heterogeneity. Stem Cell Rev 2011;7:560-568.
30. Levin S, Pevsner-Fischer M, Kagan S, Lifshitz H, Weinstock A, Gataulin D, *et al*. Divergent Levels of LBP and TGFβ1 in Murine MSCs Lead to Heterogenic Response to TLR and Proinflammatory Cytokine Activation. Stem Cell Rev 2014;10:376-388.
31. Shoshani O, Ravid O, Massalha H, Aharonov A, Ovadya Y, Pevsner−Fischer M, *et al*. Cell isolation induces fate changes of bone marrow mesenchymal cells leading to loss or alternatively to acquisition of new differentiation potentials. Stem Cells 2014;32:2008-2020.
32. Datla SR, Griendling KK. Reactive oxygen species, NADPH oxidases, and hypertension. Hypertension 2010;56:325-330.
33. Elnakish MT, Hassanain HH, Janssen PM, Angelos MG, Khan M. Emerging role of oxidative stress in metabolic syndrome and cardiovascular diseases: important role of Rac/NADPH oxidase. J Pathol 2013;231:290-300.
34. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, *et al*. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004;114:1752.
35. Touyz RM. Reactive oxygen species, vascular oxidative stress, and redox signaling in hypertension what is the clinical significance? Hypertension 2004;44:248-252.
36. Fortuño A, San José G, Moreno MU, Beloqui O, Díez J, Zalba G. Phagocytic NADPH oxidase overactivity underlies oxidative stress in metabolic syndrome. Diabetes 2006;55:209-215.
37. Greenstein AS, Khavandi K, Withers SB, Sonoyama K, Clancy O, Jeziorska M, *et al*. Local inflammation and hypoxia abolish the protective anticontractile properties of perivascular fat in obese patients. Circulation 2009;119:1661-1670.
38. Ketonen J, Shi J, Martonen E, Mervaala E. Periadventitial adipose tissue promotes endothelial dysfunction via oxidative stress in diet-induced obese C57Bl/6 mice. Circ J 2010;74:1479-1487.
39. Tchernof A, Després J-P. Pathophysiology of human visceral obesity: an update. Physiol Rev 2013;93:359-404.
40. Lassègue B, Sorescu D, Szöcs K, Yin Q, Akers M, Zhang Y, *et al*. Novel gp91phox homologues in vascular smooth muscle cells nox1 mediates angiotensin II–induced superoxide formation and redox-sensitive signaling pathways. Circ Res 2001;88:888-894.
41. Lyle AN, Griendling KK. Modulation of vascular smooth muscle signaling by reactive oxygen species. Physiology 2006;21:269-280.
42. Youn J-Y, Siu KL, Lob HE, Itani H, Harrison DG, Cai H. Role of vascular oxidative stress in obesity and metabolic syndrome. Diabetes 2014;63:2344-2355.
43. Gao Y-J, Takemori K, Su L-Y, An W-S, Lu C, Sharma AM, *et al*. Perivascular adipose tissue promotes vasoconstriction: the role of superoxide anion. Cardiovasc Res 2006;71:363-373.
44. Szasz T, Bomfim GF, Webb RC. The influence of perivascular adipose tissue on vascular homeostasis. Vasc Health Risk Manag

2013;9:105.

45. Kim K, Doi A, Wen B, Ng K, Zhao R, Cahan P, *et al*. Epigenetic memory in induced pluripotent stem cells. Nature 2010;467:285-290.

46. Sørensen AL, Timoskainen S, West FD, Vekterud K, Boquest AC, Ährlund-Richter L, *et al*. Lineage-specific promoter DNA methylation patterns segregate adult progenitor cell types. Stem Cells Dev 2009;19:1257-1266.