On the origin of human adipocytes and the contribution of bone marrow-derived cells

Mikael Rydén
Karolinska Institutet, Department of Medicine (H7), Karolinska University Hospital, Huddinge, Stockholm, Sweden

ABSTRACT
In the last decade, results in both animal models and humans have demonstrated that white adipocytes are generated over the entire life-span. This adds to the plasticity of adipose tissue and alterations in adipocyte turnover are linked to metabolic dysfunction. Adipocytes are derived from precursors present primarily in the perivascular areas of adipose tissue but their precise origin remains unclear. The multipotent differentiation capacity of bone marrow-derived cells (BMDC) has prompted the suggestion that BMDC may contribute to different cell tissue pools, including adipocytes. However, data in murine transplantation models have been conflicting and it has been a matter of debate whether BMDC actually differentiate into adipocytes or just fuse with resident fat cells. To resolve this controversy in humans, we recently performed a study in 65 subjects that had undergone bone marrow transplantation. Using a set of newly developed assays including single cell genome-wide analyses of mature adipocytes, we demonstrated that bone marrow contributes with approximately 10 % to the adipocyte pool. This proportion was more than doubled in obesity, suggesting that BMDC may constitute a reserve pool for adipogenesis, particularly upon weight gain. This commentary discusses the possible relevance of these and other recent findings for human pathophysiology.

Because white adipose tissue (WAT) is the most expandable organ in humans, the formation of new fat cells should be expected to be a continuous process not only in children but also in adult individuals. This notion is supported by the observation that, throughout the entire life span, obese subjects display twice the number of white fat cells compared with age-matched normal weight individuals. However, it wasn’t until the development of 14C-dating techniques that it could be conclusively demonstrated that there is a ~10 % annual turnover of adult human fat cells. Turnover is determined by the balance between adipocyte generation and fat cell death. Although this is independent of WAT mass, the significantly higher amounts of adipocytes in the obese state implies that the absolute number of generated fat cells per year is much larger compared with that in lean individuals.

Variations in the capacity to generate new fat cells may be of pathophysiological importance. Thus, subjects with adipose hypertrophy (few but large fat cells), display significantly reduced adipocyte turnover rates compared with age and body weight matched subjects with hyperplasia (many small fat cells). As adipose hypertrophy associates with insulin resistance/type 2 diabetes, the possibility to influence adipogenesis and adipocyte number and thereby metabolic phenotype could have therapeutic implications. This hypothesis is to some extent corroborated by the antidiabetic actions of thiazolidinediones. These agents improve systemic insulin sensitivity, in part by increasing the differentiation of adipocyte progenitor cells resulting in adipose hyperplasia. Unfortunately, their side effects, primarily mediated via actions in other tissues, have limited their clinical use in recent years.

A fundamental question in understanding fat cell formation relates to the origin of adipocytes. While it is clear that they arise from precursor cells present in the perivascular stroma, it is not yet known when, how or from where they migrate into the tissue. A major obstacle in studies of adipogenesis in vivo is the fact that adipocyte progenitors are not distinctly identifiable by cell surface markers although different panels of epitopes have been suggested. This indicates that adipocytes may arise from different progenitor cells, a notion supported by recent data in animal models demonstrating that fat cells during development and in the adult state, originate from independent adipose precursor compartments through specific molecular mechanisms.
Furthermore, in mice, adult adipogenesis appears to occur preferentially in visceral WAT depots, which suggests that subcutaneous and visceral adipocytes may also have distinct precursor cells. Identification of the spectrum of adipocyte progenitors would allow for a much better understanding of how WAT mass expands during embryonic development as well as in childhood and adulthood and possibly also explain the inter-individual variations in metabolic phenotype observed upon weight gain.

Bone Marrow (BM) contains different sets of stem cells, including the haematopoietic stem cells and the less abundant, non-haematopoietic mesenchymal stem cells (MSCs). Although it is currently a matter of debate whether the former can develop into cells outside the haematopoietic lineage, MSCs have raised substantial interest given their well-established capacity to develop into functional cells of the mesenchymal lineage e.g. osteocytes, chondrocytes and adipocytes. Following BM transplantation, several investigators have assessed the contribution of BM-derived cells (BMDCs) to different human tissues including brain, liver and buccal epithelium demonstrating that a significant proportion of the cells are donor-derived. However, in these older studies, the approach to determine the presence of donor-derived cells was to analyze the presence of Y-chromosomes in female recipients transplanted with BM from male donors. Apart from limiting the study population, the fact that most of these studies were performed on sections and/or bulk preparations of cells, cannot exclude contamination and/or cell/nuclear fusion events that could account for the detection of Y-chromosomes. In fact, a number of studies, primarily in animal models, have suggested that cell fusion is the major mechanism explaining why BM transplantation results in the presence of donor-derived sequences in neurons, hepatocytes and cardiomyocytes.

With regard to WAT, several groups have used allogeneic BM transplantation in mice to study the contribution of BMDCs to selected WAT depots. Using BM from transgenic donor animals expressing green fluorescent protein under different promoters, these investigators have come to divergent conclusions ranging from insignificant (i.e. <1%) to significant (i.e., up to 25%) proportions of donor cells in the fat cell pool. In an effort to settle this matter in human WAT, we performed a study in adult human subjects that had undergone transplantation with BM or mobilized peripheral blood stem cells (PBSCs) due to leukemia. All transplanted subjects who were fully recovered, without any ongoing immunosuppression, and lived within a reasonable distance from our hospital were identified. This enabled us to include a total of 65 subjects (transplanted 3–31 y prior to the investigation), all of which underwent fine-needle biopsies of subcutaneous abdominal WAT. We opted to use 2 independent qPCR-based methods assessing the levels of short donor-specific sequences in microsatellites or single nucleotide polymorphisms (SNPs). The advantage with this approach was that it allowed us to determine the proportion of donor-derived cells irrespective of the gender of the recipient and donor. The percentage of donor-derived cells in purified fat cells was variable and averaged around 5%. Microscopic analyses and qPCR for different non-adipocyte markers showed that our fat cell fractions were pure, without any detectable leukocytes (which by definition are all donor-derived), suggesting that our results were not an artifact due to contaminating leukocytes or other cell types. This conclusion was further supported by the observation that there was a linear increase in donor cell infiltration following time since transplantation (r = 0.34, p = 0.006). If contamination would have been an issue, the percentage of donor-derived sequences would have been evenly distributed among all samples. The proportion of obese subjects (n = 11) corresponded to that in the general Swedish population (around 15–20%) and the percentage of donor-derived cells was twice as high in this subgroup indicating that BM-derived adipocytes are present to a higher degree in the obese state.

The percentage of donor-derived adipocytes in WAT is a rough estimate and does not necessarily reflect the infiltration of BMDCs over the entire life span. To estimate this figure we developed a mathematical model to calculate the contribution of donor cells at steady state which was termed the “production ratio” and is expressed as percent of the total fat cell pool. This revealed that in the entire cohort, approximately 10% of the fat cell population was BM-derived. While this proportion was not influenced by donor/recipient age, gender and/or different transplantation-related parameters (e.g., cell dose, irradiation, graft versus host reactions etc.), body weight exerted a significant effect as there was a linear relationship between BMI and the production ratio (r = 0.44, p = 0.0002). Thus, the production ratio was more than 2-fold higher in obese compared with lean subjects. Taken together, these findings indicate that BMDCs constitute a significant, albeit not the major, reservoir for developing fat cells. This is particularly evident in obesity, a condition where there is an increased demand for adipocyte precursors in order to both enable tissue expansion and retain WAT mass. It should be pointed out that the production ratio varied significantly even between BMI-matched subjects. This could depend on several factors, e.g. the degree of vascularity in WAT which could impact on the ability of BMDCs to infiltrate.
the tissue. It is also possible that other intrinsic properties of the recipient’s WAT may facilitate or attenuate BMDC migration. In future studies we aim to determine the global gene expression in WAT from BMI-matched transplanted subjects in order to identify transcriptional signatures associated with high or low production ratios.

A major caveat with these results and most other studies in this field is that determination of donor-derived cells are based on bulk analyses of short stretches of donor-derived sequences. As discussed above, it is theoretically possible that donor cells, e.g., macrophages, may fuse with recipient fat cells resulting in the detection of donor-derived sequences in purified fat cells. To exclude this possibility we had to develop techniques to retrieve individual mature fat cells and to analyze their full content of donor/recipient DNA. A major obstacle when working with adipocytes is that they are fragile and float in suspension which makes them notoriously difficult to study at the single cell level. By embedding fat cell suspensions in low-temperature melting agarose we could identify individual adipocytes containing a single nucleus and a large triglyceride-containing lipid droplet, confirming that the cells exhibited all the morphological characteristics of a mature adipocyte. Single cells were then isolated by laser capture microdissection. We tested several approaches to detect donor and recipient-derived sequences including the trinucleotide threading technique which allows for multiplex analyses of single nucleotide polymorphisms (SNPs). However, these approaches proved to be too insensitive for the small amounts of DNA that could be isolated and resulted in allelic drop-outs.

After several attempts and technical developments, we were able to perform exome sequencing of homozygous SNPs unique for either the donor or the recipient. These were identified by comparing exome sequences of whole blood genomic DNA and the positions were then called in the single cell exome data as either donor, recipient or mixed genotypes. As laser capture microdissection is a very time consuming approach, we aimed to isolate a sufficient number of cells per subject to allow detection of at least one fat cell with donor-derived sequences. The single cells were obtained from 3 recipients with an approximate donor cell proportion of 5–10% and after quality filtering steps we had 15, 24 and 27 cells to analyze from each individual, respectively. As expected, the majority of the cells contained only recipient-specific SNPs. However, 2 cells (one cell each in 2 recipients) displayed entirely donor-derived SNPs, demonstrating that the nuclear DNA originated only from the donor. Interestingly, 2 cells (one cell each in 2 recipients) displayed mixed genotypes with both donor- and recipient-derived sequences. The presence of both donor- and mixed sequences was subsequently confirmed by genome-wide sequencing of 3 cells from one of the subjects. These results demonstrate for the first time at the single cell level the presence of mature fat cells containing a fully donor-derived DNA sequence. This suggests that BMDCs have the capacity to differentiate into mature fat cells, at least in the setting of BM/ PBSC transplantation. The mixed cells are somewhat more difficult to explain. It is possible that BMDCs may fuse with recipient cells which after reduction divisions result in mononuclear cells with heterokaryons containing sequences from both the donor and the recipient. It would therefore have been of great interest to determine ploidy in the cells. Unfortunately, this was not possible to do due to the limited material and difficulties in performing concomitant sequence and chromosome analyses. At present, the physiological/pathophysiological role of differentiation vs. cell fusion remains unclear.

Interestingly, Dwight Klemm and co-workers, a group that has done seminal work on the contribution of BMDCs to murine adipogenesis, recently reported data on flow cytometry-purified subcutaneous abdominal adipocytes from 8 subjects 12–53 months after haematopoietic stem cell transplantation. Similar to us, they observed a significant proportion (ranging from <5 to 35%) of donor-derived fat cells by quantifying microsatellite polymorphisms in bulk preparations of pure adipocytes. More importantly, using 2 independent methods for ploidy analysis (DNA fluorescence intensity by flow cytometry and FISH for 8 different chromosomes) they found no evidence of polysomy. These results confirm our conclusion that the presence of donor-derived adipocytes cannot simply be explained by cell fusion resulting in tetra- or aneuploid cells. Furthermore, they support the notion that adipocytes with a mixed genetic profile may be generated via more complex mechanisms, e.g. involving reduction division. The work by Gavin et al complements and extends our data in other aspects as well. Thus, in analogy with our findings, they found a time-dependent increase in donor cell chimerism. Moreover, in 3 subjects, repeated biopsies were obtained which enabled them to demonstrate that the fraction of donor fat cells in the same subject increased over time. However, in contrast to our results they did not observe any impact of BMI, which could depend on the relatively small sample size, the shorter follow-up and the fact that only 2 of the subjects had a BMI > 30 kg/m². Nevertheless, it is worth to mention that the highest infiltration rate in their study was observed in one of the obese individuals.

It is important to stress that neither of these studies have established whether the phenotype of fat cells containing donor-derived DNA differs from that of recipient
cells. This is relevant given that data in mice suggest that BM-derived adipocytes, in comparison with recipient fat cells, display higher expression of pro-inflammatory genes and lower expression of genes involved in mitochondrial biogenesis and lipid oxidation. It would therefore be of particular interest to determine the global gene expression to see whether cells with a fully donor-derived DNA differ from recipient cells and whether adipocytes with mixed DNA display an intermediate phenotype. Unfortunately, it is currently not possible to analyze both the genome and transcriptome from the same single cell.

Perhaps an even more relevant issue is to identify the exact BMDC that differentiates into the adipocyte lineage. Our own interpretation is that it is less probably of haematopoietic origin. As mentioned above, this notion is based on the fact that although transdifferentiation may occur in vitro, most investigators suggest that haematopoietic stem cells cannot cross lineage boundaries. Moreover, bulk preparations of fat cells expressed no detectable amounts of haematopoietic markers such as CD45 and CD11b. A more plausible origin are therefore MSCs which can be found in both BM and PBSC, although this, for the moment, remains a speculation. Additional cell sources could be endothelial cells as recently suggested in murine models. Finally, we cannot exclude the possibility that the mixed and donor-derived cells may arise from different cell types. For example, it is conceivable that the mixed cells may result from fusion with specific BMDCs that lack the capacity to differentiate into adipocytes.

Admittedly, the results discussed herein have been obtained in an artificial situation and it is not known whether they pertain also to normal physiology. However, the time-dependent increase in donor cell infiltration, in subjects without any on-going immunosuppressant therapy, suggests that BMDC-derived adipogenesis is a continuous process and that BM may contribute to WAT also outside the setting of BM transplantation. Unfortunately, this is not possible to determine at the moment as there are no available techniques in man to assess the developmental origin of mature fat cells (or any other cell for that matter). Nevertheless, the recent findings in mice discussed above, suggesting that fat cells may arise from distinct precursor pools, would be in line with the notion that BM may constitute one of several pools of progenitors in the normal growth of WAT. Future development of techniques allowing identification of cellular origin also under non-transplanted conditions will hopefully resolve these issues.

Taken together, although several questions remain unanswered due to technical limitations, our work adds the following, it suggests for the first time at the single cell level that human BMDCs contribute to the white subcutaneous fat cell population in vivo and that BMDC may constitute a clinically relevant reserve pool for adipocyte progenitors particularly upon weight gain. These data are corroborated by independent results demonstrating a time-dependent increase in diploid donor-derived adipocytes. Identification of the BMDC(s) that display adipogenic potential could enable future approaches allowing tissue engineering of genetically dysfunctional WAT, e.g. in severe forms of lipodystrophy.

Disclosure of potential conflicts of interest
No potential conflicts of interest were disclosed.

Acknowledgments
I would like to acknowledge the hard work of all co-authors that contributed to this study. In particular Joanna Hard, Jeff Mold and Erik Borgström who developed the single cell capture method and analysis. I would also like to thank all the transplanted subjects that were willing to participate.

References

[1] Knittle JL, Timmers K, Ginsberg-Fellner F, Brown RE, Katz DP. The growth of adipose tissue in children and adolescents. Cross-sectional and longitudinal studies of adipose cell number and size. J Clin Invest 1979; 63:239-46; PMID:429551; http://dx.doi.org/10.1172/JCI109295
[2] Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, Blomqvist L, Hoffstedt J, Näslund E, Britton T, et al. Dynamics of fat cell turnover in humans. Nature 2008; 453:783-7; PMID:18454136; http://dx.doi.org/10.1038/nature06902
[3] Arner E, Westermark PO, Spalding KL, Britton T, Ryden M, Frisen J, Bernard S, Arner P. Adipocyte turnover: relevance to human adipose tissue morphology. Diabetes 2010; 59:105-9; PMID:19846802; http://dx.doi.org/10.2337/db09-0942
[4] Lonn M, Mehlig K, Bengtsson C, Lissner L. Adipocyte size predicts incidence of type 2 diabetes in women. Faseb J 2010; 24:326-31; PMID:19741173; http://dx.doi.org/10.1096/fj.09-133058
[5] Weyer C, Foley JE, Bogardus C, Tataranni PA, Pratley RE. Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts type II diabetes independent of insulin resistance. Diabetologia 2000; 43:1498-506; PMID:11151758; http://dx.doi.org/10.1007/s001250051560
[6] Arner P, Arner E, Hammarstedt A, Smith U. Genetic pre-disposition for Type 2 diabetes, but not for overweight/obesity, is associated with a restricted adipogenesis. PLoS One 2011; 6.e18284; PMID:21532749; http://dx.doi.org/10.1371/journal.pone.0018284
[7] Frayn KN, Tan GD, Karpe F. Adipose tissue: a key target for diabetes pathophysiology and treatment? Horm
M. RYDÉN

Metab Res 2007; 39:739-42; PMID:17952837; http://dx.doi.org/10.1055/s-2007-990270

[8] Della-Morte D, Palmiotta R, Rehni AK, Pastore D, Capuani B, Pacifci F, De Marchis ML, Dave KR, Bellia A, Fogliame G, et al. Pharmacogenomics and pharmacogenetics of thiazolidinediones: role in diabetes and cardiovascular risk factors. Pharmacogenomics 2014; 15:2063-82; PMID:25521362; http://dx.doi.org/10.2217/pps.14.162

[9] Crisan M, Yap S, Castella L, Chen CW, Corselli M, Park TS, Andrioli G, Sun B, Zheng B, Zhang L, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 2008; 3:301-13; PMID:18786417; http://dx.doi.org/10.1016/j.stem.2008.07.003

[10] Lin G, Garcia M, Ning H, Banie L, Guo YL, Lue TF, Lin CN. Defining stem and progenitor cells within adipose tissue. Stem Cells Dev 2008; 17:1053-63; PMID:18597617; http://dx.doi.org/10.1089/scd.2008.0117

[11] Zannettino AC, Paton S, Arthur A, Khor F, Itescu S, Stoltz JF, de Isla N, Li YP, Bensoussan D, Zhang L, Hwang S, Gardner R, Neutzel S, Sharkis SJ. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 2001; 105:369-77; PMID:11348593; http://dx.doi.org/10.1016/S0092-8674(01)00328-2

[12] Berry R, Jeffery E, Rodeheffer MS. Weighing in on adipocyte precursors. Cell Metab 2014; 19:8-20; PMID:24239569; http://dx.doi.org/10.1016/j.cmet.2013.10.003

[13] Berry R, Rodeheffer MS. Characterization of the adipocyte cellular lineage in vivo. Nat Cell Biol 2013; 15:302-8; PMID:23434825; http://dx.doi.org/10.1038/nctb2696

[14] Church CD, Berry R, Rodeheffer MS. Isolation and study of adipocyte precursors. Methods Enzymol 2014; 537:31-46; PMID:24480340; http://dx.doi.org/10.1016/B978-0-12-411619-1.00003-3

[15] Jeffery E, Church CD, Holtrup B, Colman L, Rodeheffer MS. Rapid depot-specific activation of adipocyte precursor cells at the onset of obesity. Nat Cell Biol 2015; 17:376-85; PMID:25730471; http://dx.doi.org/10.1038/nctb3122

[16] Jiang Y, Berry DC, Tang W, Graff JM. Independent stem cell lineages regulate adipose organogenesis and adipose homeostasis. Cell Rep 2014; 9:1007-22; PMID:25437556; http://dx.doi.org/10.1016/j.celrep.2014.09.049

[17] Wang QA, Tao C, Gupta RK, Scherer PE. Tracking adipogenesis during white adipose tissue development, expansion and regeneration. Nat Med 2013; 19:1338-44; PMID:23995282; http://dx.doi.org/10.1038/nm.3324

[18] Stoltz JF, de Isla N, Li YP, Bensoussan D, Zhang L, Heselstein C, Chen Y, Decot V, Magdalou J, Li N, et al. Stem Cells and Regenerative Medicine: Myth or Reality of the 21st Century. Stem Cells Int 2015; PMID:26300923; http://dx.doi.org/10.1155/2015/734731

[19] Porada CD, Atala AJ, Almeida-Porada G. The hematopoietic system in the context of regenerative medicine. Methods 2015; PMID:26319943

[20] Terada N, Hamazaki T, Oka M, Hoki M, Muster DZ, Nakano Y, Meyer EM, Morel L, Petersen BE, Scott EW. Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature 2002; 416:542-5; PMID:11932747; http://dx.doi.org/10.1038/nature730

[21] Wang X, Willenbring H, Akkari Y, Torimaru Y, Foster M, Al-Dhalimi M, Lagasse E, Finegold M, Olson S, Grompe M. Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature 2003; 422:897-901; PMID:12665832; http://dx.doi.org/10.1038/nature01531

[22] Wurmser AE, Nakashima K, Summers RG, Toni N, D’Amour KA, Lie DC, Gage FH. Cell fusion-independent differentiation of neural stem cells to the endothelial lineage. Nature 2004; 430:350-6; PMID:15254537; http://dx.doi.org/10.1038/nature02604

[23] Anderson DJ, Gage FH, Weissman IL. Can stem cells cross lineage boundaries? Nat Med 2001; 7:393-5; PMID:11283651; http://dx.doi.org/10.1038/86439

[24] Krause DS, Theise ND, Collector MI, Henegarou O, Wang S, Gardner R, Neutzel S, Sharkis SJ. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 2001; 105:369-77; PMID:11348593; http://dx.doi.org/10.1016/S0092-8674(01)00328-2

[25] Wagers AJ, Sherwood RI, Christensen JL, Weissman IL. Little evidence for developmental plasticity of adult hematopoietic stem cells. Science 2002; 297:2256-9; PMID:12215650; http://dx.doi.org/10.1126/science.1074807

[26] Sera Y, LaRue AC, Moussa O, Mehrrota M, Duncan JD, Williams CR, Nishimoto E, Schulte BA, Watson PM, Watson DK, et al. Hematopoietic stem cell origin of adipocytes. Exp Hematol 2009; 37:1108-20, 20 e1-4

[27] Pittenger MF, MacKay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonett DW, Craig S, Marshall DR. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284:143-7; PMID:10102814; http://dx.doi.org/10.1126/science.284.5411.143

[28] Kobolak J, Dinnyes A, Memic A, Khademhosseini A, Mobasher I. Mesenchymal stem cells: Identification, phenotypic characterization, biological properties and potential for regenerative medicine through biomaterial micro-engineering of their niche. Methods 2015; pii: S1046-2023(15)30092-X. PMID:26384580; http://dx.doi.org/10.1016/j.ymeth.2015.09.016. [Epub ahead of print]

[29] Cogle CR, Yachnis AT, Laywell ED, Zander DS, Wingard JR, Steindler DA, Scott EW. Bone marrow transdifferentiation in brain after transplantation: a retrospective study. Lancet 2004; 363:1432-7; PMID:15121406; http://dx.doi.org/10.1016/S0140-6736(04)16102-3

[30] Crain BJ, Tran SD, Mezey E. Transplanted human bone marrow cells generate new brain cells. J Neurol Sci 2005; 233:121-3; PMID:15949500; http://dx.doi.org/10.1016/j.jns.2005.03.017

[31] Mezey E, Key S, Vogelsang G, Szalayova I, Lange GD, Crain B. Transplanted bone marrow generates new neurons in human brains. Proc Natl Acad Sci U S A 2003; 100:1364-9; PMID:12538864; http://dx.doi.org/10.1073/pnas.0336479100

[32] Weimann JM, Johansson CB, Trejo A, Blau HM. Stable reprogrammed heterokaryons form spontaneously in Purkinje neurons after bone marrow transplant. Nat Cell Biol 2003; 5:959-66; PMID:14562057; http://dx.doi.org/10.1038/nctb1053

[33] Tran SD, Pillemer SR, Dutra A, Barrett AJ, Brownstein MJ, Key S, Pak E, Leakan RA, Kingman A, Yamada KM, et al. Differentiation of human bone marrow-derived cells into buccal epithelial cells in vivo: a molecular analytical study.
[35] ten Hove WR, Verspaget HW, Barge R, Lamers CB, van Hoek B. Liver chimerism after allogeneic blood stem cell transplantation. Transplant Proc 2007; 39:231-6; PMID:17275511; http://dx.doi.org/10.1016/j.transproceed.2006.10.022

[36] Theise ND, Nimmakayalu M, Gardner R, Illei PB, Morgan G, Teperman L, Henegariu O, Krause DS. Liver from bone marrow in humans. Hepatology 2000; 32:11-6; PMID:10869283; http://dx.doi.org/10.1053/jhep.2000.9124

[37] Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, Fike JR, Lee HO, Pfeffer K, Lois C, Morrison SJ, Alvarez-Buylla A. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. Nature 2003; 425:968-73; PMID:14555960; http://dx.doi.org/10.1038/nature02069

[38] Nern C, Wolff I, Macas J, von Randow J, Scharenberg C, Priller J, Momma S. Fusion of hematopoietic cells with Purkinje neurons does not lead to stable heterokaryon formation under noninvasive conditions. J Neurosci 2009; 29:3799-807; PMID:19321776; http://dx.doi.org/10.1523/JNEUROSCI.5848-08.2009

[39] Johansson CB, Youssef S, Koleckar K, Holbrook C, Doyonnas R, Corbel SY, Steinman L, Rossi FM, Blau HM. Extensive fusion of hematopoietic cells with Purkinje neurons in response to chronic inflammation. Nat Cell Biol 2008; 10:575-83; PMID:18425116; http://dx.doi.org/10.1038/ncb1720

[40] Vassilopoulos G, Wang PR, Russell DW. Transplanted bone marrow regenerates liver by cell fusion. Nature 2003; 422:901-4; PMID:12665833; http://dx.doi.org/10.1038/nature01539

[41] Koh YJ, Kang S, Lee HJ, Choi TS, Lee HS, Cho CH, Koh GY. Bone marrow-derived circulating progenitor cells fail to transdifferentiate into adipocytes in adult adipose tissues in mice. J Clin Invest 2007; 117:3684-95; PMID:18060029; http://dx.doi.org/10.1172/JCI32504

[42] Tomiyama K, Murase N, Stolz DB, Toyokawa H, O'Donnell DR, Smith DM, Dudas JR, Rubin JP, Marra KG. Characterization of transplanted green fluorescent protein+ bone marrow cells into adipose tissue. Stem Cells 2008; 26:330-8; PMID:17975222; http://dx.doi.org/10.1634/stemcells.2007-0567

[43] Crossno JT, Jr., Majka SM, Grazia T, Gill RG, Klemm DJ. Rosiglitazone promotes development of a novel adipocyte population from bone marrow-derived circulating progenitor cells. J Clin Invest 2006; 116:3220-8; PMID:17143331; http://dx.doi.org/10.1172/JCI28510

[44] Majka SM, Fox KE, Psilas JC, Helm KM, Childs CR, Acosta AS, Janssen RC, Friedman JE, Woessner BT, Shade TR, et al. De novo generation of white adipocytes from the myeloid lineage via mesenchymal intermediates is age, adipose depot, and gender specific. Proc Natl Acad Sci U S A 2010; 107:14781-6; PMID:20679227; http://dx.doi.org/10.1073/pnas.1003512107

[45] Ryden M, Uzunel M, Hard JL, Borgstrom E, Mold JE, Arner E, Mejert N, Andersson DP, Widlund Y, Hassam M, et al. Transplanted Bone Marrow-Derived Cells Contribute to Human Adipogenesis. Cell Metab 2015; 22:408-17; PMID:26190649; http://dx.doi.org/10.1016/j.cmet.2015.06.011

[46] Zajac P, Pettersson E, Gryn M, Lundberg J, Ahmadian A. Expression profiling of signature gene sets with trinucleotide threading. Genomics 2008; 91:209-17; PMID:18061398; http://dx.doi.org/10.1016/j.ygeno.2007.10.012

[47] Gavin KM, Gutman JA, Kohrt WM, Wei Q, Shea KL, Miller HL, Sullivan TM, Erickson PF, Helm KM, Acosta AS, et al. De novo generation of adipocytes from circulating progenitor cells in mouse and human adipose tissue. Faseb J 2015; pii: fj.15-27899. PMID:26581599 [Epub ahead of print]

[48] Fernandez M, Simon V, Herrera G, Cao C, Del Favero H, Minguell JI. Detection of stromal cells in peripheral blood progenitor cell collections from breast cancer patients. Bone Marrow Transplant 1997; 20:265-71; PMID:9285540; http://dx.doi.org/10.1038/sj.bmt.1705358

[49] Kassis I, Zangi L, Rivkin R, Levdansky I, Samuel S, Marx G, Gorodetsky R. Isolation of mesenchymal stem cells from G-CSF-mobilized human peripheral blood using fibrin microbeads. Bone Marrow Transplant 2006; 37:967-76; PMID:16670702; http://dx.doi.org/10.1038/sj.bmt.1705358

[50] Tran KV, Gealekman O, Frontini A, Zingaretti MC, Morroni M, Giordano A, Smorlesi A, Perugini J, De Matteis R, Sbarbati A, et al. The vascular endothelium of the adipose tissue gives rise to both white and brown fat cells. Cell Metab 2012; 15:222-9; PMID:22326223; http://dx.doi.org/10.1016/j.cmet.2012.01.008